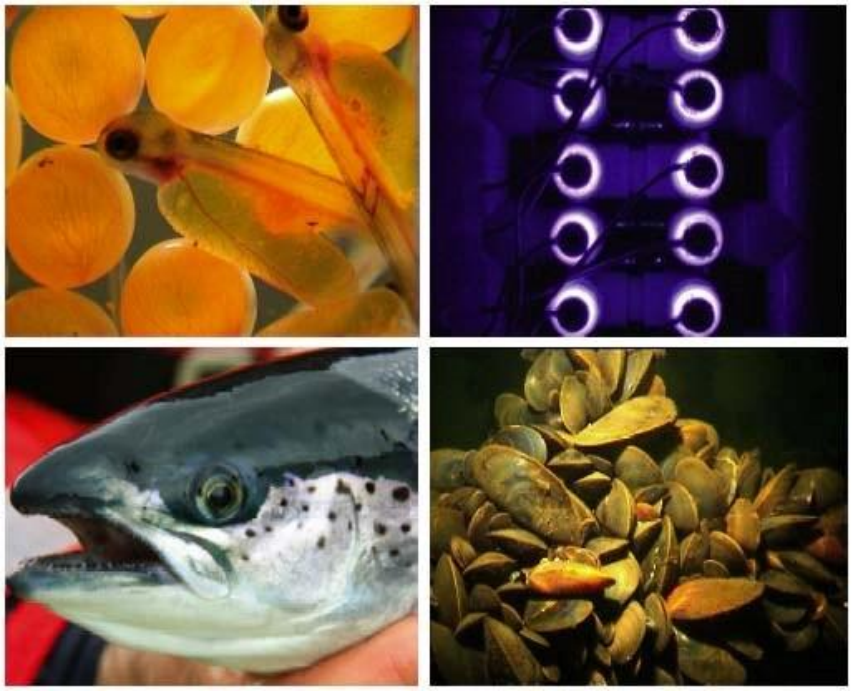
SARFSP001 - Assessment of the viability of the different life stages of Lepeophtheirus salmonis following exposure to hydrogen peroxide



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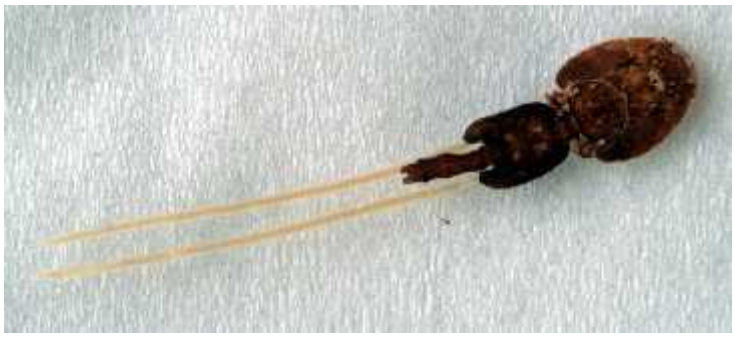
Assessment of the Viability of the Different Life Stages of *Lepeophtheirus salmonis* (Krøyer, 1837), Following Exposure to Hydrogen Peroxide



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Project Details

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1. Executive Summary

Hydrogen peroxide (H_2O_2) is one of two topical treatments licenced in Scotland for use by the Scottish salmon industry against sea lice infestations which cost the industry in excess of £30 million annually (Costello 2009). The chemical was first used in Scottish salmonid aquaculture during the early 1990s but, because of the availability of the in-feed treatment emamectin benzoate, H_2O_2 fell from favour (Bruno and Raynard 1994). More recently, with evidence of reduced efficacy among some sea lice populations to emamectin benzoate (Carmichael *et al*. 2013; Jones *et al.* 2013; Igboeli *et al*. 2013), and because hydrogen peroxide is currently the only practical treatment for AGD in Scotland, there is renewed interest in its use.

The current project, commissioned by the Scottish Aquaculture Research Forum (SARF), is intended to investigate the effect of hydrogen peroxide on the viability of different stages of *L. salmonis*. The overall goal is to fine tune criteria for the application of H_2O_2 under different temperature regimes to support existing sea lice management strategies. To achieve this goal, a number of objectives were outlined by SARF as part of the contract. These were:

1. Literature review to ascertain state of knowledge on how hydrogen peroxide affects different life stages of organisms such as *L. salmonis*: what are the target physiological processes and/or structures.
2. Design and complete lab-based experiments to validate or quantify the dose-response aspects of hydrogen peroxide treatment on different life stages of *L. salmonis*, under different temperature conditions - as likely to be experienced on Scottish salmon farms.
3. Organise and hold a workshop for industry representatives presenting data from laboratory trials, and then, after discussion with attendees, cross reference the results obtained with practical, on-farm treatment experience.
4. Develop a concise Best Practice Manual for the use of hydrogen as a treatment for *L. salmonis* in Scottish aquaculture.

In total four different trials were carried out to validate or quantify the response of *L. salmonis* lifecycle stages to different concentrations of H_2O_2 and the main findings were –

- At 96 h following exposure to 300-3000 ppm H_2O_2, the number of inactivated adult *L. salmonis* was significantly higher with increasing concentration. By 96 h all adult *L. salmonis*, regardless of H_2O_2 concentration, were active at 10 °C. In contrast at 13 °C, at all H_2O_2 concentrations, a few lice remained inactive even after 96 h and were found to be dead.
- Following exposure of adult lice to 1500 ppm H_2O_2 for 20 min at 10 °C, no significant effect on their ability to re-attach to Atlantic salmon 1 h post exposure was detected. In contrast, exposure to 1800 ppm H_2O_2 resulted in a significant decrease in attachment.
- Treatment of early chalimus with H_2O_2 delayed development from late chalimus to early pre-adult stages by approximately one developmental stage compared to untreated control lice. There appeared to be a difference between male and female lice, with development of females being delayed more than that of males at 1500 ppm.

- Hatching ability of egg strings exposed to 600-1800 ppm H_2O_2, at 10 °C or 13 °C, was significantly reduced compared to controls, even at the lowest concentration of 600 ppm and at the lower temperature of 10 °C. No significant difference was found between hatching and subsequent larval survival of *L. salmonis* from egg strings at different levels of maturity. Data also indicated there was no significant difference between the treatments, indicating that doses above the manufacturer's recommended level of 1500 ppm for 20 min are not required for inactivation.

Data generated by the four area of studies, in combination with information provided by industry at a workshop, allowed a number of recommendations to be made. These were:

- at higher temperatures exposure of adult *L. salmonis* to 1800 ppm H_2O_2 would result in an extended period of inactivation compared to a treatment at 1500 ppm. This could result in a longer period for dispersal of detached lice, reducing the risk of re-infection of the treated site, although wind and current speed/direction should be taken into account to reduce the potential risk of re-settlement on stock in neighbouring sites. It is acknowledged that this may be difficult to implement effectively.

- tank trials demonstrated that H_2O_2 did not kill the chalimus stage but did cause a one stage delay in maturation. Therefore, due to the variation in susceptibility of the different lifecycle stages found on farmed Atlantic salmon, it is recommended for effective long term lice management that sites utilise a range of therapeutants on a rotational basis.

- treatment with H_2O_2 appears to be highly effective at compromising the egg strings of *L. salmonis* resulting in high levels of hatching failure. Where hatching does occur, greatly reduced numbers of larvae successfully moult through to the infective copepodid stage. However, any larvae that do survive could form the basis of a resistant population. Therefore, post treatment counts should be carried out one week post treatment to determine how successful a treatment has been. A sample should be taken of any surviving lice for bio-assay/sensitivity analysis to determine any alterations in treatment selection.

2. Introduction

The Scottish salmon farming industry began in 1970 following a feasibility study carried out during the mid–1960s by Unilever Research Laboratories (Rae 2002). The farming of Atlantic salmon (*Salmo salar* L.) has rapidly expanded in Scotland with production increasing from 6,900 tonnes in 1985 to 163,234 tonnes in 2013 with a retail value of £700 million (Committee 2006; Anonymous 2014). With the development of the marine salmon farming industry have come infestations of ubiquitous, endemic ectoparasites generally referred to as "sea lice". The two principal reported species infecting Scottish farmed salmonids are *Lepeophtheirus salmonis* (Krøyer, 1837) and *Caligus elongatus* (Nordmann, 1832) (Laird and Needham 1991; Ritchie *et al*. 1993; Pike and Wadsworth 2000; Murray and Peeler 2005). Of the two species, *L. salmonis* is more commonly associated with infestations on Scottish farmed Atlantic salmon. The associated costs to the Scottish aquaculture industry from sea lice infestations through treatments, associated labour costs and mortalities were estimated by Costello (2009) to be in excess of £30 million in 2009.

Hydrogen peroxide (H_2O_2) is one of two topical treatments licenced in Scotland for use against sea lice infestations of cultured salmonids. The chemical was first used in Scottish salmonid aquaculture during the early 1990s but, because of issues of toxicity to the fish, particularly at higher water temperatures, and the availability of the in-feed treatment emamectin benzoate, H_2O_2 fell from favour. More recently, with evidence of reduced efficacy among some sea lice populations to emamectin benzoate, (Carmichael *et al*. 2013; Jones *et al*. 2013; Igboeli *et al*. 2013) and because H_2O_2 is currently the only practical treatment for amoebic gill disease (AGD) in Scotland, there is renewed interest in its use.

Several groups have demonstrated that H_2O_2 does not kill pre-adult/adult (mobile) stages at doses/treatment durations consistent with fish welfare, and that treated lice are able to reattach to the host (Johnson *et al*. 1993; McAndrew *et al*. 1998). However, effective doses of H_2O_2 cause the mobile stages to detach from the host so that these treatments do achieve at least temporary clearance of the most destructive life stages.

Research on mobile pre-adult and adult *L. salmonis* has found that lice are temporarily immobilised by H_2O_2 concentrations of 1000 - 2000 ppm, at temperatures off 7.5 - 11 °C and at salinities off 28 - 34.5 (Thomassen 1993; Johnson *et al*. 1993; Bruno and Raynard 1994; McAndrew *et al*. 1998; Treasurer *et al*. 2000). Higher concentrations of hydrogen peroxide can cause mortality in mobile lice, but these can also result in mortality of the salmon (Johnson *et al*. 1993). Hydrogen peroxide is more effective against lice at higher water temperatures but again, toxicity to the salmon increases with temperature and at 18 ºC, 1500 ppm resulted in 100% mortality of treated salmon (Thomassen 1993; Johnson *et al*. 1993; Bruno and Raynard 1994). Thus, as temperatures rise, it becomes necessary to counterweigh the increasing risk of toxicity with the benefit from achieving detachment of mobile lice.

The accumulation of gas bubbles, possibly oxygen, within the haemolymph is sufficient to cause lice to detach from the fish and float to the surface (Thomassen

1993; Bruno and Raynard 1994) but most lice recover swimming ability within a few hours of treatment. No data currently exist (see summary table in Appendices) from laboratory based aquarium studies at 13 and 14 ºC, typical of summer and autumn temperatures in Scotland, which may influence efficacy of H_2O_2 treatment. Some authors (Thomassen, 1993; Bruno and Raynard 1994; McAndrew *et al*. 1998; Treasurer *et al*. 2000) do suggest that lice might also be able to re-infect fish after H_2O_2 treatment, with McAndrew *et al*. (1998) reporting that pre-adult and adult *L. salmonis* successfully re-attached to Atlantic salmon after exposure to 1500 ppm H_2O_2 for 20 minutes at 7.5 ºC. Re-attachment potential at higher H_2O_2 concentrations reported to be used by aquaculture industry, and at higher environmentally relevant temperatures, have not been tested. Additionally, published findings suggest that hydrogen peroxide does not significantly reduce the numbers of attached chalimus *L. salmonis*, but instead delays maturation from the early chalimus I to late chalimus II stages (Johnson *et al*. 1993; McAndrew *et al*. 1998). Further work would be useful to establish the effects of typical current industry treatment doses and durations on this life stage to verify if it is a susceptible and legitimate target for treatment.

