



Short communication

## First report of reduced sensitivity towards hydrogen peroxide found in the salmon louse *Lepeophtheirus salmonis* in Norway

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## ABSTRACT

Reduced sensitivity towards chemotherapeutants in the salmon louse *Lepeophtheirus salmonis* (Krøyer) is an increasing problem for the fish farming industry. Most fish farmers are dependent on chemical treatments in order to maintain salmon lice numbers below permitted levels. However parasites showing reduced sensitivity contribute to complicating this task. Hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) is used as a delousing agent in bath treatments and until recently treatment failures due to reduced H<sub>2</sub>O<sub>2</sub>-sensitivity have not been documented in Norway. The aim of the current study was to develop a bioassay protocol suitable for testing H<sub>2</sub>O<sub>2</sub>-sensitivity in *L. salmonis*. If failed treatments were found to be caused by parasite insensitivity to H<sub>2</sub>O<sub>2</sub> the possibility of this reduced sensitivity being hereditary was looked into. The results show that bioassays permit differentiation between strains of salmon lice with regards to H<sub>2</sub>O<sub>2</sub>-sensitivity, coinciding with treatment efficacies. Up to ten times variance in sensitivity between two strains was recorded. The progeny of the least sensitive salmon lice also showed reduced sensitivity to H<sub>2</sub>O<sub>2</sub> in a bioassay, which indicates that reduced sensitivity towards H<sub>2</sub>O<sub>2</sub> is hereditary. The current study presents the first case report of reduced sensitivity towards H<sub>2</sub>O<sub>2</sub> in salmon lice in Norway. This change in sensitivity imposes a threat to the Norwegian fish farming industry and should be monitored closely.

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### 1. Introduction

The salmon (or sea) louse *Lepeophtheirus salmonis* (Krøyer) infests both wild and farmed salmonids in the Northern Hemisphere (Costello, 2006; Pike and Wadsworth, 2000; Torrissen et al., 2013). Control of the salmon lice in fish farms is important in order to protect the farmed fish from parasite-related stress and injuries as well as minimizing the infestation pressure on wild salmonids. Most fish farms are dependent on chemical treatments in order to keep the parasite numbers below national maximum permitted levels, but few chemical treatment agents are available (Burka et al., 1997; Roth, 2000; Westcott et al., 2004). This has subsequently led to the development of reduced sensitivity in *L. salmonis* towards most of the available chemical treatments (Jones et al., 1992; Lees et al., 2008a; Roth et al., 1996; Sevatdal and Horsberg, 2003; Treasurer et al., 2000). The geographical dispersion of the reduced sensitivity towards each treatment agent

varies (Grøntvedt et al., 2014; Jones et al., 2013; Lees et al., 2008b). Chile, the major salmonid producing country in the Southern Hemisphere is having problems with the sea louse *Caligus rogercresseyi* (Johnson et al., 2004). In Chile, chemical treatments are essential in order to control sea lice levels; however this task has become increasingly difficult as the parasites have developed reduced sensitivity towards both pyrethroids and the avermectin emamectin benzoate (Bravo et al., 2008, 2013; Helgesen et al., 2014).

In Norway, hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) was used to a certain extent as a delousing agent between the years 1993 and 1997 (Grave et al., 2004). This use was terminated due to the introduction of more efficient chemicals with larger safety margins. The emerging occurrence of reduced sensitivity towards other compounds however led to the re-introduction of H<sub>2</sub>O<sub>2</sub> for salmon lice treatment in 2009 (Norwegian Institute of Public Health, 2014). In order to delay the development of reduced sensitivity, an increased range of chemical treatment agents is desirable. This permits rotation between compounds with various modes of action subsequently postponing the development of reduced sensitivity (Brooks, 2009; Denholm et al., 2002). Unfortunately, the extensive use of H<sub>2</sub>O<sub>2</sub> as a delousing agent in various parts of Norway (Grøntvedt et al., 2014) has increased the risk of developing reduced sensitivity towards

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this compound. In Scotland, reduced sensitivity towards  $H_2O_2$  in salmon lice was reported in the year 2000, following extensive use of this treatment agent over several years (Treasurer et al., 2000). Due to the toxicity and low safety margin of  $H_2O_2$  in fish, only minor increases in treatment time and concentration can be introduced (Bruno and Raynard, 1994; Thomassen, 1993). In order to avoid unsuccessful treatments due to reduced parasitic sensitivity, a method for sensitivity testing in salmon lice is therefore required.

Incidences of reduced treatment efficacy following  $H_2O_2$ -treatments in Norway have raised the issue of possible reduced sensitivity. Treatment failure may be due to inadequate delousing procedures or reduced sensitivity in the parasites (Denholm et al., 2002). Biological assays (bioassays) are employed to test for reduced sensitivity in arthropods when the resistance mechanisms are unknown (Robertson et al., 2007). Bioassays have been developed for sensitivity testing in *L. salmonis* towards pyrethroids, emamectin benzoate and the organophosphate azamethiphos (Helgesen and Horsberg, 2013; Sevatdal and Horsberg, 2003; Westcott et al., 2008; Whyte et al., 2013). Treasurer et al. (2000) tested for  $H_2O_2$ -sensitivity using small scale treatments, however this method requires the use of fish and due to animal welfare and practical reasons should be avoided if possible.

