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Neutralisation of hydrogen peroxide after delousing events; technology development and environmental risk assessment

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Summary

The present project has conducted experiments in laboratory and pilot scale with chemical neutralisation of hydrogen peroxide, which, in combination with technological treatment pose a possible solution for onboard well boat treatment of delousing water. The suggested treatment solution has been tested on northern shrimps (*Pandalus borealis*) to assess ecotoxicological effects. By reducing hydrogen peroxide with the proposed chemical/technological solution, the swimming behaviour among shrimps were barely affected and histopathological changes in gills were mild.

If implemented onboard well boats. the suggested solution will efficiently reduce the environmental risk related to hydrogen peroxide and provide the aquaculture industry with a pharmaceutical delousing tool with low environmental impact.

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Neutralisation of hydrogen peroxide after delousing events; technology development and environmental risk assessment

Preface

This project contained development of a new solution for removal of hydrogen peroxide after delousing usage to minimise the environmental impact from one of the pharmaceutical de-lice treatments available in Norway today. Ecotoxicological experiments, modelling of spreading and risk assessment have also been included to assess the potential impact this technique could have on the environment. The treatment solution is patent pending and hence, the present report do not contain all methodological details.

The solution development was conducted by NIVA and Akvaplan-niva. Ecotoxicological experiments were conducted by NORCE, while risk assessment and modelling were conducted by Akvaplan-niva. Results and information in the present report will hopefully be a useful tool for further risk assessments of hydrogen peroxide usage, as well as providing results and technologies which after an up-scaling of dimensions can be implemented as risk reducing measurements in the aquaculture industry.

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Tromsø, 4th of May 2021

Pernilla Carlsson,
Project manager

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Summary

Hydrogen peroxide (H_2O_2) is a non-specific strong oxidant used as a de-lousing treatment against salmon lice (*Lepeophtheirus salmonis*) in the aquaculture industry. Hydrogen peroxide (H_2O_2) has been promoted as an environmentally friendly alternative to other pharmaceuticals due to rapid degradation of this chemical to water (H_2O) and oxygen (O_2). However, as a non-specific strong oxidant this substance can have effects on non-target organisms. Organisms differ in their sensitivity to H2O2, and this chemical can have negative impact on some species at much lower concentrations than is recommended for de-lousing. Potential impacts to non-target organisms depend on several environmental factors such as temperature, currents, organic matter and other oceanographical conditions. Recent research (e.g. FHF project no. 901249) has shown a high risk of distribution of H_2O_2 kilometres of distance from the release point after a delousing event. This, in combination with the large dilution needed to reach threshold values for effects (predicted no effects concentration; PNEC) call for treatment solutions of released delousing water to ensure no or acceptable environmental impact.

The aim of this project was to develop a treatment solution where H_2O_2 is removed (neutralised) before release of the delousing water into the surrounding environment. We have conducted experiments in laboratory and pilot scale with chemical neutralisation of H_2O_2, which, in combination with technological treatment pose a possible solution for reducing the potential toxicity of delousing water. The technology needs further optimisation before full-scale implementation is possible with regards to energy demand, dosing and time efficiency. Nevertheless, the method is patent-pending (prior art, July 2020, patent application number 20200773. Inventors: Pernilla Carlsson, Øyvind Garmo, Muhammad Umar and Carlos Escudero, NIVA employees).

To ensure the development of an environmentally friendly neutralisation technique, ecotoxicological experiments were conducted with neutralised delousing water. The test organism was northern shrimps (*Pandalus borealis*). For all experiments, the start concentration of H_2O_2 used was 50 mg/L. This is equivalent to a 30-times dilution of the lowest recommended treatment dose (1500 mg/L). Different concentrations of the neutralisation chemical were applied. The swimming behaviour of the shrimps were barely affected at the lowest concentration applied. Higher doses of the neutralisation agent were also tested as a worst-case scenario, where the swimming activity were affected, although less during the post-exposure period compared to exposure to only H_2O_2. Histology investigations showed some effects in this experiment, although mild. Alterations of diffuse haemolytic infiltration and swelling of gills were observed in the experiment with neutralisation chemical combined with of H_2O_2. Haemolytic infiltration was also observed at similar levels in the control experiment.

The release and distribution of H_2O_2 in the environment after treatment was modelled using the FWCOM distribution model and evaluated. It showed a reduced risk of spread and occurrence of H_2O_2 in the environment with use of the proposed neutralisation technology. Hence, the method proposed has a high industrial potential, where the released delousing water will have less risk of reaching known shrimp fields or other vulnerable species and ecosystems. Implementation of this treatment method in full-scale will provide the aquaculture industry with a pharmaceutical delousing tool, where the negative environmental impact is greatly reduced compared with the current method.

Highlights

- Development of a novel technique for neutralisation of hydrogen peroxide after de-lousing.
- An evaluation of the environmental impact of the removal technique concluded with minor impact, and high benefits by implementation.
- Use of well-boats for de-lousing combined with hydrogen peroxide removal will be a very good risk reducing action.
- Reduced impact on the swimming behaviour of shrimps compared to untreated hydrogen peroxide and mild histopathological impact.

- Utvikling av ny teknikk for å nøytralisere hydrogenperoksid etter avlusing.
- Risikovurdering av miljøpåvirkningen av metoden viste at implementering av rensing av avlusningsvannet er et godt miljøtiltak.
- Avlusning om bord i brønnbåt i kombinasjon med nøytralisering av hydrogenperoksid før utslipp vil være et meget godt risikoreduserende tiltak.
- Metoden reduserte påvirkningen på rekers svømmeadferd sammenlignet med ubehandlet hydrogenperoksid. Den histopatologiske påvirkning var mild.

Sammendrag

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Hydrogenperoksid (H_2O_2) er en sterk oksidant som brukes som medikamentell avlusingsbehandling mot lakselus (*Lepeophtheirus salmonis*) i havbruksnæringen. Hydrogenperoksid (H_2O_2) har blitt markedsført som et miljøvennlig alternativ til andre legemidler på grunn av rask nedbrytning til vann (H_2O) og oksygen (O_2). Som en sterk oksidant kan dette stoffet ha effekter på andre organismer, og denne kjemikalien kan påvirke noen arter ved mye lavere konsentrasjoner enn anbefalt for avlusing. Potensielle påvirkninger avhenger av flere miljøfaktorer som temperatur, strøm, organisk materiale og andre oseanografiske forhold. Nyere forskning (f.eks. FHF-prosjekt nr. 901249) viset en høy risiko for spredning av H_2O_2 flere kilometer fra utslippspunktet etter en avlusing. Dette, i kombinasjon med den store fortynningen som trengs for å nå terskelverdier for effekter (forventet ingen effekt; PNEC), krever behandlingsløsninger av avlusingsvannet for å sikre at det blir ingen/akseptabel miljøpåvirkning.

Formålet med dette prosjektet var å utvikle en metode for å fjerne (nøytralisere) H_2O_2 etter at det er brukt til badebehandling av fisk. Vi har utført eksperimenter i laboratorie- og pilotskala med kjemisk nøytralisering av H_2O_2, som i en kombinasjon med teknisk nøytralisering utgjør en mulig løsning for behandling av avlusingsvann om bord i en brønnbåt. Teknologien trenger optimalisering før implementering i full skala er mulig med hensyn til energibehov, dosering og tidseffektivitet. Det er søkt patent på metoden (prior art, juli 2020, patentsøknadsnummer 20200773. Oppfinnere: Pernilla Carlsson, Øyvind Garmo, Muhammad Umar og Carlos Escudero, NIVA-ansatte).

For å sikre utvikling av en miljøvennlig nøytraliseringsteknikk ble det utført økotoksikologiske eksperimenter med nøytralisert behandlingsvann. Testene ble utført med reker (*Pandalus borealis*). For alle eksperimenter var startkonsentrasjonen av H_2O_2 50 mg/L. Dette tilsvarer en 30 ganger fortynning av den laveste anbefalte behandlingsdosen (1500 mg/L). Ulike konsentrasjoner av nøytraliseringskjemikalien ble brukt. Rekenes svømmeadferd var i prinsipp upåvirket ved den laveste konsentrasjonen. Høyere doser av nøytraliseringskjemikalien ble også testet som et worst case-scenario, hvor svømmeaktiviteten var påvirket, men i mindre grad sammenlignet med kun H_2O_2 i perioden etter eksponering. Histologiundersøkelser av rekene etter eksponeringene viste noen effekter, selv om de var milde. Endringer i diffus hemolytisk infiltrasjon og hevelse i gjeller ble observert i eksperimentet med nøytraliseringskjemikalie + H_2O_2. Hemolytisk infiltrasjon var også observert i kontrollen.