There are differences in the reported sensitivity of gravid females to H_2O_2 treatment with adult females less successful at re-attaching onto a host post treatment (McAndrew *et al*. 1998; Treasurer *et al*. 2000). An important consideration in relation to treatment of gravid females is the effect of treatment on hatching success of eggs and further larval development. During hydrogen peroxide treatment, both attached and detached egg strings will be exposed to the chemical as females can shed egg strings if stressed Schram (2000). The effect of H_2O_2 on eggs has previously been investigated with Johnson *et al*. (1993) exposing egg strings still attached to the female while a later study by Aaen *et al*. (2012) exposed egg strings which had first been excised from the adult female. Both Johnson *et al*. (1993) and Aaen *et al*. (2012) found that reduced numbers of eggs hatched following hydrogen peroxide treatment and none of these succeeded in developing to copepodid stage. However, Beattie *et al*. (2012) reported similar hatch rates for control and treated gravid females, and for attached and detached egg strings, following wellboat treatment. McAndrew *et al*. (1998) reported the hatching success of egg strings at different stages of maturity removed from adult female *L. salmonis* and treated with 1500 ppm H_2O_2 for 20 minutes. They highlighted the protective effect of pigment in older eggs. There is evidence (Johnson et al. 1993; McAndrew *et al*. 1998) that hydrogen peroxide is capable of inactivating the eggs of female *L. salmonis* if applied before eggs become pigmented. McAndrew *et al*. (1998) found that the hatching success, and any subsequent development to the copepodid, from egg strings removed from adult female *L. salmonis* at different stages of maturity, and treated with 1500 ppm H_2O_2 for 20 minutes at 10 ºC, was positively correlated with egg age as defined by increasing pigment in older eggs. However, as egg development is temperature dependent, the length of the susceptible period is likely to vary depending on water temperatures. Consequently, there is a knowledge gap with regards to the treatment of *L. salmonis* egg strings at a “typical” summer water temperature of 13 ºC utilising H_2O_2 concentrations commonly administered by the Scottish aquaculture industry.

In discussions with Scottish industry representatives it became apparent that concentrations higher than those recommended (1500 ppm) by the manufacturer Solvay are commonly used. Hydrogen peroxide has the advantage of breaking down into water and oxygen so that there is no bioaccumulation or lasting effect on the

environment (Treasurer and Grant 1997). However, the effect of this chemical on different life stages of the louse is not yet fully elucidated (Treasurer *et al*. 2000).

In Scotland, the industry Code of Practice (Anon. 2006) suggests treatment criteria for sea lice burdens at different times of the year and it may be necessary to carry out lice treatments at higher temperatures. The point at which treatment efficacy is offset by toxicity, at the higher range of temperatures and H_2O_2 concentrations encountered on Scottish farms, needs further clarification in order to prevent post-treatment mortalities in fish.

This report has been prepared as part of the Scottish Aquaculture Research Forum (SARF) contract to investigate the relationship between hydrogen peroxide and the viability of different stages of the *L. salmonis* lifecycle. The overall goal was to improve recommendations for the application of hydrogen peroxide under different environmental conditions to support existing sea lice management strategies. To achieve this goal, a number of objectives were outlined by SARF as part of the contract. These were:

1. Literature review to ascertain state of knowledge on how hydrogen peroxide affects different life stages of organisms such as *L. salmonis*: what are the target physiological processes and/or structures.

2. Design and complete lab-based experiments to validate or quantify the dose-response aspects of hydrogen peroxide treatment on different life stages of *L. salmonis*, under different temperature conditions - as likely to be experienced on Scottish salmon farms.

3. Organise and hold a workshop for industry representatives presenting data from laboratory trials and then, after discussion with attendees, cross reference the results obtained with practical, on-farm treatment experience.

4. Develop a concise Best Practice Manual for the use of hydrogen peroxide as a treatment for *L. salmonis* in Scottish aquaculture.

To achieve these objectives Marine Scotland Science produced a literature review (See Objective 1) and designed and implemented a range of experiments to investigate the effectiveness of hydrogen against different *L. salmonis* lifecycle stages.

3. Scientific Objectives

1) Provide an overview of information on how hydrogen peroxide exerts its effects on organisms such as *L. salmonis* with reference to target organs and processes.

- Objective met. A literature review titled "To ascertain the state of knowledge on how hydrogen peroxide affects different life stages of organisms such as *Lepeophtheirus salmonis*: furthermore to review information on the likely target structures and physiological processes" was submitted to SARF.

2) Perform laboratory studies under industry relevant conditions, to quantify the effect of different hydrogen peroxide concentrations on:

a. Activity of pre-adult and adult lice.
- Objective met. Laboratory experiments indicated that the efficacy of a range of concentrations of H_2O_2 at 10 ºC and 13 ºC in inactivating adult sea lice is related to concentration and temperature. Trials investigating the sensitivity of adult *L. salmonis* demonstrated that the majority of adult lice are inactivated for varying periods of time if exposed to levels of 1000 ppm or higher at both temperatures.

b. Survival of eggs and subsequent developmental success.
- Objective met. Laboratory based experiments to investigate the effects of different H_2O_2 concentrations at 10 ºC and 13 ºC on *L. salmonis* egg strings and subsequent larval development to the infective copepodid stage were carried out. Trials demonstrated that H_2O_2 had a significant effect on the hatching success and subsequent development of *L. salmonis* larvae with increasing concentration and water temperature.

c. Developmental success of chalimus stages.
- Objective met. An aquarium tank based trial was carried out exposing Atlantic salmon, infested three days previously with *L. salmonis* copepodids, to 1500 ppm and 1800 ppm H_2O_2. The trial demonstrated that while chalimus *L. salmonis* were not killed following exposure to H_2O_2 a delay in development to later lifecycle stages was found although this varied according to the sex of the louse.

3) Consult with industry, using a workshop format, so that information from laboratory/aquarium experiments can be combined with that from farm experience in building an informed and practical foundation for the Best Practice Manual.

- Objective met. A one day workshop was held in Perth in November 2014 at which the experimental data generated by the Scottish Aquaculture Research Forum (SARF) funded project "Assessment of the Viability of the Different Life Stages of *Lepeophtheirus salmonis* (Krøyer, 1837) Following Exposure to Hydrogen Peroxide" was presented to industry members and discussed. The workshop allowed an exchange of information between researchers and industry participants to compare and contrast results found under experimental and field situations. This will form the foundation for a "Best Practice" manual which the SSPO (Scottish Salmon Producers Organisation) will develop using the results of this study and industry input.

4.0 Materials and Methods

Ethical Statement

Marine Scotland Science is committed to ensuring high welfare standards for all fish used in experimental trials. All handling of fish was conducted in accordance with the Animals (Scientific Procedures) Act 1986. All proposed experiments were first subject to detailed statistical review to ensure that a minimum number of fish was used which would allow statistically meaningful results to be obtained.

4.1 General Materials and Methods

Sea Lice Source and Maintenance

All *L. salmonis* used during this trial were sourced from the Machrihanish Experimental Research Laboratory (MERL) facility located on the west coast of Scotland. The *L. salmonis* used during all trials were from a strain deemed to be naïve to hydrogen peroxide treatment. The strain has been cultured under laboratory conditions through more than 40 generations and has not been exposed to any sea lice treatment since taken into culture in 2001. The lice were cultured on Atlantic salmon weighing between 500 – 1000 g in 5 m^3 tanks with a flow of ambient seawater (~ 12 ºC) of 1 L/min per kg of fish and a salinity of 34.

Sea lice and egg strings were placed into plastic bags containing ~ 3 L clean ambient seawater from MERL, in a cool box containing ice packs, for transport to the Marine Scotland aquarium facility in Aberdeen. Direct contact between icepacks and the lice/egg strings was avoided by using rolls of paper towel.

In Vitro L. salmonis Copepodid Cultivation for Challenge Experiments

Gravid adult female *L. salmonis* were collected from MERL for transport to MSS (Marine Scotland Science) where the egg strings were removed as close to the body of the *L. salmonis* female as possible using forceps. Excised egg strings were placed into 5 L beakers of fresh filtered seawater at 10 °C. Large temperature fluctuations in the culture vessels were prevented by placing each vessel in a 2 x 1 m rectangular tank containing approximately 50 L seawater (salinity of 34) at 10 °C with a flow of 2 L min^{-1}. The photoperiod regime for the louse cultivation process followed approximate ambient light levels for the time of year. Two silicone airlines with air stones (Algarde, Nottingham, UK) provided vigorous aeration to keep egg strings in suspension. Hatching, lifecycle stage and numbers of *L. salmonis* larvae were monitored daily by removing five 50 mL samples from each culture vessel, before counting and noting what stage the lice had reached. Once the majority of the lice (~ 90 %) had moulted through to the copepodid stage the lice were harvested from the culture vessels by sieving through a 100 µm mesh filter (Duncan and Associates, Manchester, UK) before washing with clean filtered seawater into a 1 L beaker for challenge experiments.

Experimental Fish Source and Maintenance

The Atlantic salmon (*Salmo salar* L.) used in experiment 1 (pilot study) came from ova of river Don origin which were hatched and held at the Marine Scotland Marine Laboratory, Aberdeen, as stock fish.

All the salmon used in trials 3 and 4 were kindly supplied by Marine Harvest as parr from a freshwater cage site and smolted at the Marine Scotland Marine Laboratory, Aberdeen site.

The salmon used in all trials were smolted after 1 year (S1s) and held in 2 m circular glass reinforced plastic (GRP) tanks under constant aeration, on a 12/12 h photoperiod regime and a flow rate of approximately 5 L min^{-1} with a running tank volume of 500 L^{-1}. Fish were maintained on 3 – 4 mm commercial pellets (Skretting, Invergordon, UK) and fed 2 % bodyweight/day.

Prior to trials commencing all experimental fish were moved into the bio-secure aquarium facility at the MSS, Aberdeen site and placed into 1 m circular GRP tanks with constant aeration, on a 12/12 h photoperiod regime and a flow rate of approximately 5 L min^{-1} with a running tank volume of 350 L. All Atlantic salmon smolts (minimum 6 week post smolt) were allowed to acclimatise to trial temperature(s) for 1 week prior to the commencement of experimental challenges.

Hydrogen Peroxide

The hydrogen peroxide (49.5 % w/w) utilised during all treatments in this study was kindly supplied by Solvay and is distributed under the commercial trade name Paramove® as a stabilised solution for fish treatment.

All the equipment used during trials was checked to ensure that it did not react adversely with H_2O_2 and had previously been placed into a tank containing 1500 ppm H_2O_2 for 24 h and thoroughly rinsed to remove any contaminants.

Hydrogen peroxide concentrations used during each trial were determined using a cerium sulphate titration method which can be found in: Paramove Analyses in Seawater by Manual Titration (Analysis-EN-1-Paramove 28/01/2013, page 2).