Possible mechanisms for reduced  $H_2O_2$ -sensitivity in salmon lice include increased antioxidant enzymes activity such as catalase, glutathione peroxidase or glutathione-S-transferase, all of which have been found in mammalian cells in conjunction with  $H_2O_2$ -resistance (Fiander and Schneider, 2000; Spitz et al., 1992; Baud et al., 2004). Increased catalase activity has also been seen in  $H_2O_2$ -resistant bacteria and fungi (Amin and Olson, 1968; Elkins et al., 1999; Nakamura et al., 2012; Uhlich, 2009). Other possible enzymes involved in reduced  $H_2O_2$  sensitivity are superoxide dismutase, superoxide reductase, glutathione reductase and thioredoxin, as they are proven to possess activity against reactive oxygen species (Nordberg and Arner, 2001).

The primary objective of the present study was to develop bioassay protocols suitable for  $H_2O_2$ -bioassays. Whether reduced treatment effects from  $H_2O_2$ -treatments were caused by reduced salmon lice sensitivity was also investigated. This required using bioassays on both field collected parasites and their laboratory reared progeny.

## 2. Materials and methods

### 2.1. Salmon lice

Salmon lice originating from seven different farms in Norway were employed in the current study (Table 1). All salmon lice with the exception of Ls A originated from the northern part of Mid-Norway. Ls V came from a farm reporting of reduced treatment efficacy in  $H_2O_2$ -treatment since one year back. The treatment performed three months prior to parasite collection for field bioassays had 74.1% efficacy in mobile stages (salmon lice from 80 fish collected from 4 fish cages were counted on the day of treatment and on the following day to calculate efficacy). The treatment efficacy was 51.4% in mobile stages in the treatment performed one month before the Ls V F0 bioassay was performed (salmon lice from 30 fish from 3 cages were counted on two consecutive days, with treatment on day 1, to evaluate treatment efficacy). Ls Ky, Ls D and Ls Aa all came from farms with a history of  $H_2O_2$ -treatments against salmon lice, but without having experienced reduced treatment efficacies. Ls S and Ls Kl originated from the same area, but from farms which had not treated with  $H_2O_2$  themselves. Ls A was a laboratory strain originally collected from the northern part of North-Norway in 2011. Bioassays and small scale treatments had

shown this strain to be sensitive to pyrethroids, azamethiphos and emamectin benzoate (Helgesen and Horsberg, 2013). Ls A had never been exposed to  $H_2O_2$ , neither in field nor in the laboratory.

The salmon lice designated for field bioassays were collected from anesthetized fish at the sea farms and transported in cooled sea water to the laboratory. These bioassays, six in total, were performed by Aqua Kompetanse AS at their laboratory in Flatanger, Norway. The other five bioassays were performed at The Norwegian University of Life Sciences in Oslo, Norway. Table 1 provides the details. The salmon lice for the five latter assays were reared on fish at the NIVA Marine Research Station in Drøbak, Norway. Ls A was kept in a continuous culture on Atlantic salmon (*Salmo salar*), while Ls V was sent to the laboratory as egg strings. After hatching and development into copepodites, 20 sea trout weighing about 150 g each were infested with the parasites. Approximately 50 copepodites per fish were employed for the infestation, which was conducted in 30 l of aerated sea water for 45 min. Pre-adult parasites and adult males were used for the bioassays depending on which instars were available at the time. All bioassays were initiated within 8 h after parasite collection.

### 2.2. Bioassays

Two types of bioassays were performed: 30-min and 24-h bioassays. The 30-min bioassays were performed according to the protocol for pyrethroid bioassays described in Sevatdal and Horsberg (2003) with some modifications. The parasites were exposed to between six and twelve different concentrations of  $H_2O_2$  using Interlox Paramove 50 ( $H_2O_2$  50%, w/w, Solvay Chemicals, Belgium) in Oslo and Eka HP T49 S ( $H_2O_2$  49.7%, w/w, Azko Nobel, Sweden) in Flatanger, diluted in sea water. Nominal concentrations ranging from 0 to 5000 mg L<sup>-1</sup> were utilized and varying concentrations within this range were chosen according to the expected sensitivity level of the respective salmon lice strain. The salmon lice were distributed in sea water filled polystyrene bioassay boxes, with approximately 10 parasites (6–13) in each box. The different concentrations of  $H_2O_2$  were prepared by adding the appropriate amount of  $H_2O_2$  to cooled sea water (10–12 °C) in one litre polypropylene containers. In each of the concentrations applied in each bioassay, two of the bioassay boxes were submerged. For the field bioassays with Ls Ky, Ls D, Ls Aa and Ls Kl only one box of parasites was used for the control group. The boxes were kept in the solution for 30 min and the results were immediately recorded. Parasites attached to the wall of the box or swimming in a straight line were considered alive. All others were regarded as immobilized or dead. For the bioassays on Ls A and Ls V F1 the boxes containing the parasites were relocated to fresh sea water after the initial evaluation, without removing any parasites, and kept at 12 °C under constant aeration for the following 24 h. The results were then re-evaluated.