En modellering (FWCOM) av spredning sammen med en risikovurdering viser at den forventede reduksjonen av hydrogenperoksidkonsentrasjoner i et utslipp etter nøytraliseringsbehandling reduserer risikoen for spredning og forekomst av H_2O_2 i miljøet. Den foreslåtte metoden har godt potensiale for Norges akvakulturindustri, da avlusningsvannet med evt. restkonsentrasjon av H_2O_2 som slippes ut vil ha mindre risiko for å nå kjente rekefelt eller andre sårbare arter og økosystemer sammenlignet med ubehandlet H_2O_2. Implementering av denne nøytraliseringsmetoden i full skala vil gi havbruksnæringen et medikamentelt avlusingsverktøy med sterk reduksjon av negative miljøpåvirkning sammenlignet med dagens praksis.

1 Introduction

1.1 Hydrogen peroxide effects and usage

The Norwegian aquaculture industry was worth 72 billion NOK in 2019 (Statistics Norway, 2021). Seafood is expected to play an increasingly important role in the future and shifts of diets toward low carbon marine sources, such as sustainably harvested fish, are expected. The Norwegian government aims at facilitating further sustainable growth in the seafood industry, as it has great ambitions for increased value creation from the ocean (Nærings- og fiskeridepartementet, 2019). However, problems related to sea-lice (*Lepeophtheirus salmonis*) are a major challenge for the aquaculture industry. It is highly resource demanding to prevent/remove salmon lice from fish farms. There is a suit of biological, technical and chemical solutions in use, each with its benefits and drawbacks. Azamethiphos, hydrogen peroxide (H_2O_2), cypermethrin and deltamethrin are bath treatments added directly to the fish cage or distributed onboard well-boats. After bath treatment, the treated water containing residual chemical is released to the surrounding marine environment. Bath treatment can also be done in well boats, which has been shown to reduce, but not eliminate the risk for environmental impacts (Refseth et al., 2019).

A total of 136 000 tonnes of (100%) H_2O_2 was used during 2010-2019 (Norwegian Institute of Public Health, 2021). The use of H_2O_2 was 4523 tonnes in Norway in 2019 alone. H_2O_2 is considered to be one of the more environmentally friendly de-lousing methods. However, H_2O_2 is a non-specific strong oxidant and can therefore affect both target and non-target organisms. The sensitivity to H_2O_2 and its effects varies between organisms, and H_2O_2 can have negative impact on some economic and ecologic important species at much lower concentrations than what is used for de-lousing. Due to the very high oxidation potential (1.8 V) of H_2O_2, it is more reactive than other delousing agents used and is eventually decomposed to oxygen (O_2) and water (H_2O). However this process may take some time and undesirable effects of the chemical may occur in the environment even at a distance from the release point (Refseth et al., 2019).

There is increased focus on the environmental risk associated with the use of hydrogen peroxide including a recent report demonstrating the need for large dilution of the chemical to reach a predicted no effect concentration (Refseth et al., 2019). It has been shown that economically and ecologically important organisms are affected by concentrations significantly lower than those in the salmon cage and released to the surrounding water (Bechmann et al., 2017; Carlsson et al., 2021; Frantzen et al., 2020; Refseth et al., 2016).

Modelling studies have shown that H_2O_2 can spread in the local environment at concentrations that may be toxic to marine life. Dispersal modelling shows that relatively high concentrations of H_2O_2 can occur close to the farm and potentially affect the ecosystem. Diluted concentrations, which can affect some species, can be found away from the release site. The size of the influence area can vary due to currents, wind and stratification. Species diversity also plays a role and a recent study concluded that there is a risk for impacts on local ecosystems (Refseth et al., 2019, Nøst, in prep.).
H_2O_2 has a higher density compared to water, and in a weakly stratified water-mass this leads to a rapid sinking after release. The sinking will occur within a few minutes and has an important impact on the spreading of H_2O_2 in the environment (Refseth et al., 2019, 2016). The risk for sinking is higher during winter when stratification between water masses is less pronounced and the problem is exacerbated

since this is also the time of the year when deep-water shrimp (*Pandalus borealis*) carry eggs (Refseth et al., 2019).

Risk assessment studies have shown that H_2O_2 remain long enough in the environment to impact ecological important species such as the deep-sea shrimp (Refseth et al., 2019, 2016). Fishermen report poorer shrimp catches in fjords where fish farms are present, and new laboratory studies have shown that deep-water shrimps are sensitive to H_2O_2 and other delousing agents even at low concentrations and short exposure times (Bechmann et al., 2019; Escobar-Lux et al., 2019; Frantzen et al., 2020; Refseth et al., 2016). Another study has shown population level effects of H_2O_2 on deep water shrimps in some fjords (Moe et al., 2019). Many other studies have also reported negative effects of H_2O_2 on crustaceans, algae and fish (Brokke, 2015; Dummermuth et al., 2003; Urbina et al., 2019).

Viable measures that reduce the potential risk from delousing chemicals are desirable to ensure sustainable development of aquaculture and fisheries.

1.2 Aim of project

H_2O_2 presents a larger potential for neutralisation or on-site removal in well-boats compared to some other delousing agents. The overall goal of this project was to develop a method for neutralising H_2O_2 to minimise negative effects on the environment from discharge.

The project consists of three parts:
1). Proof of concept: initial laboratory experiments followed by up-scaling of any promising results and cost-benefit analysis of them.
2). Ecotoxicological experiments to investigate potential negative impact on deep-water shrimps from the suggested experimental solution.
3). Model the dispersion of (partially) neutralised hydrogen peroxide.

2 Methods

2.1 Chemical experimental design

A suit of chemicals were identified as potential «neutralisers» based on the following criteria:

- Reactivity: Potential to reduce H_2O_2 quickly.
- Potential environmental toxicity: Expected products of the reaction and impacts on physical conditions, i.e. temperature, pH and oxygen level in the sea water.
- Industrial potential: Availability, price, health and safety for people and equipment (e.g. gases).

In addition to these, potential catalyst compounds paired with neutralising agents were also tested and evaluated regarding their efficiency on H_2O_2 removal. The initial experiments were designed as «quick screening» to be able to test several chemicals alone and in combination with catalysts. This was carried out in both 45 L and 1 L experiments. The latter was mainly used as a quick screening experiment to test physical parameters such as pH modification.

The experiments were carried out at Akvaplan-niva's research station FISK (Forsøks- og Innovasjons Stasjon Kraknes) in Kvaløya, Tromsø during Summer 2019. Sea water was sourced from the inlet at a depth of 60 m in Sandnessundet, Tromsø. Water was analysed at NIVA with accredited methods for a suite of water parameters: nitrite + nitrate (NO_2 + NO_3), dissolved organic carbon (DOC), total organic carbon (TOC), pH, total nitrogen and total phosphorous. Further information about analyses can be found in Fagerli et al. (2019). Temperature (6°C), pH (7.7-8.0) and oxygen saturation (98-108%) were stable throughout the experimental period. The experimental area was kept as dark as practically feasible to avoid light induced degradation of H_2O_2.

The test protocol for 45 L experiments was as follows: 45 L sea water was filled into the experimental buckets, temperature, pH and oxygen were measured. Then, 135 g of Paramove®, which is one of the commercially available delousing agents with H_2O_2 was added, at a concentration of 1500 mg/L (the lowest recommended treatment dose). Concentrations were confirmed by analysis of H_2O_2 before the experiment began with a handheld SAM Single Analyte Photometer (ferric thiocyanate). Measured concentrations were within acceptable limits of the nominal (expected) concentration.