Hydrogen peroxide was added to each exposure tank to achieve the desired concentration and thoroughly mixed, before water samples were taken from various points within the tanks and titrated to ensure that a uniform distribution was achieved.

4.2 Adult *Lepeophtheirus salmonis* Inactivation Post Hydrogen Peroxide Treatment

The susceptibility of adult male and female *L. salmonis* to a range of H_2O_2 concentrations and the time to recovery post exposure, if at all, was investigated. Six concentrations were selected that were thought likely to give inactivation rates

ranging from low to close to 100% and the effectiveness of these concentrations were tested at 10 °C and 13 °C. The concentrations were initially set at 300, 600, 1000, 1500, 1800 and 3000 ppm plus a seawater (SW) control. However, after the first experiment at 13 °C, it was apparent that the lower concentration of 300 ppm did not produce a visible effect on sea lice activity. Thus, the concentrations for the experiment at 10 °C were set as 600, 1000, 1500, 1800, 2300 and 3000 ppm plus a SW control. Each treatment condition was replicated three times to account for any random experimental effects.

The number of lice to be used in the treatments was determined by assuming that the inactivation rate was a linear logistic function of log concentration and that the size concentrations were such that the true inactivation rates were (0.04, 0.20, 0.53, 0.80, 0.95, 0.99). Data were simulated from this model, a linear logistic function of log-concentration fitted to the data, and the concentration giving an inactivation rate of 95% estimated from the fitted model. This was repeated 1000 times. With 20 lice per concentration, the concentration giving an inactivation rate of 95% is estimated with a coefficient of variation of 8%. To allow for equal triplication of the experiment, 21 lice were recommended per treatment, assigning seven lice to each replicate.

Adult *L. salmonis* were carefully removed utilising curved forceps from Atlantic salmon which had been previously infested by copepodids by MERL staff. The lice were collected by MSS staff and transported directly from MERL to the MSS Marine Laboratory in Aberdeen. On arrival at the MSS aquarium the lice were placed into 1 m circular tanks (inflow of 330 L/h^{-1}) in their transport bags to allow slow acclimatisation to their respective experimental temperature (10 °C and 13 °C) for 12 h. Prior to exposure to hydrogen peroxide, sea lice were carefully removed from the bags with forceps and checked to ensure they were motile before being sorted randomly into groups.

Seven groups, each containing seven lice, were placed into a stainless steel sieve and each group was exposed for 20 minutes to its corresponding H_2O_2 concentration and a seawater control, with tanks stirred intermittently to ensure uniform distribution of H_2O_2 throughout the exposure tank. Two separate experiments were conducted in triplicates (one at 10 °C and the other 13 °C) with the tanks randomised using outputs from *R* software (R core team, 2014). After exposure, lice were placed into tanks of clean SW at the experimental temperature to remove any residual H_2O_2 before the lice were subsequently transferred to randomised 5 L holding tanks with constant aerated seawater supply and the outlet water was screened to prevent lice escaping, using a 0.5 mm mesh. The number of inactive and active lice (exhibiting some motor response to gentle stimulation) was recorded immediately after and at 1 h, 3 h, 6 h, 12 h, 24 h, 48 h and 72 h post-treatment and the data were transferred into an Excel® spreadsheet.

The data were the proportion of inactivated lice (out of a total of 21 lice) in each control and treatment group at each experimental time point. However, as the same lice were assessed at each time point, the data were dependent, so for simplicity, the data for each time point were modelled separately.

There were two explanatory/independent variables: concentration (SW, 300, 600, 1000, 1500, 1800 2300 and 3000 ppm H_2O_2) and temperature (10 ºC and 13 ºC).

Both of these variables were modelled as categorical variables. In principle, concentration could have been modelled as a continuous variable (and that was the original intention), but the observed proportions of inactivated lice were often so low that it was simpler to regard concentration as categorical.

The data at each time point were modelled using generalised linear models using the binomial distribution and the logistic link function.

Each treatment was triplicated to investigate possible tank effects. Therefore, replicate was a potential random effect. However, variation between replicates was not significant ($p > 0.05$ in all cases) so there was no evidence of over dispersion and thus replicate was not included in the analysis.

Forward step-wise model selection was conducted to find the best fitting model for each time point, starting with the simplest model in which inactivation rate did not depend on concentration or temperature. The explanatory variables were then iteratively added to the model, followed by the interaction term between the two variables. The final model was the model with the fewest parameters within 2 units of the lowest Akaike Information Criterion (AIC).

4.3 Adult *Lepeophtheirus salmonis* Infestation Potential Post Hydrogen Peroxide Treatment

The ability of adult male and female *L. salmonis* to re-infest Atlantic salmon after exposure to H_2O_2 concentrations typically used on Scottish marine salmon farms was investigated. Statistical modelling was carried out at the experimental design stage to ensure the numbers of fish and lice used during the trial would be sufficient to demonstrate if any effects of H_2O_2 treatment reduced immobilisation of lice significantly compared to control conditions. This was done by model simulations of expected outcomes and proposed acceptable limits of confidence levels (95%) and the accepted significance level of 0.05.

The model identified that for the treatment groups, with a sample size of 60 lice, the observed estimate of re-attachment will be within +/- 13% of the true value of immobilisation with 95% confidence (Thompson, 1992). For the control, with a sample size of 30, the estimated re-attachment will fall to within 18% of the true value with 95% confidence. If all lice in a treatment group are immobilized then, with a sample size of 60 lice, the true immobilisation probability will be at least 0.95 with 95% confidence.

The number of Atlantic salmon required was dependent on the number of lice and tank size; there needed to be a sufficient number of fish for the lice to find and attach to if they were capable of doing so. In this case, 1 fish per 3 lice was sufficient totalling 20 fish for each concentration and 10 for the control.

Immediately prior to the trial, adult male and female *L. salmonis* were collected from MERL (See 4.1) and transported back to MSS, Marine Laboratory, Aberdeen where they were acclimatised in an aerated 1 m diameter GRP tank at the experimental temperature of 10 °C for 12 h. Prior to the start of trials all the animals were carefully

examined to ensure only healthy and motile individuals were used during the trial, with dead or moribund lice carefully removed with forceps.

One week prior to the trial 50 Atlantic salmon (mean weight 208.89 g) were transferred into the MSS disease aquaria and separated into five 1 m circular tanks each containing ten fish and left to acclimatise. Each tank had an inflow of seawater (34) at a flow rate of 330 L/h^{-1} at 10 °C with constant aeration.

Prior to the lice being introduced into the tanks all fish were starved for at least 48 h. Two tanks (duplicate tanks) were randomly selected using a random "lottery" style draw for each of the two H_2O_2 concentrations (1500 and 1800 ppm) with one tank of controls.

At the start of the trial 30 lice per tank were carefully removed from their holding tank and exposed to their corresponding concentration of H_2O_2 (1500 ppm and 1800 ppm) for 20 minutes. An equal number of males and females were included in each treatment. The control group was exposed to SW only. After 20 minutes exposure, lice were removed from treatment tanks and placed into tanks of clean SW at correct temperature to rinse any residual H_2O_2.

After rinsing, lice were transferred into tanks of clean seawater for 1 h before adding to the 1 m tanks containing the salmon, with the aquarium lights turned off to minimise the chance of initial predation on the lice by the fish at point of addition. The number of inactive lice immediately following treatment was observed, counted and recorded. Fish were observed daily and the number of lice attached to the fish and or the walls of the tank was recorded at 24, 72 h and 96 h post treatment as well as removing and noting any lice mortalities from the outflow mesh.

The data were the proportion of lice attached to a host in control condition and in treatment conditions of 1500 ppm and 1800 ppm H_2O_2, split by gender. The lice exposed to H_2O_2 concentrations were split into replicate tanks to identify any tank effects. There was low variation in attachment rate between the replicates so replicate was not included in the analysis.

Due to the small sample size of this experiment and the simplicity of the experimental design, a Fisher's 1-sided exact test was sufficient to test for any significant relationship between lice settlement and H_2O_2 dosage. This was done in StatXact V10.1 (Cytel, Cambridge, Massachusetts, USA) to calculate the exact P value. The asymptotic P value is commonly used for statistical analysis due to the high computational demand required for exact P value calculation. However, with small datasets, the exact P value can be determined and, as it is more accurate than the asymptotic P value, can detect significant relationships between variables that may not be detected by the asymptotic P value.

4.4 Chalimus *Lepeophtheirus salmonis* Development Post H_2O_2 Treatment

A trial was designed to investigate if chalimus *L. salmonis* were susceptible to H_2O_2 and therefore, a potentially suitable lifecycle stage to target using H_2O_2 by treating

them with commonly used industry dosages of 1500 and 1800 ppm for 20 minutes. At the experimental design stage sample size was determined under the assumption that the mean number of copepodid lice attached per fish is 7.25 under control conditions. This mean was derived from Pert *et al*. (2009) in which the mean number of lice attached to Atlantic salmon was 3.62 after being exposed to infected water with a concentration of 1.5 copepodids/mL and left in sea water for 96 hours. The fish in this experiment were exposed to double the number of copepodids; thus mean attachment per fish for the control in this experiment was assumed to be twice that observed by Pert *et al*. (2009).

Limited information was available to predict the mean number of lice per fish following each treatment. Therefore, a 25% reduction in lice burdens per fish was set as the target for detection between the control and the lower concentration treatment and between the lower and higher concentration treatments. With a control mean infestation of 7.25 lice per fish, 25% reductions equate to mean burdens of 5.44 and 4.08 lice per lice per fish for the 1500 and 1800 ppm H_2O_2 treatments respectively.

The number of fish used (60 for each treatment and 30 for the control) was determined via a power analysis in which a reduction in mean settlement between the control (at a settlement value of 7.25 per fish) and a treatment of at least 25% would be detected with 90% power.

One week prior to the start of the trial 150 smolts were transferred into Ellis building aquarium for acclimatisation to the trial temperature of 10 °C. Fish were divided between 2 tanks of 30 per treatment (duplicate tanks) and a single tank of 30 fish for the control.

Twenty four hours prior to the start of the trial approximately 200 adult female *L. salmonis* bearing egg strings were collected from MERL for transport back to MSS where the egg strings were removed and placed into 5 L culture vessels (No more than 50 egg strings per beaker) and aerated as described in 4.1.

Daily observations were carried out to determine hatching progress and identify when greater than 90% of lice had developed to the copepodid stage which should take ~ 7-8 days at 10 °C from “pale” egg strings. The number of copepodids per beaker was determined by stirring the beaker and taking 5 x 10 mL sub samples and counting under a dissecting microscope. Once sufficient lice had moulted through to the copepodid stage four 1.5 L infection baths were prepared with 3 copepodids/mL.

The method used to infect fish was a modified variation of Sevatdal’s protocol (Sevatdal, 2001). Briefly, two fish were netted and anaesthetised simultaneously using 100 mg/L ethyl 3-aminobenzoate methanesulfonate salt (MS222; Sigma-Aldrich, Germany) in seawater. The fish were washed in fresh seawater to prevent anaesthetic contamination of the infection tank before immersion in the infection bath for 30 seconds. Fish were replaced in their relevant tanks with the flow stopped and aerated to allow recovery. The water flow was re-started after 4 hours.

Three days post infection the water level was reduced by half and the fish exposed to H_2O_2 at either 1500 ppm or 1800 ppm for 20 min. The tanks were flushed and the

initial starting volume (330 L) and flow rate (330 L h^{-1}) re-established and treated fish were left for 10 days (See 4.1 for H_2O_2 application and exposure method). A control challenge involved the same procedure but the fish were only exposed to SW.