The 24-h bioassays were performed according to the protocol for pyrethroids, azamethiphos and emamectin benzoate bioassays described in Helgesen and Horsberg (2013), with some modifications. Between eight and seventeen parasites from Ls A were exposed to six or seven different nominal concentrations of  $H_2O_2$ , ranging between 0 and 120 mg L<sup>-1</sup>, in one litre glass bottles. The bottles were kept at 12 °C for 24 h and supplied with constant aeration. Ls V was exposed to 11 different nominal  $H_2O_2$ -concentrations between 0 and 1800 mg L<sup>-1</sup>. Between 30 and 61 parasites were used for each concentration. After the exposure period the results were recorded by turning the bottles upside down three times and then moving them in circles with a diameter of 20 cm 10 times. When the water had settled it was poured out into a beaker. All parasites remaining in the bottle or able to attach to the beaker wall or swim in a straight line were considered alive. All other parasites were regarded as immobilized or dead.

**Table 1**

Bioassays. All salmon lice strains were collected in the northern part of Mid-Norway with the exception of Ls A which came from the northern part of North-Norway. Ls V F0 and Ls V F1 were the same strain, but bioassays were performed both with parasites from the field (F0) and with laboratory-reared parasites from field collected egg strings (F1). Two types of bioassays were performed, with both 30 min and 24 h parasite exposure to hydrogen peroxide. Six to eleven different concentrations were employed for each bioassay and the dose-response curve were modelled in JMP (SAS Institute Inc., Cary, NC, USA). The concentrations in  $\text{mg L}^{-1}$  immobilizing 50% of the parasites ( $\text{EC}_{50}$ ) with 95% confidence intervals (CI) are given.

Strain	Origin	Date	Place	Assay	$\text{EC}_{50}$	Lower 95% CI	Upper 95% CI
Ls A	Laboratory	10.10.2013	Oslo	30-min	216	153	305
Ls V F1	Laboratory	13.11.2013	Oslo	30-min	1767	1494	2090
Ls V F0	Field	11.10.2013	Flatanger	30-min	2127	1253	3610
Ls S	Field	03.02.2014	Flatanger	30-min	539	386	754
Ls Ky	Field	03.02.2014	Flatanger	30-min	693	483	993
Ls D	Field	31.01.2014	Flatanger	30-min	563	435	730
Ls Aa	Field	30.01.2014	Flatanger	30-min	541	403	727
Ls Kl	Field	30.01.2014	Flatanger	30-min	538	373	776
Ls A	Laboratory	12.02.2014	Oslo	24-h	45.9	29.8	70.9
Ls A	Laboratory	23.06.2014	Oslo	24-h	64.7	43.4	96.4
Ls V F1	Laboratory	13.11.2013	Oslo	24-h	138	112	171

The bioassay results were modelled using probit-analysis in JMP 10.0.0 (SAS Institute Inc., Cary, NC, USA) to find  $\text{EC}_{50}$ -values, which is the concentration immobilizing 50% of the parasites, with 95% confidence intervals (CI).

### 3. Results

Ls A had the lowest  $\text{EC}_{50}$ -values for both the 30-min and the 24-h bioassays with 216 and 45.9 to 64.7  $\text{mg L}^{-1}$  respectively. Ls V had the highest  $\text{EC}_{50}$ -values for both types of bioassays, with  $\text{EC}_{50}$ -values of 1767 and 2127  $\text{mg L}^{-1}$  in the 30-min bioassays and 138  $\text{mg L}^{-1}$  in the 24-h bioassay.

The five other strains tested in the 30-min bioassay showed intermediate  $\text{EC}_{50}$ -values, ranging from 538 to 693  $\text{mg L}^{-1}$ . The dose-response curves for Ls A and Ls V F1 with 90% CI are presented in Fig. 1 (30 min exposure) and Fig. 2 (24 h exposure). All results after modelling are displayed in Table 1.

No control group mortality was seen in any of the bioassays with 30 min exposure and immediate evaluation. In the 24-h bioassay, 0 and 8.3% mortality was observed in the Ls A control groups, while 7.5% of the parasites in the Ls V-bioassay-control group were dead after 24 h.

With respect to the results for the 30-min Ls A and Ls V F1 bioassays, fewer parasites were regarded as dead or immobilized at the re-evaluation 24 h after exposure than in the first evaluation. With respect to Ls A the total percentage immobilized parasites decreased from 68 to 51% when the results from all concentrations were included. For Ls V F1 the same group decreased from 48 to 21%. The attempts to model the re-evaluation results gave illogical dose-response curves.