Paramove® contains 49.5% H_2O_2, which is stabilised by disodium dihydrogen phosphate, nitric acid and demineralised water. The water was stirred during the whole experiment to avoid sinking of H_2O_2 and to maintain good contact between H_2O_2 and the neutralising agent. The neutralising chemicals were added in a 1:1 molar ratio (2:1 where the stoichiometry indicated that 2 mol H_2O_2 would be neutralised by 1 mol neutralising agent) during stirring. Concentrations of H_2O_2, pH, temperature and oxygen were recorded at 1, 1.5, 2, 3, 5, 10 and 20 minutes. Additional measurements at 1-2 hours were also taken if needed. The time it took to add the neutralising agents was noted, and this step was kept as short as possible (<1 min, most often <30 s). When needed, the sample was diluted 1000-5000 times due to analytical restrictions for the SAM photometer. The collected sample was stirred until the analysis could be performed to avoid any sinking of H_2O_2 which could result in under-/over-estimation of the concentration.

Oxygen was not measured during the first trials due to sensor issues (too high concentration of H_2O_2). Those experiments were re-run later (sometimes as 1 L experiments) to record any effects of H_2O_2 on oxygen level in the water with the multi-sensor WTW Multi 3430 Handy Polaris 2 (also used for pH and temperature in those experiments).

One neutralising agent (sodium ascorbate, recommended by Dag Hongve, retired chemist) was also tested in a 400 L experiment after promising initial results in 45 L experiments. The experimental protocol followed the protocol for 45 L, but the amount of H_2O_2 and sodium ascorbate was scaled-up to provide the correct industrial concentrations. The experiment was replicated only twice due to limitations on accessible chemicals and equipment.

2.1.1 Neutralising agents and catalysts used and evaluated

The chemicals used (Table 1) were evaluated based on time, pH (both in the early 0-20 min and at longer time scale; 20-120 min), temperature, oxygen saturation in the water and price for the chemical. The evaluation scale had four categories: very good, good, sufficient (ok) or bad. The scale is somewhat subjective and there were not always firm borders between each category. The criteria for "OK" and "Very Good" temperature was defined for conditions showing no significant increase during the experiment. Oxygen and pH should stay within levels used as common practice for fish welfare during experiments (>80% saturation of oxygen and pH between 6-9). Any values outside this was categorised

as “bad”. Stable values compared to the starting points were set as “very good”. Time for neutralisation is set to good-very good when the major part of H_2O_2 was neutralised in the first 5-10 minutes.

Table 1. Chemicals used in neutralisation experiments.

Neutralising agent	Neutralising agent: H_2O_2 (mol)
A	1:1
B	1:2
(C) Sodium ascorbate ($C_6H_7NaO_6$)	1:1
(D) Sodium ascorbate ($C_6H_7NaO_6$)**	1:2
Catalysts	
K5	
(K1) Ferric chloride heptahydrate ($FeCl_3$ x 7 H_2O)	
(K2) Iron oxide, II, III (Fe_3O_4)	
E	
(K4) KOH (pH effect)	

**Sodium ascorbic acid was also tested to investigate the impact of the acidic form.

To test the effect of different catalysts, small (1L) experiments were also performed where KOH, EDTA-acid and the neutralisation chemical were dissolved in 10 mL seawater and thereafter added to a beaker with 1L H_2O_2 (3 g of Paramove®) (Table 2). These experiments were conducted to investigate the effect of pH and usage of a chelate (EDTA).

Table 2. Experiments with chelates and modification of pH

	1.	2.	3.	4.	5.
Amount EDTA-acid (g)	0.079	0.045	0.025	-	0.048
Amount KOH (g)	0.58	0.383	0.059	0.96	0.045

2.2 Technological experimental set-up

Since chemicals alone were not enough to neutralise H_2O_2 and maintain a good water quality, new techniques were investigated and initial experiments with technological treatment alone and in combination with the neutralisation chemical were conducted. Preliminary experiments using a chemical treatment followed by a technological one
where H_2O_2 is efficiently reduced without large reductions in water quality. The most promising combination(s) were applied in experiment #2, where a larger reactor was used.

The reactor has a volume of 115 L. Seawater from 60 m depth was used in the experiments. Two experimental set-ups were used; one with technology only and one where technology was combined with chemical treatment. Both experiments used Paramove® (49.5% H_2O_2) at the lowest treatment concentration; 1500 mg/L sea water. Different molar ratios were investigated at lab-scale and the most suitable ratio was selected for the pilot-scale experiments in m^3 scale. Concentrations of H_2O_2 were determined by Peroxide Vacu-vials® Kit, K5543. Temperature, salinity, pH and oxygen concentration was continuously measured during the experiments.

2.2.1 Stand-alone technological treatment

In technology-alone experiments, water containing H_2O_2 was subjected to treatment and samples collected after various intervals for determination of H_2O_2 concentration. Treatment was carried out in a recirculatory mode with first sample collected directly from the tank (600 L volume) after mixing of about 3-5 min. The reactor was then started and run until almost 100% neutralisation of H_2O_2.

2.2.2 Hydrogen peroxide neutralisation by combining technology and chemistry

In the next set of experiments**,** pre-dissolved chemicals in water were added to seawater containing H_2O_2 (1500 mg/L) and a control experiment was performed without technological treatment, i.e., water containing H_2O_2+neutralisation chemical. Water samples were collected over a 30 min period to determine the concentration of H_2O_2. In the second step, water containing H_2O_2+ neutralisation chemical (in a preferred molar ratio), was subjected to pilot-scale technology treatment and samples collected after different times for determination of H_2O_2 concentration.

2.3 Ecotoxicological experiments on shrimps (*Pandalus borealis*)

Two experiments were conducted and are thoroughly described below. The *Swimming behaviour experiments* consisted of two-hour pulses of diluted Paramove® with and without the addition of neutralisation chemical . Observations were made on swimming behaviour responses and post exposure survival. A total of 4-8 individual shrimp were used for each treatment. The *Survival and effects on gill-tissue* consisted of a two-hour pulse of treatments selected from preliminary exposures with cumulative mortality recorded over eight days and gill tissue samples taken for subsequent analysis. Adult male shrimp (n=12) were used for each treatment.

2.3.1 Animal collection and maintenance

Northern shrimp (*Pandalus borealis*) were collected by trawl from Hillefjord (North of Åmøy, Rogaland County, Norway (59° 04' 00'' N, 5° 45' 00' E) in January 2020 using a net with a cod end modified with the addition of a barrel to minimise damage to the shrimp. Trawling depth was 100 m. Shrimp were transferred from the trawl to aerated seawater holding tanks. On arrival at the laboratory shrimp were randomly distributed among eight independent 500 l tanks, each continually refreshed with flow through seawater pumped from the fjord adjacent to the laboratory from a depth of 75 m and passed through a sand filter prior to delivery to the tanks. Seawater temperature in the holding tanks was controlled at 7 ± 0.5°C and salinity was recorded at 34 ± 0.5. Shrimp were acclimated to laboratory conditions for 2 weeks and fed daily *ad libitum* on a diet of fish feed pellets (Spirit supreme, Skretting, Norway). Dead or moribund individuals found in the tanks were removed during daily inspections. Experimental exposures were approved by the Norwegian Animal Research Authority (FOTS).

2.3.1.1 Continuous recording of swimming and walking activity

Following the acclimation period, individual adult egg bearing shrimp (size range 10 -12.5 cm length) were selected at random from the holding tanks and placed into a smaller test tank. Each of four identical test tanks was fed a constant flow of filtered seawater via a header tank at a rate of 680 ml min $^{-1}$ to give a standing volume of 6.3 L (Figure 1). Seawater temperature was controlled at 7 ± 0.5°C (approximate water temperature at trawling depth). The test room housing the test tanks and recording equipment was held at a constant low light level with an average intensity above the tanks of two lux. Disturbance of the animals during the monitoring period was limited to short daily system checks. A fresh single pellet of commercial fish feed was added each day to those tanks where the