Atlantic salmon were destructively sampled 10 days post treatment and all fish examined for lice burdens, lifecycle stage and position on the fish. Data were subsequently transferred onto Excel® spreadsheet.

The data were explored for outliers and none were found. The data were the counts of attached chalimus on each individual fish within a tank treated with either 1500 ppm or 1800 ppm H_2O_2 plus a control. There were two tanks for each treatment to identify any tank effects and one tank for the control. Lice were divided into groups based on gender and by their moult stage of which there were four: Chalimus I, Chalimus II, Pre-adult I and Pre-adult II.

The response variable was the count of lice attached in each moult category and was therefore an ordered factorial response variable. The data were therefore modelled using a cumulative-link mixed model, which accounts for the multinomial nature of the data and retains the structural integrity of the factorial response. The fixed explanatory variables were concentration and gender of the chalimus, both of which were factors. Both fish ID and tank were considered as potential random effects.

Forward-stepwise model selection was done to determine the best fitting model, starting with the simplest model in which the counts in each category did not depend on concentration or gender and in which there were no random effects. At each stage, the main effects of the explanatory variables treatment and gender were considered and, if already included in the model, their interaction. The random effects fish ID and tank were also considered for inclusion at each stage. Model selection was based on AIC, with the chosen model being that with the fewest parameters within 2 units of the minimum AIC.

The modelling process was applied to the full data set (i.e. with both the control and the treatments 1500 ppm and 1800 ppm). To determine if there was any difference between treatments, the modelling process was repeated without the control.

4.5 *Lepeophtheirus salmonis* Egg string/Larval Development Post H_2O_2 Exposure

Approximately 100 gravid female lice were collected from Atlantic salmon at MERL. Both egg strings were carefully removed on site, and placed into separate bijou tubes containing ambient seawater from the site and labelled identifying the left and right egg string. Tubes were placed into tube racks and transported to the MSS aquaria in cool boxes containing icepacks. Direct contact between icepacks and the egg strings was avoided by using rolls of paper towel.

On arrival at MSS the tubes containing egg strings were carefully unpacked and placed into a 1 m tank with a flow of 330 L h^{-1} to acclimatise to the experimental temperature (trials were run at both 10 °C and 13 °C) for at least 12 h post transport

from Machrihanish. Randomized left and right egg strings were transferred into separate aerated hatching chambers (Fig. 1) (5 paired egg strings – one of the pair into SW control and the other into H_2O_2). To ensure conditions replicated as near as possible to those in the field, egg strings were picked at random for each H_2O_2 concentration. Their maturity was recorded by noting the degree of pigmentation (light, medium, dark) of each egg string as it was placed into a hatching chamber. Multiple tanks (randomised) were set up so triplicates could all be done at the same time.

Figure 1: Egg strings were removed from adult female *L. salmonis* and placed into individual hatching chambers (left) before being exposed to either H_2O_2 or a seawater control, and placed back into flow through tanks with aeration (right).

Egg strings in hatching chamber holder units were submerged in an exposure tank containing one of four different test H_2O_2 concentrations (600, 1200, 1500, and 1800 ppm) and the control egg string was placed into a exposure tank containing only clean seawater. Both H_2O_2 and control egg strings were left in their respective exposure tanks for 20 min before transfer to tanks of clean seawater to wash hydrogen peroxide from egg strings and equipment.

Following H_2O_2 exposure and washing in clean seawater, each hatching chamber holder was transferred into a 7 L flow through tank of clean SW maintained at the experimental temperature with suitable aeration to ensure water movement. Tank number and the treatment status of the egg strings were recorded, and tanks were monitored at least twice per day post exposure checking for adequate water flows, suitable aeration, temperature, hatching and the lifecycle stage any hatched lice had reached. Egg strings were allowed to hatch and the proportion which developed through to copepodid stage (~ 6-8 days at 10 °C and 5-7 days at 13 ºC) assessed and recorded. Once all the larvae appeared to be at the copepodid stage each hatching chamber was removed from its holder, filtered through a 68 µm mesh before all the material was fixed with 70% ethanol for later counting under a dissecting microscope. Successful development and survival to copepodid stage between treated and untreated groups was recorded and data were transferred onto Excel® spreadsheet.

Data for this experiment were the number of hatched copepodids from each egg string from each tank, with each tank being treated with a different concentration of H_2O_2 and exposed to one of two temperature conditions. As each female produces

two egg strings, from each female one egg string was placed into H_2O_2 with the remaining string used as a control placed in a tank containing only sea water. This was to account for the high natural variation in hatching rate between individual females. Data were explored for outliers and none were found.

The response variable was the count of hatched lice from each egg string and was modelled using a generalised linear mixed model assuming poisson errors and a log link. The explanatory variables were concentration (control, 600 ppm, 1200 ppm, 1500 ppm and 1800 ppm), temperatures (10 °C and 13 °C), and egg string colour, all treated as categorical. Three random effects were considered: tank, string ID and individual ID. String ID paired the strings from the same female to account for variation in hatching rate between females and individual ID accounted for individual string variation (over-dispersion).

Forward step-wise model selection was applied, starting with a simple model with no explanatory variables or random effects. Explanatory variables were then iteratively added followed by interaction terms between the variables. Random effects were also added. Model selection was based on AIC with the final model being that with the fewest parameters within two units of the minimum AIC

5.0 Results

5.1 Adult *Lepeophtheirus salmonis* Inactivation Post Hydrogen Peroxide Treatment

Concentration had a significant effect on the number of inactive lice at all time points (Table 1), with the proportion of inactive lice generally increasing with concentration (Figures 2 and 3). Concentration had a larger effect upon lice inactivation at the beginning of the experiment relative to the last time point of 96 hours (Table 1). Temperature became more influential upon the number of inactive lice at later time intervals with more lice inactive at 96 h post exposure at 13 °C than 10 °C (Table 1, Figures 2 and 3).

Immediately after treatment (time 0), between four and all (21) lice became immobilised in all treatments at both temperatures (Figures 2 and 3). The level of immobilisation at time 0 was significantly related to concentration with more lice immobilised at higher concentrations of H_2O_2 ($p<0.001$). By the first hour post-exposure, lice had begun to reactivate (Figures 2 and 3). The reactivation rate appeared to be greater at 13 °C with more lice reactivating by 1 h post exposure compared to reactivation levels at 10 °C (Figures 2 and 3). After this large increase in active lice between 1 and 3 h post exposure at 10 °C and 0 and 1 h post exposure at 13 °C respectively, reactivation occurred at a slower rate for the remainder of the experiment. By 72 hours, all lice had reactivated at 10 °C regardless of concentration (Figure 2). At 13 °C there was some fluctuation in the numbers of active lice from 6 h post exposure onwards, with some lice that were active at this time point being inactive again later on. By the end of the experiment, there were still some lice inactive in 4 treatments at 13 °C (Figure 3). Overall, concentration appeared to have a stronger effect on lice inactivation than temperature. However, temperature did have a significant effect on the inactivation level after the first hour of the experiment (Table 1). This is likely due to the difference in numbers of reactivated adult *L. salmonis* between temperatures at this time point. As time progresses, due to the eventual reactivation of all lice at 10 °C and the continued inactivation of some lice at 13 °C, temperature becomes a more important explanatory variable and has a more significant effect than concentration at 48 hours and beyond (Table 1).

Table 1. Best fitting models for each time step for adult inactivation trials.

Time	Model	P-values
0	Rate ~ Conc	Conc <0.001
1	Rate ~ Conc + Temp + Conc:Temp	Conc <0.001, Temp <0.001, Conc:Temp 0.03
3	Rate ~ Conc	Conc <0.001
6	Rate ~ Conc	Conc <0.001
12	Rate ~ Conc + Temp	Conc=0.001, Temp=0.02
24	Rate ~ Conc + Temp	Conc=0.002, Temp=0.02
48	Rate ~ Conc + Temp	Conc=0.01, Temp=0.003
72	Rate ~ Conc + Temp	Conc=0.02, Temp=0.001
96	Rate ~ Conc + Temp	Conc=0.008, Temp<0.001

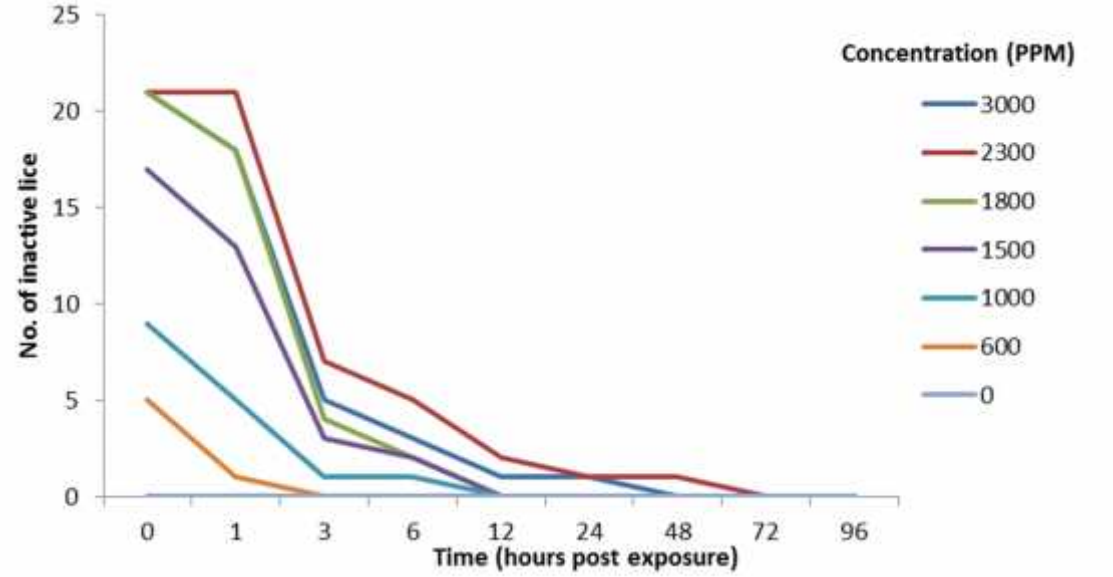

Figure 2. Number of inactivated lice at increasing time intervals for different concentrations of H_2O_2 at 10 °C.

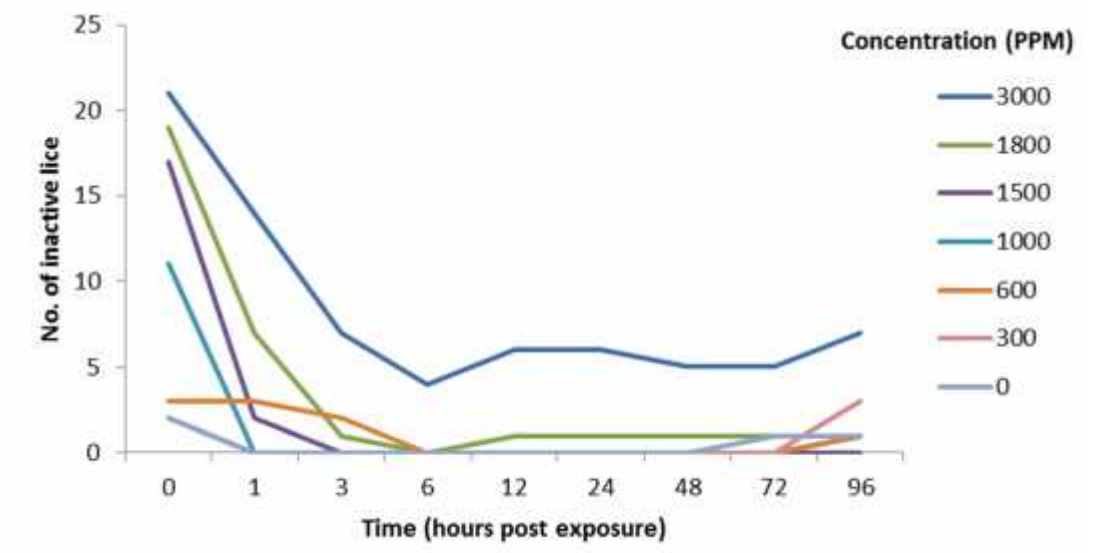

Figure 3. Number of inactivated lice at increasing time intervals for different concentrations of H_2O_2 at 13 °C.