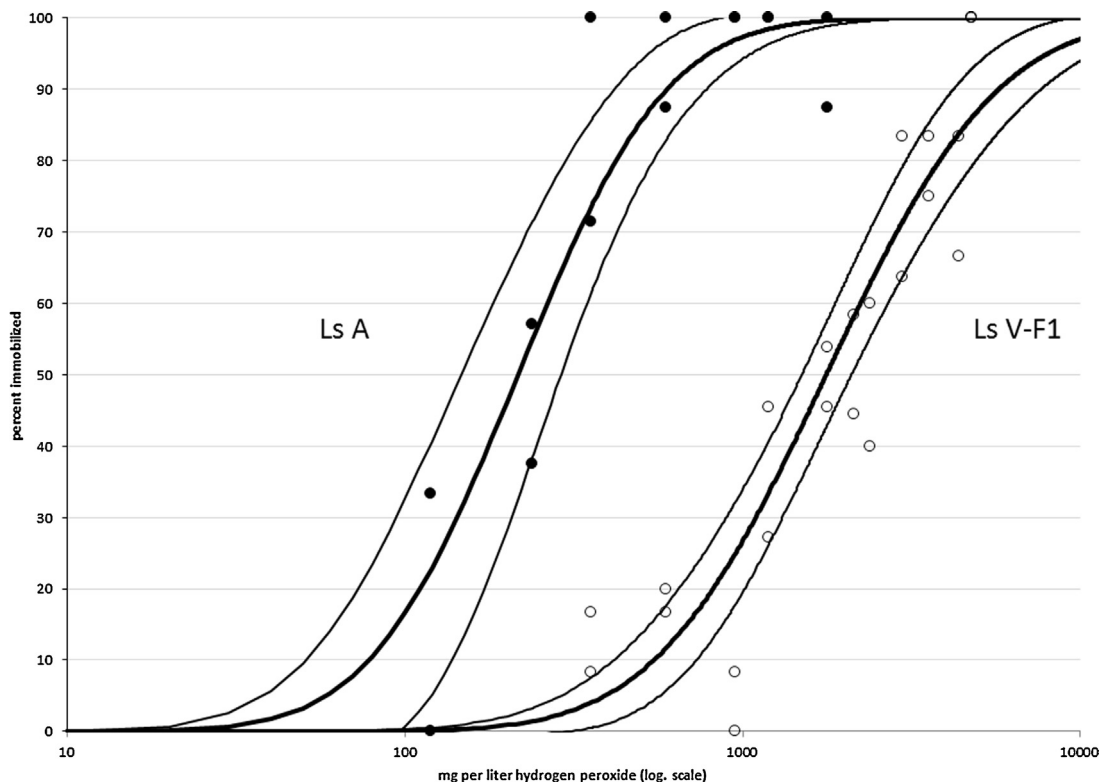
### 4. Discussion

Assuming that  $\text{H}_2\text{O}_2$ -sensitivity is similar in both attached and free-swimming parasites; one would expect reduced treatment efficacy from treatment of fish infested with Ls V. The treatment regime, outlined in the summary of product characteristics for Paramove (49.5%  $\text{H}_2\text{O}_2$ , Solvay chemicals), is exposure to 1500  $\text{mg L}^{-1}$   $\text{H}_2\text{O}_2$  for 20 min (Norwegian Medicines Agency, 2014). In the Ls V bioassays, less than 50% of the parasites were immobilized when exposed to this concentration for 30 min. The results from the bioassays may not however be directly interpreted into treatment results. The bioassays were performed on parasites detached from the fish and sensitivity to chemicals may differ between parasites attached to and detached from the fish (Sevatdal and Horsberg, 2003). Furthermore during treatment, a constant  $\text{H}_2\text{O}_2$ -level is maintained through-out the exposure time. In contrast the bioassays were performed by adding the substance

to sea water only at the beginning of exposure. In order to develop bioassays into an accurate prediction tool for treatment efficacy, the correlation between bioassay results and treatment results need to be elucidated.

The intermediate bioassay results found in five of the tested strains could be a sign of reduced sensitivity. This hypothesis is supported by the fact that all of the lower 95% CI values in these strains were above the upper 95% CI for the fully sensitive strain, Ls A. The intermediate results could also represent the range of  $\text{EC}_{50}$ -results found in sensitive parasites. This latter theory is supported by the results obtained by Bruno and Raynard (1994). Evaluating their bioassay results using probit analysis in JMP, an  $\text{EC}_{50}$ -value of 890  $\text{mg L}^{-1}$  could be calculated for pre-adult parasites and 503  $\text{mg L}^{-1}$  for adult salmon lice. In an experiment where salmon lice infested salmon were treated with different concentrations of  $\text{H}_2\text{O}_2$ , an  $\text{EC}_{50}$ -value of 800  $\text{mg L}^{-1}$  was obtained (Thomassen, 1993). In both trials results were evaluated immediately after exposure. However the shorter exposure period in these trials (20 min) would have increased the  $\text{EC}_{50}$ -values. This in comparison to a protocol with 30 min exposure time. Furthermore both experiments were performed at lower temperatures (10 °C in Bruno and Raynard's experiment and 6–9 °C in Thomassen's experiment) than in the current study, which might also have affected the results. The toxic effect of  $\text{H}_2\text{O}_2$  on salmon lice increases at higher temperatures (Treasurer and Grant, 1997).  $\text{EC}_{50}$ -values would therefore be expected to be higher at lower temperatures. Thomassen's experiment was performed on parasites attached to fish. Given that salmon lice are more susceptible to pyrethroid bath treatments when detached from fish, (Sevatdal and Horsberg, 2003) and if this is also the case for  $\text{H}_2\text{O}_2$ , then the same parasites would have shown a lower  $\text{EC}_{50}$ -value if Thomassen's experiment had been performed on parasites which had been removed from the fish. Whether the differences in  $\text{EC}_{50}$ -values between different life stages of salmon lice, as seen by Bruno and Raynard (1994), were present in the current study, was not evaluated. If differences were present this might have biased the results as the parasitic instars were not allocated evenly within or between the experiments. Future research should look into the possible differences of  $\text{H}_2\text{O}_2$ -sensitivity between instars of *L. salmonis* as this might influence both sensitivity assessments and treatment effects.