previous pellet had been consumed. Swimming and walking activity in shrimp were logged using an infrared light beam system that allowed simultaneous continuous recording of individual shrimp held under low-light conditions over several days. Each tank had four beams set across its width. Infrared light was provided by light emitting diodes (LED) (Farnell 121-2749) and was detected on the far side of the tank by a matched wavelength phototransistor (Farnell 161-2659). All electronic components were housed in 8 mm diameter plastic tubes that were inserted into matched drilled blocks to assist accurate alignment of the beams and phototransistors. A 5 v DC supply provided power to the LED and phototransistor system. An optical filter (Farnell 177-143) was attached to the end of the phototransistor tubes to minimise potential interference from ambient light. Figure 1 illustrates the positioning of the four beams set up across each tank. The beams were stacked, with the lower pair positioned to detect walking activity and the upper pair swimming in the shrimp. Movement of shrimps that disturbed the beam of light reduced the light intensity arriving at the phototransistor causing its output voltage to drop. Voltage output from the phototransistors was recorded using a National Instruments USB-6009 data logger (Austin, USA), connected to a PC. The data logger was set to record voltage every 0.2 seconds. Data files were saved to the computer hard drive every twelve hours throughout the recording period. The data sets were processed and analysed using a spreadsheet (Microsoft Excel). A reduction in voltage greater than 10% of the uninterrupted voltage for each beam was considered a breakage. Stationary 'resting' shrimp have the potential to register multiple events on adjacent single beams from repeated movements of appendages that would distort any subsequent plots of activity. Data sets were processed to avoid this problem by registering only those breakage events that were preceded by the breakage of a different beam. Thus, sequences of breaks on single beams were excluded from the analyses and lateral and vertical movements of shrimp within the tank were readily recognised. It was also considered reasonable to assume that when only the lower beams were broken in sequence that this likely represented walking activity whereas when the upper beams were broken within any sequence of breakages then swimming activity had taken place.

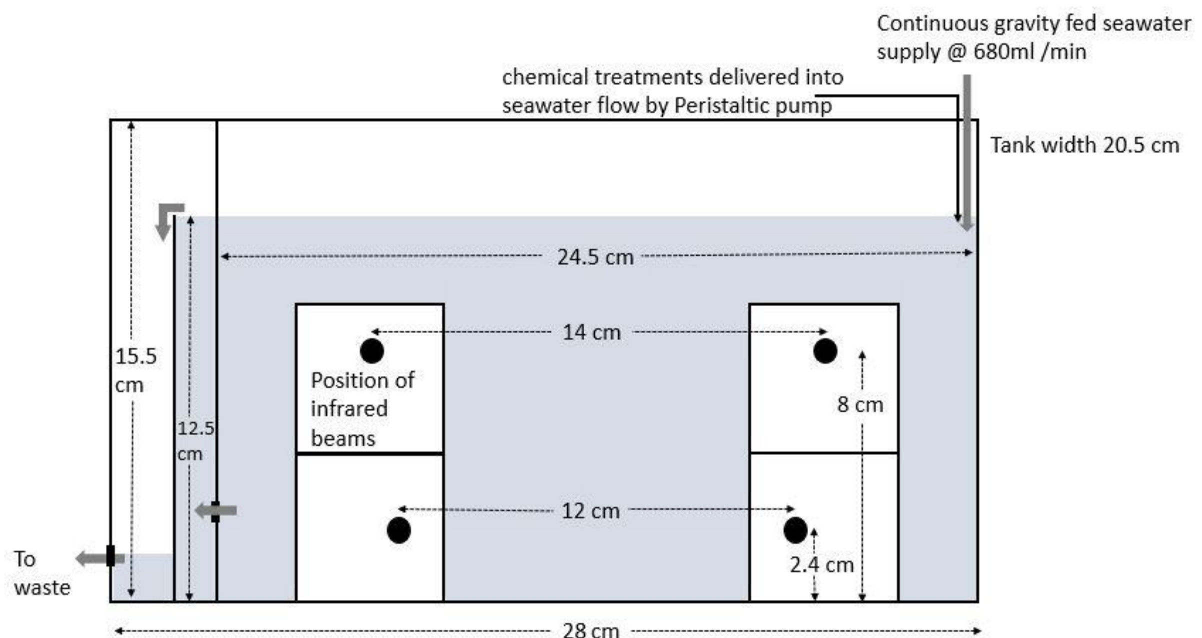

Figure 1. Test tank used to house individual shrimp, showing dimensions and positioning of the infrared light beams used to monitor and record activity.

2.3.2 Chemical treatments

Test shrimp were exposed to H_2O_2 (Paramove® formulation) at a 30-fold dilution of salmon treatment concentration (to give 50 mg/L H_2O_2 in the test tanks). A further series of treatments, that combined H_2O_2 at this concentration with the neutralising agent at the preferred molar ratio treatment between neutralisation chemical and H_2O_2, as well as two higher ratios with concentrations of 92.5 and 185 mg/L were carried out, with the highest concentration theoretically providing full neutralisation of the H_2O_2.
All test solutions were mixed in filtered seawater to give a total volume of 45 L within a 50-litre volume header tank 30 minutes prior to introduction to the test tanks with physical stirring provided to assist in the dissolution of the neutralisation chemical.

2.3.3 Sequence of exposure

Each exposure trial for individual chemical treatments lasted approximately 3.5 days, with shrimp first given several hours to acclimate to test tank conditions. Thereafter continuous recording of their activity commenced. The first 42 hours of recording established a baseline of activity, after which the chemical treatments were delivered for 2 hours at a rate of 23 ml/min via a peristaltic pump (Model 520S Watson and Marlow, Cornwall, UK) into the main seawater flow into the tanks (680 ml/min[1]), with a further period of 40 hours recorded following the end of treatment delivery to identify any post treatment changes in behaviour. Four exposure tanks, each containing a single shrimp were used for each exposure run. In order to increase the number of replicates for some chemical treatments, exposure trials were repeated.

2.3.4 Exposure of male adult shrimps to selected test treatments to generate histological samples

Adult male shrimp were exposed to the same three chemical concentration treatments as described in section 2.3.2 "Chemical treatments" and a control with the selection of concentrations based on the findings of the behavioural assay experience. The primary purpose of these exposures was to provide tissue samples for histopathological analysis and to examine survival rate. Four groups of 12 shrimp were each exposed for 2 h to each the treatments within the behavioural assay tank system (3 per tank) and then transferred to larger 44 litre volume plastic tanks for daily observations on survival (one tank per treatment). All tanks were fed with a continuous flow of filtered seawater at 7 C. After 8 days all surviving shrimp were sacrificed with gill tissue sampled and subsequently scored for a range of histopathological conditions. This was a smaller exposure than originally planned but delays in obtaining a key chemical meant that there were insufficient egg carrying females remaining to carry out the original experimental plan.

2.4 Gill histopathology

At the end of the exposure experiment described in 2.3.2 "Chemical treatments", gills from control shrimp and shrimp exposed to Paramove® solution alone (H_2O_2), H_2O_2 + neutralisation chemical and neutralisation chemical alone as described in the section 2.3 "Ecotoxicological experiments on shrimps (*Pandalus borealis*)" were sampled for gill histopathology (n = 10 samples per treatment). Gill samples were dissected out, fixed in Davidson's fixative for 48 h and transferred to formalin free Fine Fix® solution. Later, the samples were processed by serial alcohol dehydration, embedded in Thechovit

7100 a plastic embedding system based on HEMA (2-hydroxyethyl methacrylate) and cut into 8 µm transactions by a Leica RM 2165 rotary microtome before being stained by toluidine blue staining observation by the light microscope according to Landers et al., (2020). The degree of histological damage was scored in four fields on each of the 10 slides from each treatment. Scores were based on the number of fields in which histological changes were observed with (class 0) no histopathology in any field, (class 1) = mild histopathology present in < 25% of the fields, (class 2) = moderate histopathology present in 25%–75% of the fields, and (class 3) = severe histopathology present in > 75% of the field, following the scale suggested by Zodrow et al. (2004) and Beckmann et al. (2019). Parasites were scored as 0 = absent or 1 = present.

2.5 Modelling

The Finite Volume Community Ocean Model, FVCOM (Chen et al., 2003), was used to model dispersion concentrations of H_2O_2 in the environment. Concentration modelling was performed with and without treatment (90% reduction in H_2O_2 concentration) after a delousing of 4-cages and delousing by wellboat. Due to its unstructured grid, FVCOM is particularly suited to model oceanic flows in regions with fractured coastlines and archipelagos. FVCOM is used all over the world for aquaculture related challenges (Adams TP et al., 2016; Aleynik et al., 2016; Foremen et al. 2015). Predicted environmental concentrations (PEC) were calculated, and aspects such as sinking, number of hours with concentrations in the environment above threshold values for effects (PNEC) were estimated in Nøst et al., (in prep) and Refseth et al. (2019). For details on modelling methods, spreading and sinking of H_2O_2, see (Refseth et al., 2019). The study area is from an aquaculture location (Jakobsteinvika) located on the east side of the island Leka in Lekafjorden (Norway). This is a deep fjord with depth of more than 200 m, and it is exposed to the open ocean towards the west. The release point is located on the steep western slope of the fjord. For more information on location and how modelling was performed in this area see (Refseth et al., 2019).