5.2 Adult *Lepeophtheirus salmonis* Infection Potential Post Hydrogen Peroxide Treatment

No significant difference in re-attachment levels under control conditions was detected between females (0.533) and males (0.733) (Fisher's exact test: F=1.26, p=0.44). Similarly, no significant difference in re-attachment levels under either treatment condition was detected between females (0,533, 0.366) and males (0.3, 0.3) (Fisher's exact test: 1500 ppm: F=3.30, p=0.12; 1800 ppm: F=0.32, p=0.78). As there was no difference between males and females, analyses were performed with all data combined.

Resettlement level was reduced by more than 10% when treating lice at 1500 ppm and by 30% when treating lice at 1800 ppm (Figure 4). A Fisher's exact test confirmed a significant decrease in attachment at 1800 ppm but not at 1500 ppm (1500 ppm: F=3.71, p=0.07, 1800 ppm: F=7.22, p=0.007). The difference in resettlement between treatments was less than 10% and not significant (F=0.89, p=0.45).

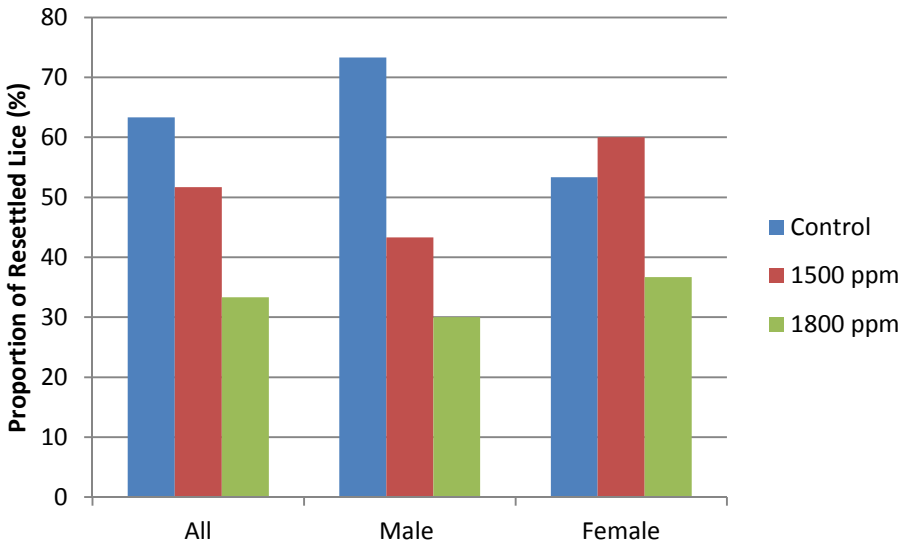

Figure 4. Adult *L. salmonis* resettlement rate for all, male and female lice under control conditions and two treatment conditions.

5.3 Chalimus *Lepeophtheirus salmonis* Development Post H_2O_2 Treatment

A model comparison of the full model and a binomial 'treatment', 'no treatment' model indicated a significant difference between the treatments of 1500 and 1800 ppm (df=2, p=<0.001) thus treatment was modelled as a 3-factor variable. There was an interaction term between treatment and gender with random effects fish ID and tank. Thus, for simplicity, genders were modelled separately.

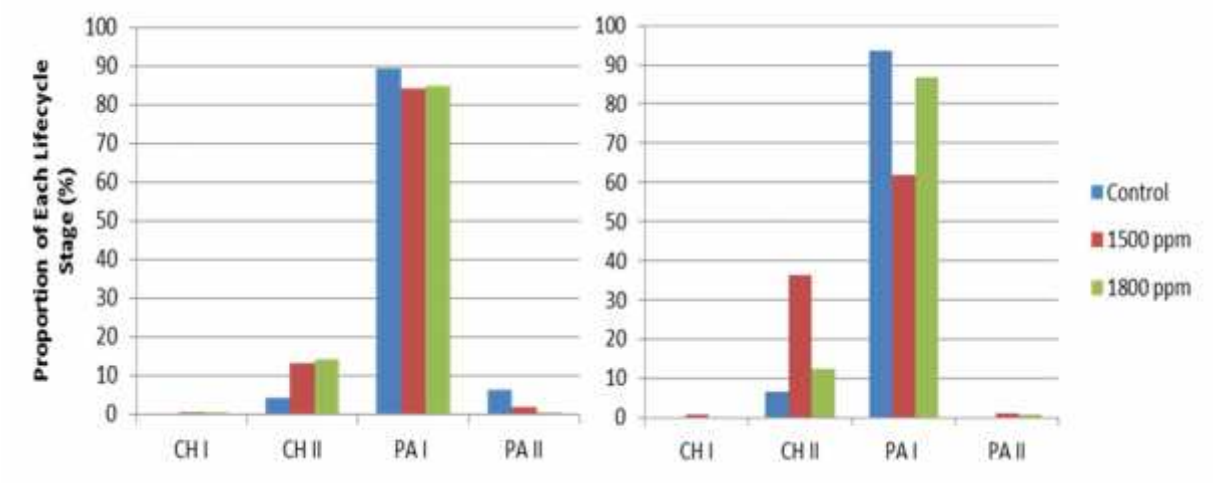

Figure 5. Proportions of the four *L. salmonis* life stages of female (left graph) and male (right graph) in control conditions and treated with two concentrations of H_2O_2 (1500 ppm and 1800 ppm) at 10 ºC. CH I = Chalimus I, CH II = Chalimus II, PA I = Pre-Adult I, PA II = Pre-Adult II.

When considering males, both H_2O_2 treatments are significantly different from the control in reducing the number of chalimus which moulted to pre-adults (1500 ppm: $z = -2.71$, $p = 0.007$, 1800 ppm: $z = -3.09$, $p = 0.002$). For all treatments and in each tank, male pre-adult I proportions were lower than the control and chalimus II proportions higher than control conditions (Table 2) demonstrating that there was a delay in moulting between the treated and untreated male chalimus and pre adult stages.

For females, the proportion of chalimus II reaching pre-adult stage was significantly reduced following 1500 ppm H_2O_2 treatment ($z=-2.09$, $p=0.04$; Table 2). The proportions of stages were not significantly different from the control at a H_2O_2 concentration of 1800 ppm.

Table 2. Proportions of four *L. salmonis* life stages of chalimus treated with sea water (control) and two concentrations of H_2O_2 (1500 ppm and 1800 ppm). Stages are coded as: CH I = Chalimus I, CH II = Chalimus II, PA I = Pre-adult I, PA II = Pre-adult II.

Treat	Sex	CH I	CH II	PA I	PA I
Control	F	0	0.064	0.936	0
Control	M	0	0.044	0.892	0.064
1500(1)	F	0	0.571	0.286	0.143
1500(2)	F	0.007	0.357	0.629	0.007
1500(1)	M	0	0.308	0.692	0
1500(2)	M	0.006	0.127	0.848	0.019
1800(1)	F	0	0.084	0.916	0
1800(2)	F	0	0.538	0.385	0.077
1800(1)	M	0	0.124	0.873	0.002
1800(2)	M	0.029	0.343	0.600	0.029

5.4 *Lepeophtheirus salmonis* Egg string/Larval Development Post H_2O_2 Exposure

A generalised linear mixed model was the most appropriate model for these data with concentration and temperature as interacting explanatory variables and tank ID, String ID and individual ID markers as random effects. Egg string colour was not found to significantly improve model fit. Concentration and temperature as interacting explanatory variables were demonstrated to have a significant effect on the hatching success and development of *L. salmonis* eggs and larvae with significantly less larvae developing to the infective copepodid stage with increasing H_2O_2 concentration and water temperature ($F=65.06$, $df=10$, $p<0.001$; Figure 6). At each concentration, the estimated number of hatched larvae decreases with hatching levels significantly lower at the higher temperature (Figure 6). There was a slight increase in hatching rates at both temperatures at a H_2O_2 concentration of 1500 ppm, but a model comparison of the full model and a binomial 'treatment', 'no treatment' indicated no significant difference between the H_2O_2 concentration treatments ($df=6$, $p=0.22$). Therefore, the difference in the hatching and subsequent development to the infective copepodid stage is driven by the marked decline in hatching ability when treated by any concentration of H_2O_2 compared to control conditions and not between different concentrations of H_2O_2.

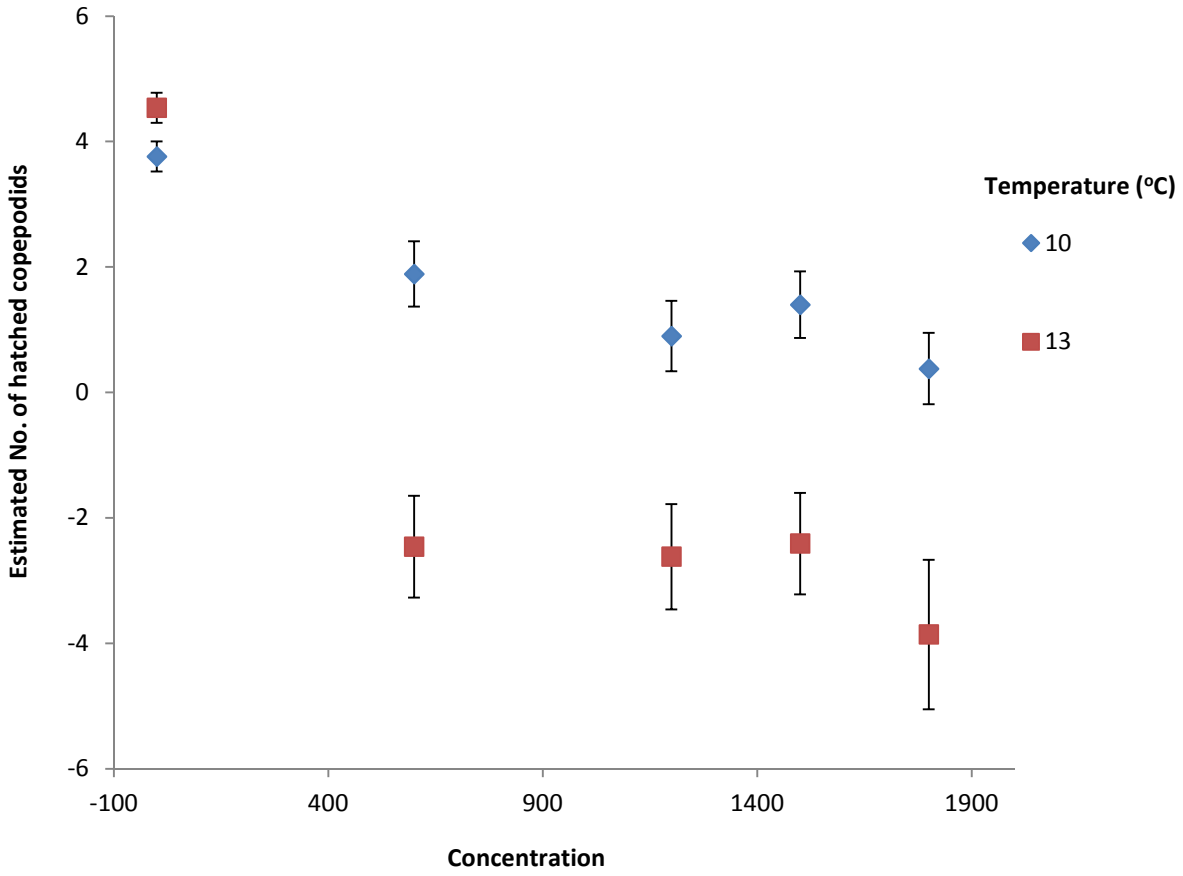

Figure 6. Estimated number of hatched copepodids at each H_2O_2 concentration for two temperature conditions. H_2O_2 concentrations = 0 (control), 600, 1200, 1500, 1800 ppm. Note that negative estimates exist due to large number of zeroes in the dataset for treated egg strings but represent an estimate of zero copepodids.