A large proportion of the parasites immediately characterized as immobilized, were 24 h later re-evaluated as alive. Recovering parasites after  $\text{H}_2\text{O}_2$ -exposure were also observed by Hodneland et al. (1993), Johnson et al. (1993) and Treasurer and Grant (1997). This implies that salmon lice have mechanisms to fight the effect of  $\text{H}_2\text{O}_2$ . These mechanisms seem to be induced by the treatment, as sensitive parasites are immediately knocked down and



**Fig. 1.** 30-min bioassay. The dose-response curves (90% CI) and the observed immobilization rates for two of the salmon lice strains tested for sensitivity towards hydrogen peroxide in a 30-min bioassay. The observed rates for the sensitive strain (Ls A) are indicated by filled circles, while the corresponding values for the strain with reduced sensitivity (Ls V) are indicated by open circles.

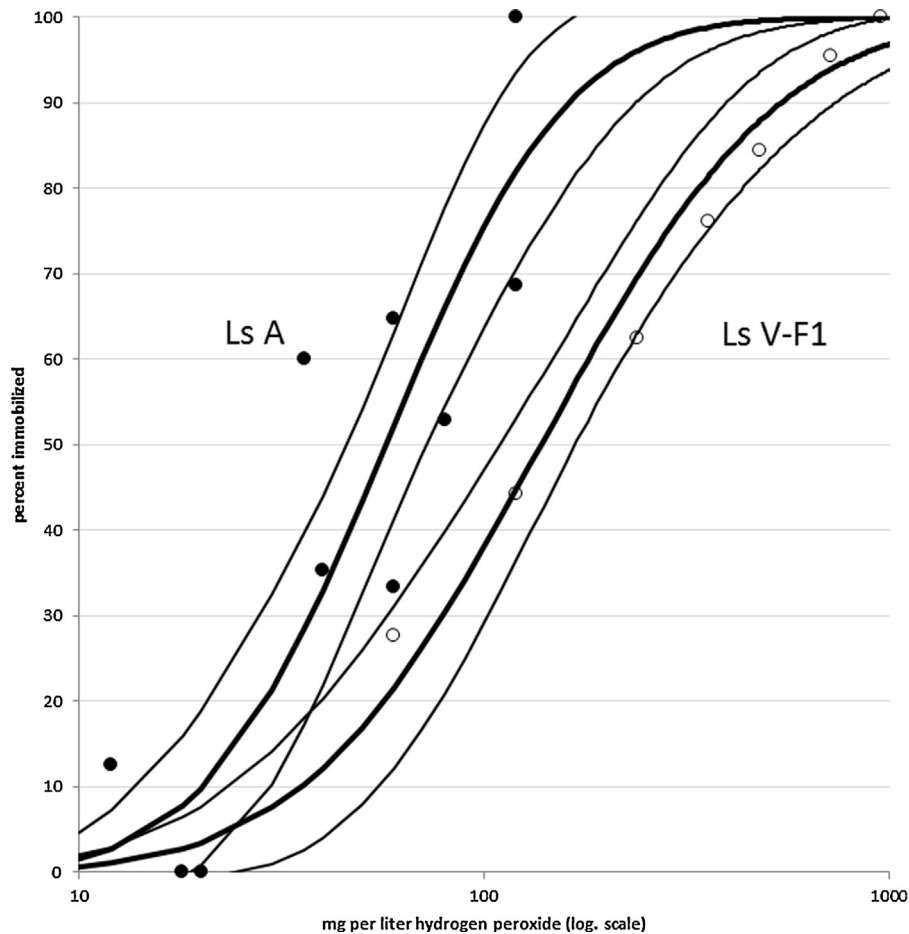
later recover. Recovery, following treatment-induced immobilization, was observed to a greater extent in parasites from the least sensitive strain. This was in addition to the fact that a higher concentration of  $H_2O_2$  was necessary for their immobilization. The cause of this might be dual; both an increase in the already existing inducible mechanism and an additional mechanism that is present prior to exposure. The exact mechanisms behind reduced sensitivity towards  $H_2O_2$  in salmon lice are not yet clarified, and research into this field is therefore required. The knowledge on mechanisms for reduced sensitivity is important in order to develop mechanism-specific tools for sensitivity testing and -surveillance, as well as avoiding pursuing existing mechanisms when developing new chemical treatment agents (Denholm et al., 2002).

Due to the revitalization of the parasites the results from the 30 min-exposure bioassays could not be evaluated in the same manner as the Sevatdal and Horsberg (2003) pyrethroid bioassays. Sevatdal and Horsberg allowed a waiting period of 24 h in fresh sea water following the exposure period. When this protocol was applied to the  $H_2O_2$ -bioassays, it resulted in illogical dose-response curves. The immediate evaluation of the bioassays however gave plausible dose-response curves in addition to low control group mortalities. This was therefore chosen for the  $H_2O_2$ -bioassay protocol. The chosen protocol was considered a good proxy for lice survival under farm treatment conditions due to similar exposure periods. The parasites that recovered after first being immobilized were not considered important with regards to field treatments, as they were not seen to re-infest fish in the study by Treasurer and Grant. This coincides with the Norwegian field treatment experience. However, a laboratory study showed that both adult males and pre-adult parasites have the ability to re-infest salmon independent of the parasites' previous exposure to  $H_2O_2$  (McAndrew et al., 1998).