2.6 Risk evaluation

To assess the risk of an area reaching harmful concentrations after H_2O_2 delousing and applied neutralisation, illustration maps were developed to show distribution of H_2O_2 concentration from a 4-cage release and from well boat de-lousing four cages from an area in mid-Norway (Jacobsteinsvika), before and after neutralisation of H_2O_2. The modelled concentrations were compared to threshold levels for effects, both for a whole community and also for relevant Norwegian species separately. For details around risk evaluation process, see (Refseth et al., 2019). The principles are briefly described below:

Species generally show different sensitivities to chemicals and a species sensitivity distribution curve (SSD) is a commonly used tool for environmental risk assessment (ERA). The variation in sensitivity between species can be described by statistical distribution. A threshold value for effects for a whole biological community, i.e. a predicted no effect concentration (PNEC) is derived. The PNEC-values used in risk assessment procedures are normally derived from SSD-curves with a safety factor, or from ecotoxicological information from single species (i.e. no effect concentration; NEC, or LC_{50} values with a safety factor) or lowest reliable endpoint value is used with an assessment factor (AF) (Kooijman, 1987). In Refseth et al. (2019), ecotoxicological experiments were conducted and combined with data available in the literature to establish a SSD curve and PNEC value. This value in addition to threshold values for single species were compared to predicted concentrations in the environment (PEC). Areas

were threshold levels of effects are exceeded (either PNEC or singles important commercial and ecological important species) has a risk for negative environmental effects, and risk reducing measures should be implemented.

Herein, we compare maps with distribution of concentrations in the environment by showing distribution of H_2O_2 with and without neutralisation process (assuming 90% reduction of H_2O_2 concentration), and the concentrations are compared to threshold values. The threshold value for effects on biological communities is 0.14 mg/L (Refseth et al., 2019). The SSD curve from which the PNEC levels were derived from is shown in Figure 2.

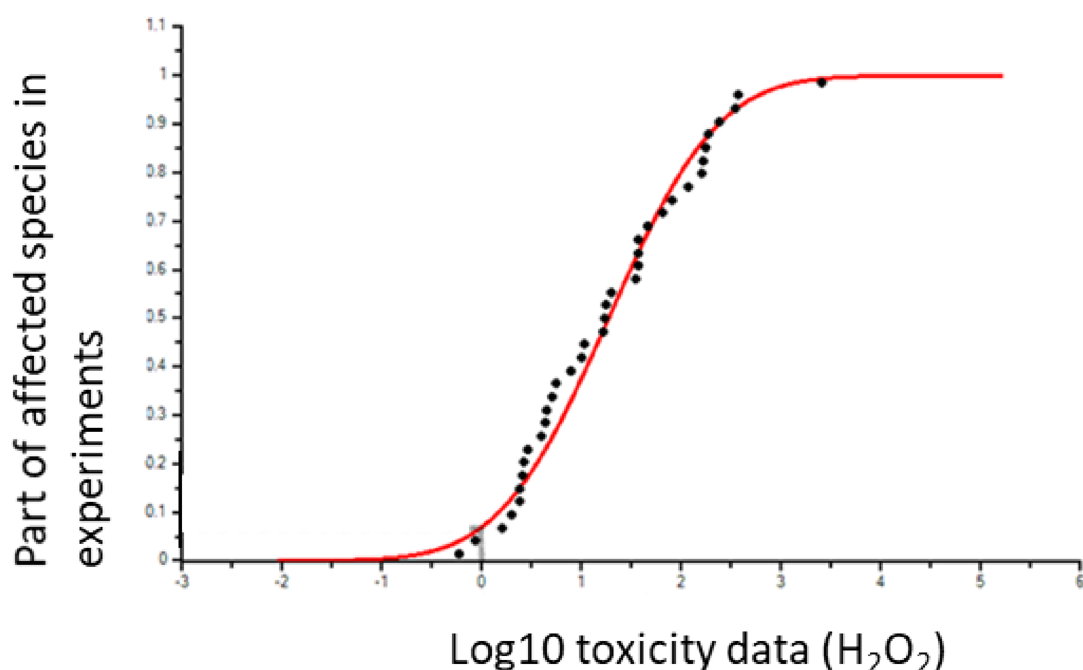

Figure 2. Species sensitivity distribution (SSD) of H_2O_2 based on acute toxicity data derived from 34 species representing seven different phyla. There is no apparent difference in sensitivity between fresh-water and marine species. Algal species from the phyla cyanobacteria and bacillariophyta represent the most sensitive species while marine vertebrates represent the least sensitive trophic level. Data from Akvaplan-niva, Institute for Marine Research (IMR) and from the literature (Figure created by Nouryon and first published in Refseth et al. (2019)).

3 Results and discussion

The following water quality parameters were measured at the intake water: nitrite + nitrate (NO_2 + NO_3): 107 µg N/L. DOC: 1.0 mg C/L. TOC: 0.9 mg C/L. pH: 7.89 ($\pm$0.2), total nitrogen: 220 µg/L, total phosphor: 26 µg P/L (method uncertainty were 20% for these analyses). Temperature (6°C), pH (7.7-8.0) and oxygen saturation (98-108%) were measured before the experiments began and were within these values throughout the experimental period. An evaluation of all chemicals investigated are presented in Table 3.

3.1 Chemical experiments

Table 3. Evaluation of effectiveness of tested compounds, including catalysts. The evaluation criteria for good/very good are pH: 6-9, Oxygen: >8 mg/L, stable temperature (less than 1°C fluctuation during the experiments). Time for neutralisation should be short, i.e. notable difference within the first few minutes. Price is a relative evaluation among the compounds used.

	Time neutralisation	**pH 0-20 min**	**pH 20-120 min**	**Temperature**	**Oxygen**	**Price**
A	Very good	Very good	Very good	Ok	Bad	Very good
B	Very good	Bad	Bad	Very good	Bad	Very good
C	Bad	Bad	Bad	Very good		Bad
D	Good	Good	ok	Very good	Good	Bad
D-400L 1h	ok	Good	Good	Very good	Good	Bad
D400L 24h	Bad	Good	Bad	Very good	Bad	Bad
E	Good	Bad/ok		Ok	Ok	Good
Catalysts and combinations						
K1		Bad	Bad			
K2	Bad	Very good	Very good	Very good	Very good	Good
A + K1	Very good	Good	Ok	Ok	-	
B + K2	Very good	Bad		Very good	Bad	
D + K3	Very good	Good		Very good	Good	Bad
K4	Good	Good		Bad	Good	Good
B + K4	Good	Good		Good	Bad	Good
A + K5	Good	Good		Ok	Bad	Good

3.1.1 Sodium ascorbate

Since sodium ascorbate ($C_6H_7NaO_6$) provided interesting results in the 45 L experiments, it was chosen for a 400 L experiment as well (appendix, Table A1) in a 1:2 molar ratio to H_2O_2. However, ascorbate was the most expensive of the neutralising agents tested. This, in combination with a slight lowering of the pH and less removal effect of H_2O_2 compared to the other agents led to discard of sodium ascorbate after the 400 L experiments. The experiment was evaluated on short-term basis (1h) where the neutralisation process of H_2O_2 went slightly slower than in the smaller experiments. Water qualities were also measured after 24h, where e.g. pH had dropped below 6, which was set as one requirement to maintain good water quality and H_2O_2 was still present.

3.1.2 Chemical B

Even though H_2O_2 were efficiently removed with (B), it also reduced the pH and oxygen saturation within minutes (Figure 3).