Overall, concentration and temperature had a significant effect upon the hatching ability egg strings and subsequent (If any) development into copepodids ($F=65.06$, $df=10$, $p<0.001$). While there was a significant relationship between egg hatching rates and temperature, concentration and an interaction between these two variables compared to control conditions, there was no significant difference between the H_2O_2 concentrations.

6.0 Discussion

6.1 Adult *Lepeophtheirus salmonis* Inactivation Post Hydrogen Peroxide Treatment

The results reported here indicate that the efficacy of a range of concentrations of H_2O_2 at 10 ºC and 13 ºC in causing the inactivation of adult sea lice is related to concentration and temperature.

Trials investigating the sensitivity of adult *L. salmonis* demonstrated that the majority of adult lice are inactivated for varying periods of time if exposed to levels of 1000 ppm or higher at both temperatures. The rate of immobilisation immediately post exposure was found to be significant ($p<0.01$) with greater numbers of lice becoming inactivated with increasing H_2O_2 concentration. However, by 3 h post exposure, more than 70% of these lice had recovered and by 12 h post exposure greater than 90% of all the lice exposed had recovered, except those treated with the highest dose of 3000 ppm at 13 ºC.

For both temperatures, the decrease in proportion of adult lice inactivated was much greater for concentrations of 1000 ppm and below. These findings suggest that while H_2O_2 is effective at removing lice, particularly at concentrations above 1000 ppm, it does not kill the lice but causes them to become temporarily immobilised and release from their host. In this study approximately 25% of lice were inactivated by exposure to 600 ppm for 20 min at 10 ºC although all had recovered within 3 h. In contrast, at 13 ºC approximately 10% were still inactive at 3 h although all had recovered by 6 h post exposure. These findings are not entirely consistent with trials carried out in Scotland by Treasurer and Grant (1997) who reported that at 9 ºC and 600 ppm, 70% of pre-adult and adults were inactivated and at 15 ºC the inactivation level was 90%. However, in those laboratory trials the lice were exposed for 1 h compared to the 20 min in the current study which suggests that length of exposure may also play a part in the inactivation of pre adult and adult *L. salmonis* and should be investigated in any future study. Previous studies on attached lice (Treasurer & Grant, 1997; Johnson et al. 1993) reported that up to 100% of pre-adult and adult *L. salmonis* were removed from salmon after H_2O_2 treatment at 1500 ppm for 20 min although up to 90% of these lice had recovered by 24 h post exposure.

The H_2O_2 dose recommended by Solvay is 1500 ppm for 20 min although 1800 ppm is often used with the length of exposure often reduced, particularly at higher water temperatures (direct discussion with industry). During the current study all lice recovered following exposure to all concentrations of H_2O_2 at 10 ºC, although at 13 ºC a number of lice failed to reactivate or reactivated only temporarily, and were found to be dead at the end of the 96 h trial – particularly at 1800 and 3000 ppm. These findings suggest that at higher temperature the effects of H_2O_2 may be more detrimental to the lice and compromise them in such a way that their survival is reduced. This was particularly evident following exposure to 3000 ppm which resulted in more than 35% of lice remaining inactive at 96 h. However, this dose is twice that recommended by the supplier and would likely result in heavy mortalities among treated fish, as reported in a number of studies which have investigated the effects of H_2O_2 concentration, exposure time and water temperature on Atlantic

salmon (Johnson *et al*. 1993; Bruno & Raynard 1994; Kiemer & Black 1997). At temperatures of 13.5 ºC mortalities occurred in Atlantic salmon exposed for 20 min, with Johnson *et al*. (1993) recording a 7.7% mortality at 1500 ppm while Bruno & Raynard (1994) demonstrated a 65% mortality at 1230 ppm 24 h post exposure and 100% mortality at 4760 ppm. Kiemer & Black (1997) also found temperatures above 14 ºC and doses in excess of 1500 ppm could cause significant mortalities, and reported 100 % mortality after exposing fish to 2580 ppm for 20 min at 16 ºC.

Results of exposure experiments at 10 ºC indicate that use of concentrations above 1800 ppm do not result in an increase in sea lice inactivation, though the period of inactivation may be increased at higher doses. Therefore, benefit from using concentrations higher than 1800 at 10 ºC compared with increased risk to fish may depend on how well the detached sea lice are dispersed from site by water flow, or how quickly they are removed by various filtration/collection systems which are being developed.

Periods of inactivation post H_2O_2 exposure at 13 ºC increased, particularly at concentrations of 1800 ppm and 3000 ppm with greater number of lice remaining inactivated and potentially killed by treatment. However the benefit of this difference in inactivation potential on sea lice numbers directly post treatment, and on subsequent infection cycles would need to be modelled to determine if beneficial when impairment to host welfare is taken into account. Levels of 3000 ppm are unlikely to be used at higher temperatures due to fish health issues. Future studies could investigate if these concentrations were equally effective at shorter exposure durations which may highlight potential applications – should modelling of effect mentioned previously show a benefit to reducing lice levels within the system.

Though sea lice inactivation levels and time for recovery are discussed here in relation to different H_2O_2 concentrations, other effects which may augment the benefit of higher concentrations, though not directly visible - such as effect on ability to re-infect, even if swimming activity has been regained must also be assessed.

The *in-vitro* trials carried out on adult *L. salmonis* suggest that at the manufacturer recommended dosage (1500 ppm) and at a higher level (1800 ppm) sometimes used by industry temporary inactivation of lice occurs, although it is not known how viable these animals may be on recovery, which is discussed in the next section.

6.2 Adult *Lepeophtheirus salmonis* Infection Potential Post Hydrogen Peroxide Treatment

The experiments reported here describe the ability of *L. salmonis* male and female lice which have been exposed to either 1500 ppm or 1800 ppm H_2O_2 for 20 min at 10 ºC to re-infect Atlantic salmon.

Aquarium tank based trials that were carried out after *in-vitro* laboratory trials demonstrated (See 5.1) that H_2O_2, even at concentrations considerably below the manufacturer's recommended treatment dose of 1500 ppm for 20 min, resulted in the inactivation of a medium to high proportion (temperature dependent) of adult *L. salmonis* immediately post exposure. However, greater than 80% recovered motor

activity by 3 h post treatment, even when a higher than recommended concentration of H_2O_2 was used. Consequently, trials were carried out to investigate the re-infection potential of male and female *L. salmonis* post exposure to concentrations of H_2O_2 commonly used in Scottish aquaculture.

The resettlement level of adult *L. salmonis* was reduced by over 10% and 30% following exposure to 1500 and 1800 ppm respectively when compared to the untreated control lice. However, while there was found to be a statistically significant difference ($p = 0.007$) at 1800 ppm, the difference was insignificant ($p = 0.07$) between lice treated with 1500 ppm H_2O_2 and the control. When taken in conjunction with the previous trial where 100% of the lice had recovered at 12 h post exposure to concentrations of both 1500 and 1800 ppm at 10 ºC (Figure 5), these results suggest that the higher concentration has sub-lethal effects not accounted for by activation ability. Again, whether this level of difference in re-attachment potential would have a large scale effect on subsequent infection cycles remains to be tested. The results however also indicate that the majority of lice are not sub-lethally compromised to such an extent that they cannot re-infect fish. Additionally, in previous studies by Hull (1997) and Hull *et al*. (1998) it was demonstrated that just removing mobile stages of *L. salmonis* from the host and returning them to tanks containing Atlantic salmon resulted in a reduction of approximately 20 % in re-settlement rates which is consistent with the observations from this trial.

An interesting observation made during the trial was that adult females appeared to have a lower re-attachment rate than males in the untreated controls although in both the 1500 ppm and 1800 ppm treatments the reverse was found to be true. When these observations were tested statistically no significant difference was found making drawing meaningful conclusions difficult.

Previous work reported from the literature has demonstrated that husbandry procedures on marine aquaculture sites, such as grading or H_2O_2 bath treatments could potentially dislodge mobile *L. salmonis* into the water column (Ritchie 1997; Treasurer and Grant 1997). Mobile *L. salmonis* stages have also been shown to transfer between hosts if dislodged (Ritchie 1997). Therefore, these findings suggest that the release of large numbers of deactivated mobile *L. salmonis* following H_2O_2 treatments has potential implications for re-infection of the treated site, neighbouring sites or even wild salmonid hosts within the locality. Consequently, treatments, where possible, should be carried out in accordance with optimal water flows in the locality to minimise the risk of re-infection by released sea lice.

6.3 Chalimus *Lepeophtheirus salmonis* Development Post H_2O_2 Treatment

Studies carried out, including the current study, examining the efficacy of H_2O_2 have demonstrated that pre-adult and adult *L. salmonis* can be removed from Atlantic salmon using bath treatments of H_2O_2. As part of this project we exposed Atlantic salmon three days post infestation with copepodid *L. salmonis* to 1500 ppm and 1800 ppm H_2O_2 for 20 min at 10 ºC to determine if H_2O_2 was effective against the attached chalimus stages.

One of the main findings from the statistical analysis was the proportions of *L. salmonis* moulting successfully through to the pre-adult stage after treatment with 1500 ppm and 1800 ppm H_2O_2 was significantly different when compared to the control, indicating a treatment effect. Additionally, settled louse burdens from both treatments showed that male chalimus II proportions were higher and male pre-adult I proportions were lower than those on the control fish indicating a delayed moulting in the males. This difference in stage progression was significant (1500 ppm $p = 0.007$, 1800 ppm $p = 0.002$) for exposure to both 1500 ppm and 1800 ppm H_2O_2 treatments. Similarly, when the female settlement data were examined it was found that at 1500 ppm H_2O_2 treatment resulted in a significantly higher proportion of chalimus II compared to the control while the proportion of pre-adult I were significantly ($p = 0.04$) reduced. On fish exposed to 1800 ppm the proportions of the female chalimus and pre-adult stages were not significantly different from the control.