The 24-h bioassay also differentiated between parasite strains with differences in sensitivity. The  $EC_{50}$ -value for the least sensitive strain was two to three times higher than for the most sensitive strain. The lower difference in  $EC_{50}$ -value, compared to the 30-min bioassays, might be caused by the mechanisms for reduced sensitivity and/or  $H_2O_2$ -degradation. Since exposure time does not exceed 30 min in normal  $H_2O_2$  field treatments, it may be concluded that the parasite has not undergone selection in order to develop mechanisms to withstand long term  $H_2O_2$ -exposure. Developing a 24-h bioassay is an attempt to standardize all salmon lice bioassays to the set up by Helgesen and Horsberg (2013), thereby making field bioassays easier to perform. To accomplish this task a degradation curve of  $H_2O_2$  under the circumstances given in the bioassay protocol should be employed and the correlation between bioassays and treatment results should be established.

The development of reduced sensitivity towards  $H_2O_2$  in *L. salmonis* was expected, as reduced sensitivity towards pyrethroids, azamethiphos and emamectin benzoate has previously been described by bioassays performed in Norway (Grøntvedt et al., 2014; Helgesen and Horsberg, 2013; Sevatdal et al., 2005; Sevatdal and Horsberg, 2003). As a result of the development of reduced sensitivity towards other available chemical treatments, the use of  $H_2O_2$  for anti-salmon lice treatments has increased since the product was re-introduced to the Norwegian market in 2009. In 2013, 8262 metric tonnes of  $H_2O_2$  was applied in treatment while the figure was 2538 metric tonnes the previous year (Norwegian Institute of Public Health, 2014). The outbreak of amoebic gill disease (AGD) may also have contributed to the increased use of  $H_2O_2$  in 2013. Treatments against AGD will nonetheless simultaneously combat infested salmon lice and therefore impose selection pressure on both parasitic species. Grøntvedt et al. (2014) showed that the most intense  $H_2O_2$  treatment regime against salmon lice was found in the northern part of Mid-Norway and in the southern part of





**Fig. 2.** 24-h bioassay. The dose-response curves (90% CI) and the observed immobilization rates for the two strains of salmon lice tested for sensitivity towards hydrogen peroxide in a 24-h bioassay. The observed rates for the sensitive strain (Ls A) are indicated by filled circles, while the corresponding values for the strain with reduced sensitivity (Ls V) are indicated by open circles. The dose-response curve for Ls A is modelled with results from two different bioassays.

North-Norway. The development of reduced sensitivity towards  $H_2O_2$  in these particular areas was therefore not unexpected as repeated treatments provide fast lane evolution towards reduced sensitivity. In the current study reduced  $H_2O_2$ -sensitivity was found in one strain of salmon lice in combination with reduced treatment efficacy. To decide if reduced sensitivity is a settled trait in the salmon lice population more sensitivity tests should be conducted on farms reporting of reduced treatment efficacy.

Reduced  $H_2O_2$ -sensitivity in *L. salmonis*, due to extensive use of the chemical as a delousing agent, was first reported from Scotland in 1999, seven years after  $H_2O_2$ -treatment was introduced to the Scottish market (Treasurer et al., 2000). Using experimental bin treatments, 15% effect of treatment was recorded for the gravid females and 25% effect for the other mobile stages. Fish, infested with salmon lice previously unexposed to  $H_2O_2$ , were treated and used as a control with an effect of 87% or more for all groups. The study by Treasurer et al. was performed on the F0-generation of salmon lice, while the current study also included the F1-generation, which had not previously been exposed to  $H_2O_2$ . This structure provided stronger evidence for the heritability of reduced  $H_2O_2$ -sensitivity.

The bioassay results presented in the current paper show differences in the sensitivity level towards  $H_2O_2$  between various strains of *L. salmonis*. These differences coincide with the differences in treatment efficacies. 30-min bioassays revealed up to tenfold variations in  $EC_{50}$ -values between the different strains of salmon lice. Reduced sensitivity towards  $H_2O_2$ , evident in both reduced treatment efficacy and increased bioassay  $EC_{50}$ -values, is

most likely an inherited trait. This is suggested by the fact that the F1-generation showed a relatively high  $EC_{50}$ -value, despite having not been exposed to  $H_2O_2$ -treatment. The current study presents the first case report of reduced  $H_2O_2$ -sensitivity in salmon lice in Norway. This reduced sensitivity imposes a threat to the Norwegian fish farming industry and should be monitored closely.

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#### References

- Amin, V.M., Olson, N.F., 1968. Influence of catalase activity on resistance of coagulase-positive staphylococci to hydrogen peroxide. *Appl. Microbiol.* 16, 267–270.
- Baud, O., Greene, A.E., Li, J.R., Wang, H., Volpe, J.J., Rosenberg, P.A., 2004. Glutathione peroxidase-catalase cooperativity is required for resistance to hydrogen peroxide by mature rat oligodendrocytes. *J. Neurosci.* 24, 1531–1540.
- Bravo, S., Nuñez, M., Silva, M.T., 2013. Efficacy of the treatments used for the control of *Caligus rogercresseyi* infecting Atlantic salmon, *Salmo salar* L., in a new fish-farming location in Region XI, Chile. *J. Fish Dis.* 36, 221–228.
- Bravo, S., Sevattal, S., Horsberg, T.E., 2008. Sensitivity assessment of *Caligus rogercresseyi* to emamectin benzoate in Chile. *Aquaculture* 282, 7–12.