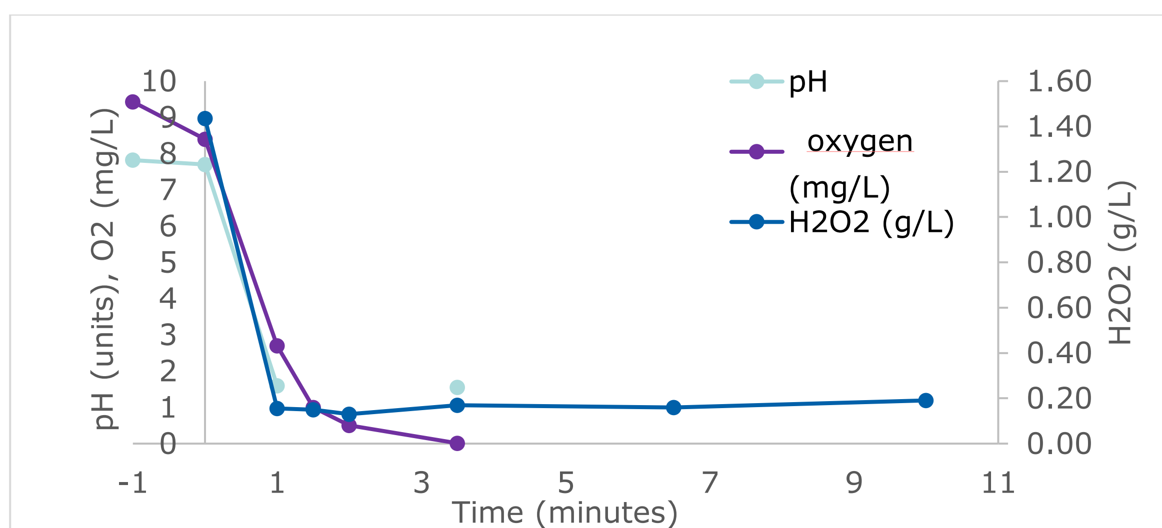

Figure 3. Experiments with chemical B and H_2O_2. t=-1 are measurements before adding of H_2O_2 to the experiment (t=0). Both oxygen and pH dropped below critical values (8 mg/L) and pH=6 within the first minutes of the experiment.

3.1.2 Catalysts

Experiments with catalysts (alone and in combination with neutralisation agents were performed (Table 4). The main focus was on optimalisation of the neutralisation chemical since it showed the most promising results in experiments with neutralisation agents only. In general, little help from the catalysts were observed. The effect of the neutralisation chemical (both benefits and backdraws) were larger than the effect of the catalyst.

Experiments where pH is increased (e.g. by potassium hydroxide; KOH) showed good stability on all parameters except pH (pH >9 is considered undesirable) and temperature may also be a problem since dissolving of KOH is an exotherm reaction.

Table 4. Experiments (1L) on the efficiency of catalysts. Green oxygen and pH boxes indicate measurements with >8 mg/L O_2 and pH within 6-9. It was used neutralisation chemical and 3 g of H_2O_2 in each experiment, as described in Table 2.

	1.	**2.**	**3.**	**4.**	**5.**
Combinations of neutralisation chemical, K5 and KOH				**KOH and H_2O_2**	
Amount K5 (g)	0.079	0.045	0.025	-	0.048
Amount KOH (g)	0.58	0.383	0.059	0.96	0.045
O_2 (mg/L)	11.6	12.25	8.6		7.04
pH	12.98	12.94	10.81		10.73
Temperature (ºC)	20	20	20		20
Measurements 5 min after reaction with H_2O_2					
O_2 (mg/L)	11.33	10.78	2	11.58	0.01
pH	10.4	10.1	8.52	10.4	8.28
Temperature (ºC)	16.1	16.8	17.3	14.6	16.4
H_2O_2, g/L (3 min)	0.4	0.01	0.84		0.27
H_2O_2, g/L (5 min)				1.6	

3.1.3 Neutralisation chemical A

The initial 45 L experiments with showed an efficient reduction of H_2O_2 (on average 31% reduction of initial H_2O_2 concentration after 3 minutes). However, oxygen concentrations were also reduced (Figure 4) to anoxic conditions and it was therefore evaluated as to large side effects to be a suitable treatment. Hence, it was decided to include a technological treatment in the project, even though it was not planned in the initial project description.

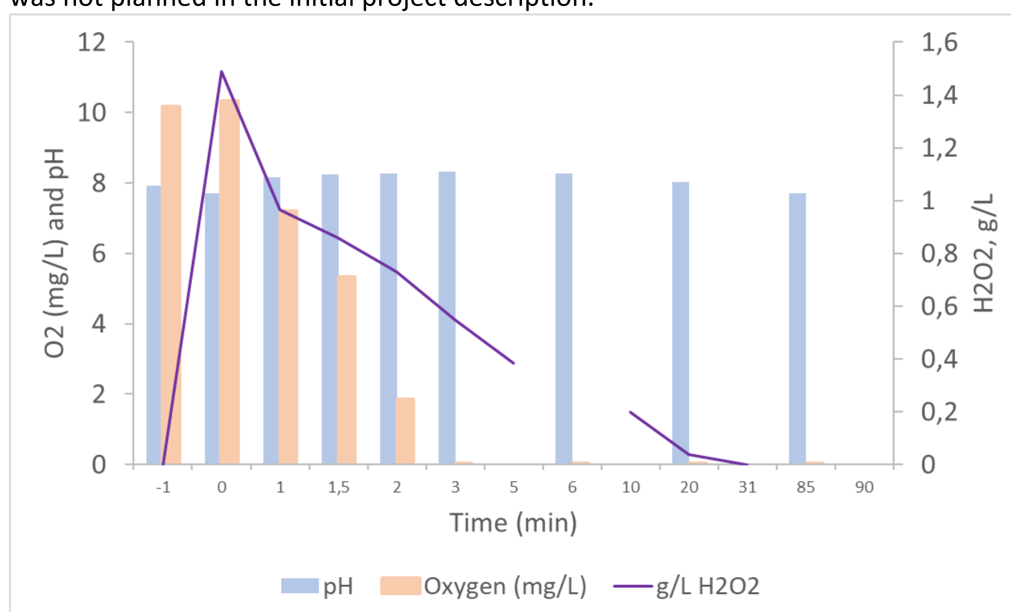

Figure 4. Removal of H_2O_2 by neutralisation chemical. pH remains constant and removal of H_2O_2 is efficient. However, the oxygen is reduced.

3.2 Chemical and technological treatment

The initial experiments were used to establish an optimised ratio between the neutralisation chemical and H_2O_2 (Figure 5). A patent application was sent on the combination as a neutralisation process (prior art; July 2020, application number 20200773. Inventors: Pernilla Carlsson, Øyvind Garmo, Muhammad Umar and Carlos Escudero, NIVA employees).

Figure 5. Experimental set-up for initial experiments at Akvaplan-niva's test facilities at Kraknes, Tromsø.

Neutralisation of H_2O_2 was investigated also by using technology as a stand-alone treatment. The experiment was carried out until almost 100% neutralisation of H_2O_2 that occurred over a period of 7 h to investigate the time-based removal (Figure 6). The reduction of H_2O_2 was 21% in the first 60 min and increased to about 51% after 3.5 h. To achieve greater reduction in the concentration of H_2O_2 and to test the efficiency of the system, the operation was prolonged and almost complete (99.7%) removal of H_2O_2 was observed after 7 h. It must, however, be noted that also a non-complete removal of H_2O_2 would still decrease the concentrations of H_2O_2 released into the environment and help to reduce the environmental risk (as described in section 3.4-3.5; modelling and risk assessment) by lower mass H_2O_2 released and hence, the size of the area with probability for concentrations above the PNEC level for the environment (0.14 mg/L; Refseth et al. (2019)) will be reduced. This in combination with lower reduction (10%) in the last 100 min indicates the time of treatment could be reduced if, for example, a target of 90% H_2O_2 reduction is set. Furthermore, the time could be reduced using lower flow rates or longer residence time in the treatment container.