This latter finding in female chalimus *L. salmonis* exposed to 1800 ppm H_2O_2 makes it difficult to interpret what was occurring biologically with regards the other treatment groups. However, there was considerable difference in lice numbers between the two 1800 ppm tanks therefore the result could be as a result of variations between tanks rather than a true biological effect. Previous studies by Johnson *et al*. (1993) reported there was no significant effect on the chalimus stages after exposure to 1000 to 2000 ppm H_2O_2 at 11 ºC for 20 min. As such further study may be required in this area as the data from this study would suggest that H_2O_2 does have a delaying effect on the development time of chalimus *L. salmonis* to moult through to the later chalimus and pre-adult stages, with the effect varying depending on the sex of the louse.

6.4 *Lepeophtheirus salmonis* Egg string/Larval Development Post H_2O_2 Exposure

The egg strings from adult female *L. salmonis* were used to carry out laboratory based experiments to investigate the effects of a range of concentrations of H_2O_2 at 10 ºC and 13 ºC on egg strings and subsequent larval development to the infective copepodid stage.

Trials demonstrated that H_2O_2 had a significant effect ($p < 0.001$) on the hatching success and subsequent development of *L. salmonis* larvae, with significantly less larvae hatching and developing to the infective copepodid stage with increasing concentration and water temperature. These findings are consistent with a study by Toovey & Lyndon (2000) who reported that egg strings exposed to 1500 ppm H_2O_2 at 10 ºC produced over 80% less larvae compared to untreated egg strings. Similarly, a study by Aaen *et al.* (2014) during field based treatment reported that there was no hatching observed when *L. salmonis* egg strings were exposed to 1750 ppm for 32 min at 6 ºC.

Egg strings appeared to be highly sensitive to treatment with any concentration of H_2O_2 with the hatching success almost halved even when exposed to levels as low as 600 ppm at 13 ºC. These observations are similar to those of a recent study by Aaen *et al*. (2014) who reported that in a laboratory study egg strings treated with 470 ppm H_2O_2 for 36 min at 8 ºC resulted in virtually no hatching. Aaen *et al*. (2014)

attribute the success of their study to the fact they thoroughly stirred the H_2O_2 into the treatment water to mimic field treatment conditions and this was deemed to be a crucial part of the laboratory based study. Similarly, during the current study exposure tanks were mixed before and even during exposure ensuring thorough mixing and distribution of H_2O_2 throughout the exposure tank before treating the egg strings.

There was a slight increase in the hatching success of egg strings exposed to 1500 ppm at both 10 ºC and 13 ºC although when this was investigated statistically no significant difference was found between the treatments and the increase may just be an anomaly within the data. Studies by McAndrew *et al*. (1998) and Aaen *et al*. (2014) both report differences in hatching success of *L. salmonis* egg strings in relation to the level of pigmentation. Aaen *et al*. (2014) describe how egg strings with a low level of pigmentation hatched more successfully than highly pigmented egg strings when exposed to hydrogen peroxide, with McAndrew *et al*. (1998) reporting the inverse. The observations from the current study are therefore more consistent with those of McAndrew *et al*. (1998) who described how pale immature egg strings failed to hatch after treatment, whereas dark mature egg strings successfully hatched and developed through to the copepodid stage, although in significantly fewer numbers than those of the control. The findings of this study and those of McAndrew *et al*. (1998) would suggest that a difference in egg string susceptibility to H_2O_2 exists between immature and mature egg strings. This might be as a result of differences in egg string permeability between early and late developmental stage egg strings.

A study by Schram (2000) observed that gravid females shed eggs on exposure to treatments, and so there may be an advantage to specifically targeting this development stage during wellboat treatments with added filtration added to prevent egg string discharge post treatment. Any eggs that remain viable may result in treatment efficacy issues in future populations.

The data from this study suggest that use of H_2O_2 is highly effective at compromising the egg strings of *L. salmonis*, resulting in high levels of hatching failure. Where hatching does occur greatly reduced numbers of larvae successfully moulted through to the infective copepodid stage. However, further studies are required to determine the viability of these copepodids to successfully infect a salmonid host. In addition, in relation to egg strings and H_2O_2, it would also be useful to determine the effect of H_2O_2 treatment on adult female lice at different concentrations on subsequent egg string production, and the hatching success and subsequent development and infectivity of larval stages. Such information would highlight added benefit from H_2O_2 treatments and help determine its contribution to controlling overall sea lice numbers in the dispersal zone for sea lice larvae.

7. Recommendations

A one day workshop was held in Perth on the 18th November 2014 at which the experimental data generated by the Scottish Aquaculture Research Forum (SARF) funded project "Assessment of the Viability of the Different Life Stages of *Lepeophtheirus salmonis* (Krøyer, 1837), Following Exposure to Hydrogen Peroxide" were presented to representatives of the Scottish salmon farming industry, fish health professionals and ancillary companies. The workshop was intended to allow an exchange of information between researchers and participants to compare and contrast results found under experimental and field situations. It was also intended to identify practices in the field which are already successfully supporting best practice for H_2O_2 treatment of sea lice, or areas where improvements could be made or developed based on the results of the current study.

From the discussions it was apparent that participants already had in place a range of checks and procedures with regards H_2O_2 treatment for sea lice to ensure good pest management strategies. As such we have broken down the main questions into four sections which are –
1) Environmental Parameters
2) Treatment Efficacy with Regards Lifecycle Stage
3) Stock Infestation and Potential Resettlement Post Treatment
4) Strategy/General Questions

We have, where possible, addressed these questions (below) based on the data generated from our study as well as additional information from the literature with regards the use of H_2O_2 for sea lice management.

1) Environmental Parameters

What environmental variables should be monitored prior to and during H_2O_2 treatments?

The discussion focused on oxygen and turbidity in the water – the latter in relation to irritation of the gills during treatment, organic matter loading and accelerated degradation of hydrogen peroxide and therefore inadequate dosing of fish which might compromise the treatment.

Experimental Finding – While the scope of this study did not cover gill irritation due to organic matter within the water column during H_2O_2 treatment, some work was carried out by MSS researchers (see SARFSP005 report) investigating the effects of organic loading on H_2O_2 concentrations. To investigate this parameter waters from Stonehaven Bay, Aberdeenshire, Scotland with heavy biological loading (Secchi disk of 2.5 m) were used as well as additional laboratory trials involving the addition of Humic and Tannic acid. The trials carried out by MSS demonstrated there was no reduction in H_2O_2 concentration due to organic loading. However, a study by Lyons *et al*. (2014) carried out in Canada reported that H_2O_2 degradation in the formulation Interox Paramove™50 was slower in natural unfiltered seawater than in filtered seawater.

Recommendation - It was agreed that maintaining oxygen levels during treatment was essential and this is in line with recommendations by H_2O_2 manufacturers. During treatment it is strongly advised that oxygen levels should be monitored with additional aeration added throughout the treatment at a level of 8 -12 mg /L.

Additionally, it is also advised that a Secchi disk reading should be taken prior to treatment to measure the turbidity or to give an indication of plankton blooms, with a minimum reading of 3.5 m recommended before proceeding with a H_2O_2 treatment in line with the guidance from Salartec and Solvay (Paramove).

2) Treatment Efficacy With Regards To Lifecycle Stage

What is the best stage to target*?*

A considerable amount of discussion and anecdotal observations revolved around treatment efficacy, and variations in the different *L. salmonis* lifecycle stages to exposure to H_2O_2.

Experimental Findings - The laboratory trials demonstrated that, particularly with very immature and very mature *L. salmonis* egg strings, the H_2O_2 resulted in a high level of hatching failure among egg strings. Of those that did hatch there was a considerable mortality among the nauplius stages to the copepodid stage. Additionally, data from this study indicate that the lower doses (~ 1200 to 1300 ppm) of H_2O_2 used for the treatment of AGD would also be effective at preventing the hatching and/or moulting through infective stages of most larval lice. This could be used as a method of strategic intervention although this could potentially increase the likelihood of resistance building up within lice populations.

H_2O_2 was largely found to be ineffective against the chalimus stages, although it was shown to cause a delay in moulting through the chalimus and pre-adult stages but this also varies by the sex of the louse. It may be worth noting that during moulting pre-adult I and II stages do temporarily attach onto the host via a frontal filament (Pike and Wadsworth 2000). This means that some pre-adults may not be removed from the fish during treatment resulting in them developing into adults shortly post treatment. This should be detected during the weekly lice count procedure.

The use of H_2O_2 was found to be effective at immobilising adult *L. salmonis.* However the majority of these lice do recover within 3 h and approximately 50% have been demonstrated to be able to re-infect a salmon host in tank trials.

Recommendation - Due to the variation in susceptibility of the different lifecycle stages that can be found infesting farmed Atlantic salmon it is recommended that, for effective long term lice management, sites utilise a range of therapeutants on a rotational basis. Such a combination could include utilising a treatment, such as SLICE®, which offers an extended period of protection post treatment prior to a H_2O_2 application. This would ensure the efficacy of each product used is optimised during each treatment and to reduce the risk of resistance building up in lice populations.

Is there any preference in using H_2O_2 (first or second year of production) or seasonality?

Some questions centred around when H_2O_2 should be used, both in relation to the point in the production cycle when administrated, as well as seasonality. In relation to the latter, treatment concentrations higher than the manufacturers' recommended 1500 ppm are often used which, particularly in relation to higher water temperatures, can result in gill damage.

Experimental findings – Laboratory trials on *L. salmonis* egg strings demonstrated that H_2O_2 had a significant effect ($p < 0.001$) on the hatching success of egg strings and subsequent development of the larvae. Significantly less larvae hatched and developed to the infective copepodid stage with increasing H_2O_2 concentration and water temperature.

Trials exposing adult lice to a range of H_2O_2 concentrations showed that all the lice recovered at all concentrations at 10 ºC, although at 13 ºC a number of lice failed to reactivate by the end of the trial at 96 h – particularly at 1800 and 3000 ppm. These findings suggest that at higher temperature the effects of H_2O_2 may be more detrimental to the adult lice and compromise them in such a way that their later viability and subsequent survival are reduced.

Recommendations – The data showed for both egg strings and adults that at higher H_2O_2 concentrations and temperatures H_2O_2 treatment was more effective. At 13 ºC H_2O_2 compromised egg strings resulting in either complete failure in egg string hatching or a significantly reduced number of larvae moulting to the copepodid stage compared to controls. Therefore the manufacturers recommended H_2O_2 dosage of 1500 ppm and above is particularly effective when water temperatures are 10 – 13 ºC. Treatment with H_2O_2 appears to be highly effective at compromising the egg strings of *L. salmonis* resulting in high levels of hatching failure from egg strings. Where hatching does occur, greatly reduced numbers of larvae successfully moult through to the infective copepodid stage.

Similarly, higher water temperature, even at low concentrations, inactivated adult *L. salmonis* for longer periods or, at 1800 and 3000 ppm, resulted in some mortalities. These findings indicate that at higher temperatures and at the higher concentration of 1800 ppm that is often used the treatment would result in mortalities among the adult lice population of approximately 10% (0% at 1500 ppm) and an extended period of inactivation compared to a treatment at 1500 ppm. An extended period of inactivation post treatment could result in a greater period of dispersal of detached lice. This may reduce the risk of re-infection of the treated site although wind, current speed and direction should be taken into account to reduce the potential risk of re-settlement on stock in neighbouring sites.