- Brooks, K.M., 2009. Considerations in developing an integrated pest management programme for control of sea lice on farmed salmon in Pacific Canada. *J. Fish Dis.* 32, 59–73.
- Bruno, D.W., Raynard, R.S., 1994. Studies on the use of hydrogen peroxide as a method for the control of sea lice on Atlantic salmon. *Aquac. Int.* 2, 10–18.
- Burka, J.F., Hammell, K.L., Horsberg, T.E., Johnson, G.R., Rainnie, D.J., Speare, D.J., 1997. Drugs in salmonid aquaculture – a review. *J. Vet. Pharmacol. Ther.* 20, 333–349.
- Costello, M.J., 2006. Ecology of sea lice parasitic on farmed and wild fish. *Trends Parasitol.* 22, 475–483.
- Denholm, I., Devine, G.J., Horsberg, T.E., Sevatdal, S., Fallang, A., Nolan, D.V., Powell, R., 2002. Analysis and management of resistance to chemotherapeutants in salmon lice, *Lepeophtheirus salmonis* (Copepoda: Caligidae). *Pest Manage. Sci.* 58, 528–536.
- Elkins, J.G., Hassett, D.J., Stewart, P.S., Schweizer, H.P., McDermott, T.R., 1999. Protective role of catalase in *Pseudomonas aeruginosa* biofilm resistance to hydrogen peroxide. *Appl. Environ. Microbiol.* 65, 4594–4600.
- Fiander, H., Schneider, H., 2000. Dietary ortho phenols that induce glutathione S-transferase and increase the resistance of cells to hydrogen peroxide are potential cancer chemopreventives that act by two mechanisms: the alleviation of oxidative stress and the detoxification of mutagenic xenobiotics. *Cancer Lett.* 156, 117–124.
- Grave, K., Horsberg, T.E., Lunestad, B.T., Litlestak, I., 2004. Consumption of drugs for sea lice infestations in Norwegian fish farms: methods for assessment of treatment patterns and treatment rate. *Dis. Aquat. Organ.* 60, 123–131.
- Grøntvedt, R.N., Jansen, P., Horsberg, T.E., Helgesen, K.O., Tarpai, A., 2014. The Surveillance Programme for Resistance to Chemotherapeutants in Salmon Lice (*Lepeophtheirus salmonis*) in Norway 2013. Norwegian Veterinary Institute, Oslo.
- Helgesen, K.O., Bravo, S., Sevatdal, S., Mendoza, J., Horsberg, T.E., 2014. Deltamethrin resistance in the sea louse *Caligus rogercresseyi* (Boxhall and Bravo) in Chile: bioassay results and usage data for antiparasitic agents with references to Norwegian conditions. *J. Fish Dis.* 37, 877–890.
- Helgesen, K.O., Horsberg, T.E., 2013. Single-dose field bioassay for sensitivity testing in sea lice, *Lepeophtheirus salmonis*: development of a rapid diagnostic tool. *J. Fish Dis.* 36, 261–272.
- Hodneland, K., Nylund, A., Nilsen, F., Midttun, B., 1993. The effect of Nuvan, azame-thiphos and hydrogen peroxide on salmon lice (*Lepeophtheirus salmonis*). *Bull. Eur. Assoc. Fish Pathol.* 13, 203–206.
- Johnson, S.C., Constible, J.M., Richard, J., 1993. Laboratory investigations on the efficacy of hydrogen peroxide against the salmon louse *Lepeophtheirus salmonis* and its toxicological and histopathological effects on Atlantic salmon *Salmo salar* and Chinook salmon *Oncorhynchus tshawytscha*. *Dis. Aquat. Organ.* 17, 197–204.
- Johnson, S.C., Treasurer, J.W., Bravo, S., Nagasawa, K., Kabata, Z., 2004. A review of the impact of parasitic copepods on marine aquaculture. *Zool. Stud.* 43, 229–243.
- Jones, M.W., Sommerville, C., Wootten, R., 1992. Reduced sensitivity of the salmon louse, *Lepeophtheirus salmonis*, to the organophosphate dichlorvos. *J. Fish Dis.* 15, 197–202.
- Jones, P.G., Hammell, K.L., Gettinby, G., Revie, C.W., 2013. Detection of emamectin benzoate tolerance emergence in different life stages of sea lice, *Lepeophtheirus salmonis*, on farmed Atlantic salmon, *Salmo salar* L. *J. Fish Dis.* 36, 209–220.
- Lees, F., Baillie, M., Gettinby, G., Revie, C.W., 2008a. The efficacy of emamectin benzoate against infestations of *Lepeophtheirus salmonis* on farmed Atlantic salmon (*Salmo salar* L.) in Scotland, 2002–2006. *PLoS ONE* 3, e1549.
- Lees, F., Baillie, M., Gettinby, G., Revie, C.W., 2008b. Factors associated with changing efficacy of emamectin benzoate against infestations of *Lepeophtheirus salmonis* on Scottish salmon farms. *J. Fish Dis.* 31, 947–951.