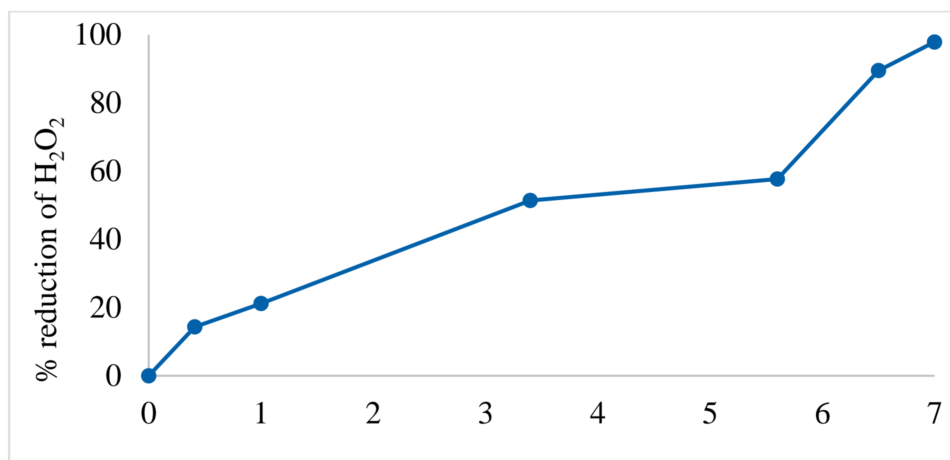

Figure 6. H_2O_2 neutralisation after technological treatment alone.

Figure 7 shows the reduction of H_2O_2 after the addition of the neutralisation chemical at optimised molar ratios during the first 30 min. As indicated, the reduction (14%) occurred in the first minutes after addition. Since high concentrations lead to deoxygenation of water, the oxygen concentration was continuously measured and was not impacted at the concentration used in these experiments, which is very important from the viewpoint of practical use and industrial implementation. The other parameters measured were temperature, pH and salinity which all remained unchanged during the treatment period.

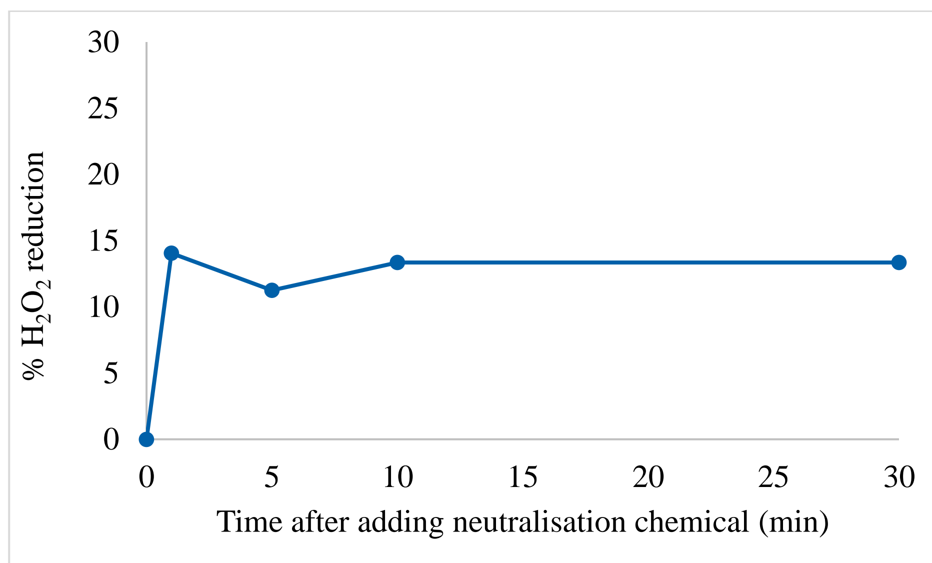

Figure 7. H_2O_2 removal after addition of neutralisation chemical.

Neutralisation of H_2O_2 by technological treatment with and without neutralisation chemical showed a fairly similar rate of reduction under comparable technological treatment fluence but a marginal difference was observed at lower technological treatment effect, i.e., 44% and 51%, after combined technological treatment + neutralisation chemical and stand-alone technological treatment, respectively. Since the neutralisation chemical removed about 14% of H_2O_2 and some difference in the technological treatment was noted (5%), both the difference in the initial concentration of H_2O_2 and technological treatment could be causing the difference in the neutralisation of H_2O_2 at lower

technological treatment effect. However, as the treatment progressed, the difference in the neutralisation decreased with comparable rate of H_2O_2 removal. It is possible to increase the concentration of neutralisation chemical to reduce the initial concentration of H_2O_2, which would facilitate the technological treatment and achieve a faster H_2O_2 removal. Since, the concentration of oxygen in water when using the optimised neutralisation chemical mole ratio (in comparison to mole H_2O_2) was high (>100% saturation), it is possible to increase the concentration of neutralisation chemical as long as it does not cause oxygen depletion. This issue may also be solved by aeration/oxygenation of the treated water before release into the sea.

3.3 Shrimp experiments

Figure 8-12 represent the patterns of activity presented by the exposed shrimps. In each case the vertical bars represent activity measured in beam breaks per hour throughout the monitoring period. The 2 h exposure period for delivery of each treatment is indicated below the x-axis. The darker sections of the columns represent swimming activity and the lighter sections at the base of the columns represent walking. The solid continuous line indicates the number of shrimps recorded as active within each hour. Three treatments were applied with 30x diluted H_2O_2 treatment solution (50 mg/L H_2O_2; Figure 8) alone and in combination with high, medium and low neutralisation chemical concentrations (Figure 9-11). An exposure of neutralisation chemical alone as well as to a new batch of H_2O_2 were also performed (Figure 12-13).

In the experiment with H_2O_2 only (50 mg/L, Figure 8), there is a clear increase in activity observed within the first hour of delivery of the H_2O_2 which diminishes over time. This suggests the shrimp detected the H_2O_2 and this triggered an intense period of activity that could represent avoidance behaviour.

The treatment with H_2O_2 in combination with high neutralisation chemical concentration should neutralise the activity of the H_2O_2 and when water samples from the tank were measured, it showed an efficient reduction in H_2O_2 concentrations. This combination reduced the H_2O_2 measured in the tank from 50 to approximately 2 mg/L. However, there was an increase in activity as the treatment was delivered. There were also additional peaks of activity during the post exposure period. The neutralisation chemical reduces oxygen concentration when added to water and measurements taken from the mixer tank during the exposure clearly indicated that this was the case. In addition, there was a substantial quantity of undissolved neutralisation chemical within the mixture tank throughout the exposure and particles of this compound could have been carried through the pump to the test tanks. It is possible that the decrease in oxygen in the test tanks, (albeit much lower than in the mixer tank due to dilution by the filtered seawater continuous flow input) could be responsible for triggering this increased activity.

An increase in activity was once again observed in the first hour of the exposure to H_2O_2 in combination with medium concentration of the neutralisation chemical (Figure 10), though this increase was less than that observed in the experiment with H_2O_2 alone and in combination with high concentration of the neutralisation chemical. The activity levels returned to close to pre-exposure levels shortly after the delivery. The neutralisation chemical appears to reduce the concentration of H_2O_2 in the test tanks by approximately 50% but does not reduce dissolved oxygen concentration to such an extent to trigger a further response in the shrimp.

The lowest concentration of neutralisation chemical tested (Figure 11) in combination with H_2O_2 resulted in an increase in activity during the first hour of exposure, followed by a return to pre-exposure activity levels thereafter. The increase in activity was approximately the same as recorded for H_2O_2 alone, although activity in the post exposure period is more similar to what was observed in pre-exposure for these shrimps than found in the H_2O_2 exposure. This combination reduced the H_2O_2 measured in the tank from 50 to approximately 37.5 mg/L.

There was little detectable change in activity when shrimp were exposed to neutralisation chemical alone at the lowest concentration. Unlike the two higher neutralisation chemical concentrations tested, this treatment was fully dissolved in the mixing tank before delivery into the test tanks. When this volume of neutralisation chemical was used in combination with the H_2O_2 treatment it reduced the concentration of the H_2O_2 in the test tank by approximately 25%.

The response seen with the new batch was very similar to the original batch of H_2O_2, with an initial increase in activity followed by a return to pre-exposure level soon after. Concentration measured in the test tanks was close to the 50 mg/L target concentration in both this batch and the original.
None of the adult female egg carrying shrimp tested in any of the exposures described above died within 14 days from the end their exposures. The neutralisation effect of neutralisation chemical on H_2O_2 was as predicted with an expected proportional reduction of H_2O_2 measured in the test tanks (Figure 14).