While the authors cannot advocate utilising concentrations higher than the manufacturers' guidelines it became apparent through dialogue with industry representatives that this regularly occurs although exposure times are usually reduced accordingly. However, it must be highlighted with regard to temperature/seasonality that the toxicity of H_2O_2 to stock increases with temperature so its use at temperatures above 14 ºC is not usually carried out although occasional

use was reported but with reduced exposure time and close observation of the fish during treatment.

There was no overall preference reported regarding the use of H_2O_2 to year of production although as the product does not require a withdrawal period prior to harvesting for human consumption then its use may be preferable in the latter stages of grow-out compared to other treatments.

What is considered as an effective H_2O_2 treatment with regards a reduction in infestation levels on stock?

Experimental Findings – Aquarium tank based experiments determined the ability of adult *L. salmonis* exposed to either 1500 ppm or 1800 ppm H_2O_2 for 20 min at 10 ^oC to re-infect Atlantic salmon. The resettlement level of adult *L. salmonis* was reduced by over 10% and 30% following exposure to 1500 and 1800 ppm respectively when compared to the untreated control lice. However, while there was found to be a statistically significant difference ($p = 0.007$) at 1800 ppm, when data were tested at 1500 ppm the difference was insignificant ($p = 0.07$) between lice treated with 1500 ppm H_2O_2 and the control. The results indicate that the majority of lice are not sub-lethally compromised to such an extent that they cannot re-infect fish.

Recommendation – Lice counts should be done 24 - 48 h post treatment as this should indicate how successful a treatment has been. A sample should be taken of any surviving lice and, where possible, submitted for bio-assay/sensitivity analysis. A combination of these data would give an indication of any efficacy issues and/or re-settlement levels post H_2O_2 exposure.

Additionally, treatment efficacy could also be improved by removing floating lice within 1-3 h post treatment to avoid dispersion & re-infection. Some sites report that this is carried out using small meshed hand nets although on larger circle cages the proportion of immobilised lice removed is likely to be small. Another observation reported is that the lice floated for only 2-3 minutes after treatment commenced before they sank so therefore any removal would have to involve a small meshed (100 µm) net dragged over the surface like a sweep net. How effective this would be is unclear and the effect this may have on already stressed fish is questionable.

3) Stock Infestation & Potential Resettlement Post Treatment

Considerable infestation levels of chalimus has been observed post treatment and could this be linked with H_2O_2 treatment?

A number of workshop delegates reported elevated levels of *L. salmonis* chalimus stages on farmed Atlantic salmon post H_2O_2 exposure and there was some discussion on how this could be linked to the treatment.

Experimental Findings – As previously reported above during laboratory based trials the only detrimental effect detected on the chalimus stages was a delay in moulting to the pre-adult stages so we cannot draw direct comparison from the trials

carried out under this project. However, the literature, observations by Schram (2000) would indicate that treatments can result in the release of egg strings and observations during the current study would indicate that very mature egg strings often hatched on immersion to H_2O_2 baths. Despite data from the current study identifying a high efficacy against the egg strings it should be noted that they were of a naïve strain so may be more susceptible than in areas where there have been regular H_2O_2 treatments. Therefore these reports may indicate that treatments could result in the "co-ordinated" release of large numbers of egg strings of which, despite many being compromised, the remaining viable egg strings would all hatch over a short time period as the development time to the infective copepodid stage is 63 h at 11 ºC (Johannessen 1978). The occurrence of large numbers of chalimus in the 3 – 5 days post treatment may therefore arise from higher infestation pressure as a result of the treatment and synchronised egg hatching. Alternatively alterations in the skin mucus layer due to H_2O_2 exposure may facilitate/promote higher infestation but further investigation would be needed for clarification.

Recommendations - Where a bath treatment uses tarpaulins then a fully enclosed tarpaulin should be deployed rather than an open "skirt" treatment to ensure fish and lice are exposed to optimal levels of H_2O_2 for the full treatment period, reducing the likelihood of a resistance build up, and reducing the risk of detached egg strings or immobilised lice sinking below the "skirt", dispersing from the cage and on recovery either infecting fish in the same cage or neighbouring cages or sites.

Ideally if sites have the necessary licences then it is recommended that a well boat is used, with the treatment water retained and treated to kill the lice or mechanical filtration used to remove lice prior to discharge. Consequently this will ensure as much material of all lifecycle stages is retained; some workshop attendees reported an increase in settled larval stages in the period after a treatment which is likely due to a simultaneous egg string release or egg hatching event as a result of the female lice being stressed by the treatment.

4) Strategy/General Questions

Should gill condition be considered prior to H_2O_2 treatment?

A number of instances were reported where the gills of fish had become compromised by other events such as AGD infection, jellyfish or the mechanical damage caused by the silica frustules from some species of phytoplankton during blooms. Fish subsequently treated have been reported to react badly to H_2O_2 with mortalities recorded in a number of instances as a result.

Experimental Findings and Recommendations – While this question fell outwith the remit of this study a complementary study "SARFSP005 Assessment of the viability of *Neoparamoeba perurans* following exposure to hydrogen peroxide" did look at some elements of gill pathology and gill scoring and should be consulted.

However, on a more general level stock identified for H_2O_2 treatment should first be examined by well-trained staff pre-treatment with a gross scoring carried out across a representative sample of fish within the cage.

- Prior to a H_2O_2 treatment a representative fish sample should be taken and gills histologically examined by a qualified pathologist. These recommendations are consistent to those proposed by some industry Fish Health Managers to be performed on a weekly basis to monitor the health status of the fish to be treated.

- No H_2O_2 treatment should be carried with a gill score of over three (As recommended by Salartec) to reduce the risk of welfare issues or mortalities.

- Check deleterious effects of H_2O_2 post treatment within 24 - 48 h post treatment.

How often should titrations be carried out to ensure H_2O_2 levels are at the correct level to remove lice without any detrimental effects on stock?

Experimental Findings and Recommendations – Again this question fell outwith the remit of the study although, from the authors' personal experiences carrying out the trials in aquaria, it is recommended that water samples should be taken at 0 and 10 minutes during H_2O_2 treatment and titrations carried out utilising the method outlined in the Solvay (Paramove) product guidance manual. Samples should be taken at various locations and depths in treatment tarps to ensure good mixing was achieved and fish are adequately exposed to the correct dose. A final sample taken 15 minutes after flushing and tarpaulin removal according to guidance provided by Salartect which advises that in cages H_2O_2 levels should be below 100 ppm after 10 min post treatment and in well boats below 200 ppm within 15 min and 0 ppm within 30 min post treatment.

8.0 Future Work

The work carried out under SARFSP001 has provided data on the efficacy of H_2O_2, at a range of concentrations, on the main phases of the *L. salmonis* lifecycle. However, the work has also identified some knowledge gaps that require additional study to provide a greater understanding of *L. salmonis* biology and to allow more effective pest management strategies to be utilised by the Atlantic salmon farming industry, supporting future sustainable growth and expansion of the industry.

Data from this study demonstrated that the egg strings of *L. salmonis* are compromised by exposure to H_2O_2 even at relatively low concentrations resulting in low numbers of larvae moulting through to the copepodid stage. Therefore, it would be useful to determine the effect of H_2O_2 treatment at a range of concentrations on adult female lice on subsequent egg string production, and the hatching success and subsequent development to, and infectivity of, the copepodid stage. These studies would investigate, with the use of bio-assays and tank challenges, the development of resistance among lice populations, with data forming the basis of predictive population models which could be used to identify different treatment strategies to limit future efficacy issues.

Trials exposing adult *L. salmonis* to concentrations of H_2O_2 utilised by industry demonstrated that while H_2O_2 temporarily deactivated adult *L. salmonis* the vast majority recovered, and aquaria based infections trials confirmed they were able to re-infect salmon. Therefore, these findings suggest that post H_2O_2 treatment there is likely to be a release of large numbers of deactivated mobile *L. salmonis* which will likely recover, resulting in potential re-infection implications for any local salmonid populations. Modelling work conducted by MSS for Scotland's largest sea loch system, Loch Linnhe (Salama *et al.* 2013, Salama & Rabe 2013) has produced initial results demonstrating that sea lice released from sites within the system can be transported in the marine environment, with the majority being transported within 15 km (Salama *et al*., 2015) over a 19 day period. Laboratory based trials by Johnson and Albright (1991) reported that adult *L. salmonis* survived for 18 days off host. Modelling by Salama *et al.* (2014) has also demonstrated that the majority of sites self-expose, that is to say lice emanating from release sites return to the farm as infective copepodids. The work contained in this report demonstrates that a large proportion of lice remain viable after a H_2O_2 treatment event, and ability to reattach is also high. However, there is no indication of whether these lab based observations would occur either at a commercial farm site or at neighbouring sites, despite the substantial proportion of viable mobile sea lice. This is an important knowledge gap and field experiments would provide further estimates of the efficacy of using H_2O_2 as a control method for lice burdens on farmed fish. It is proposed that, to address this knowledge gap, the infestation pressure for recovered infective adult sea lice in the marine environment surrounding a site might be monitored for 3 days post H_2O_2 treatment (as current study found most adult sea lice recovered at 72 h post treatment with H_2O_2 concentrations commonly used on Scottish farms). A transect of sentinel cages containing Atlantic salmon could be located away from sites and the re-settlement rate estimated. Additionally, dispersal modelling could be used to estimate the distance in which dislodged, viable lice may be theoretically transported over a 3 d period.

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Appendices

Table 3.
Summary of previous work exposing a range of lifecycle stages to hydrogen peroxide at a range of temperatures, concentrations and durations.

Author (s)	Concentration (ppm)	Temp (°C)	Exposure Time (Min $^{-1}$)	Lifecycle Stage
Johnson *et al*. (1993)	1000 1500 2000 3000 4000	11 °C	20	Chalimus Pre-adult Adult
Bruno & Raynard (1994)	500 1250 2000 3000	10 °C	20	Copepodid (1250 and 3000 only) Pre-adult Adult
Treasurer & Grant (1997)	400 600 800 1000 1500	10 °C	20	Chalimus (1500 only) Pre-adult Adult
McAndrew *et al*. (1998)	1500	7.5 °C	20	Egg strings Nauplii Copepodids Chalimus Pre-adult Adult
Aaen *et al*. (2014)	470 (Lab) 1000 (Lab) 1500 (Lab) 2000 (Lab) 1800 (Field)	8.0 °C 6.4 °C	36 31	 Egg strings Nauplii Copepodids
*Current Study	300 600 1000 1200 1500 1800 2300 3000	 10 °C 13 °C		Egg strings Nauplii Copepodids Chalimus Pre-adult Adult

* In the current study –
Egg strings were exposed to 600, 1200, 1500 and 1800 ppm at 10 °C and 13 °C.
Chalimus (Infesting Atlantic salmon) were exposed to 1500 and 1800 ppm at 10 °C.
Adults were exposed to 300, 600, 1000, 1500, 1800, 2300 and 3000 ppm at 10 °C and 13 °C.

Scottish Aquaculture Research Forum

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SARF - Member Organisations

Industry

SEAFISH
Association of Scottish Shellfish Growers
Scottish Salmon SSPO
British Trout Association

Government and Regulators

Scottish Natural Heritage
SEPA Scottish Environment Protection Agency
marinescotland
The Scottish Government
HIE Highlands and Islands Enterprise
Iomairt na Gaidhealtachd 's nan Eilean

Non-Governmental Organisations

The Crown Estate
Scottish Environment LINK
Rivers & Fisheries Trusts of Scotland
Safeguarding Scotland's Rivers & Lochs
ASFB The Association of Salmon Fishery Boards