- McAndrew, K.J., Sommerville, C., Wootten, R., Bron, J.E., 1998. The effects of hydrogen peroxide treatment on different life-cycle stages of the salmon louse, *Lepeophtheirus salmonis* (Krøyer, 1837). *J. Fish Dis.* 21, 221–228.
- Nakamura, K., Kanno, T., Mokudai, T., Iwasawa, A., Niwano, Y., Kohno, M., 2012. Microbial resistance in relation to catalase activity to oxidative stress induced by photolysis of hydrogen peroxide. *Microbiol. Immunol.* 56, 48–55.
- Nordberg, J., Arner, E.S.J., 2001. Reactive oxygen species, antioxidants, and the mammalian thioredoxin system. *Free Radic. Biol. Med.* 31, 1287–1312.
- Norwegian Institute of Public Health, 2014. Wholesaler-based Drug Statistics. <http://www.fhi.no/artikler/?id=109432> (accessed 20.10.14).
- Norwegian Medicines Agency, 2014. Summary of Product Characteristics for Paramove. <http://www.legemiddelverket.no/layouts/Preparatomtaler/SpC/11-8486.pdf> (accessed 20.10.14).
- Pike, A.W., Wadsworth, S.L., 2000. Sealice on salmonids: their biology and control. *Adv. Parasitol.* 44, 233–337.
- Robertson, J.L., Savin, N.E., Preisler, H.K., Russel, R.M., 2007. *Bioassays in Arthropods*, 2nd ed. CMC Press, New York.
- Roth, M., 2000. The availability and use of chemotherapeutic sea lice control products. *Contrib. Zool.* 69, 109–118.
- Roth, M., Richards, R.H., Dobson, D.P., Rae, G.H., 1996. Field trials on the efficacy of the organophosphorus compound azamethiphos for the control of sea lice (Copepoda: Caligidae) infestations of farmed Atlantic salmon (*Salmo salar*). *Aquaculture* 140, 217–239.
- Sevatdal, S., Copley, L., Wallace, C., Jackson, D., Horsberg, T.E., 2005. Monitoring of the sensitivity of sea lice (*Lepeophtheirus salmonis*) to pyrethroids in Norway, Ireland and Scotland using bioassays and probit modelling. *Aquaculture* 244, 19–27.
- Sevatdal, S., Horsberg, T.E., 2003. Determination of reduced sensitivity in sea lice (*Lepeophtheirus salmonis* Krøyer) against the pyrethroid deltamethrin using bioassays and probit modelling. *Aquaculture* 218, 21–31.
- Spitz, D.R., Adams, D.T., Sherman, C.M., Roberts, R.J., 1992. Mechanisms of cellular resistance to hydrogen peroxide, hyperoxia, and 4-hydroxy-2-nonenal toxicity – the significance of increased catalase activity in H<sub>2</sub>O<sub>2</sub>-resistant fibroblasts. *Arch. Biochem. Biophys.* 292, 221–227.
- Thomassen, J.M., 1993. In: Reinertsen, H., Dahle, L.A., Jørgensen, L., Tvinneim, K. (Eds.), *Fish Farming Technology*. Balkema, Rotterdam, pp. 233–236.
- Torrissen, O., Jones, S., Asche, F., Guttormsen, A., Skilbrei, O.T., Nilsen, F., Horsberg, T.E., Jackson, D., 2013. Salmon lice – impact on wild salmonids and salmon aquaculture. *J. Fish Dis.* 36, 171–194.
- Treasurer, J.W., Grant, A., 1997. The efficacy of hydrogen peroxide for the treatment of farmed Atlantic salmon, *Salmo salar* L. infested with sea lice (Copepoda: Caligidae). *Aquaculture* 148, 265–275.
- Treasurer, J.W., Wadsworth, S., Grant, A., 2000. Resistance of sea lice, *Lepeophtheirus salmonis* (Krøyer), to hydrogen peroxide on farmed Atlantic salmon, *Salmo salar* L. *Aquac. Res.* 31, 855–860.
- Uhlich, G.A., 2009. KatP contributes to OxyR-regulated hydrogen peroxide resistance in *Escherichia coli* serotype O157:H7. *Microbiology* 155, 3589–3598.
- Westcott, J.D., Hammell, K.L., Burka, J.F., 2004. Sea lice treatments, management practices and sea lice sampling methods on Atlantic salmon farms in the Bay of Fundy, New Brunswick, Canada. *Aquac. Res.* 35, 784–792.
- Westcott, J.D., Stryhn, H., Burka, J.F., Hammell, K.L., 2008. Optimization and field use of a bioassay to monitor sea lice *Lepeophtheirus salmonis* sensitivity to emamectin benzoate. *Dis. Aquat. Organ.* 79, 119–131.
- Whyte, S.K., Westcott, J.D., Elmoslemany, A., Hammell, K.L., Revie, C.W., 2013. A fixed-dose approach to conducting emamectin benzoate tolerance assessments on field-collected sea lice, *Lepeophtheirus salmonis*. *J. Fish Dis.* 36, 283–292.