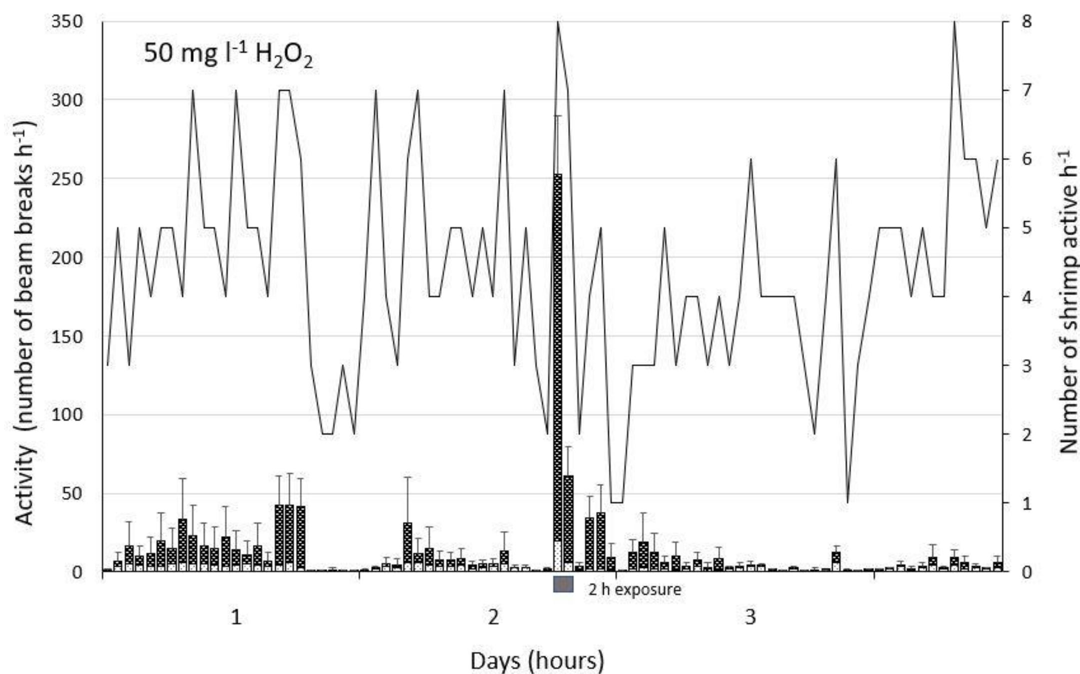

Figure 8. Shrimp activity during three days in response to exposure to H_2O_2 (original solution) at 50 mg/L + standard error of means (SEM). The 2 h exposure period for delivery of each treatment is indicated below the x-axis. The vertical bars show activity as beam breaks per hour and the dark sections of them show swimming activity while the lighter sections at the base of the columns represent walking behaviour. The solid continuous line indicates the number of shrimps recorded as active within each hour.

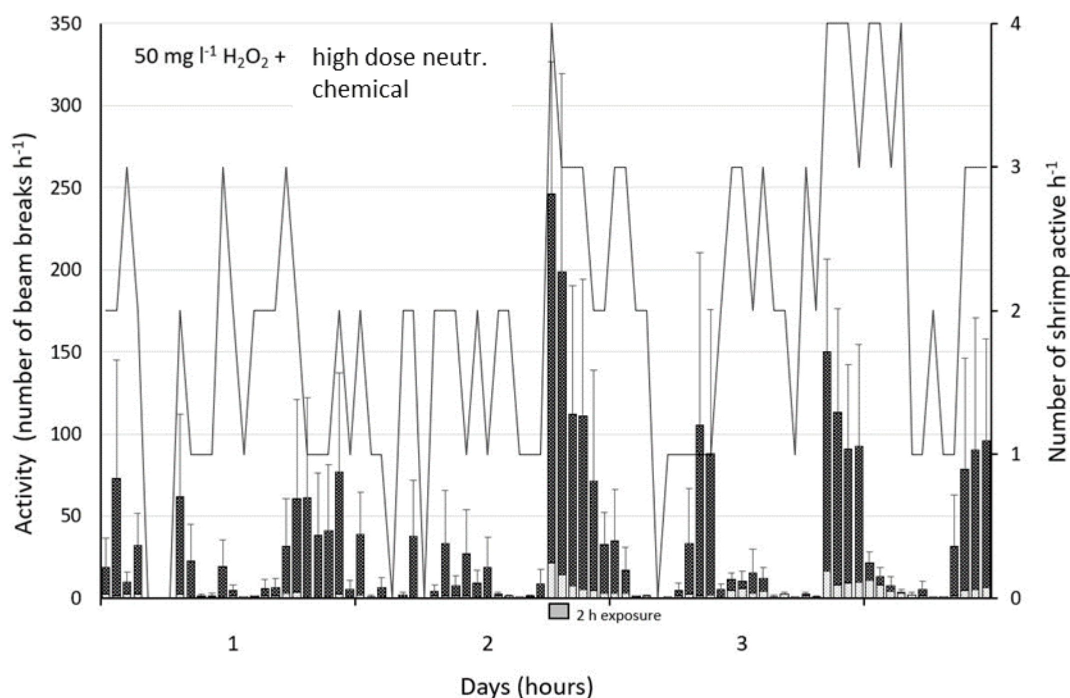

Figure 9. Shrimp activity during three days in response to exposure to H_2O_2 at 50 mg/L combined with high concentration neutralisation chemical (+SEM). The 2 h exposure period for delivery of each treatment is indicated below the x-axis. The vertical bars show activity as beam breaks per hour and the dark sections of them show swimming activity while the lighter sections at the base of the columns represent walking behaviour. The solid continuous line indicates the number of shrimps recorded as active within each hour.

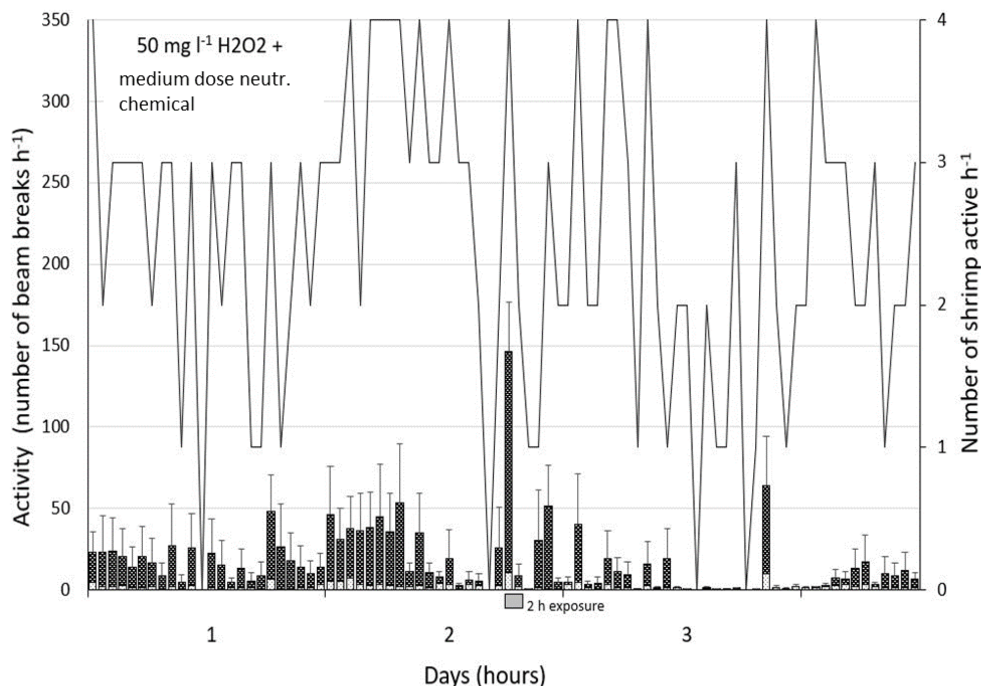

Figure 10. Shrimp activity during three days in response to exposure to H_2O_2 at 50 mg/L combined with medium concentration of neutralisation chemical (+SEM). The 2 h exposure period for delivery of each treatment is indicated below the x-axis. The vertical bars show activity as beam breaks per hour and the dark sections of them show swimming activity while the lighter sections at the base of the columns represent walking behaviour. The solid continuous line indicates the number of shrimps recorded as active within each hour.

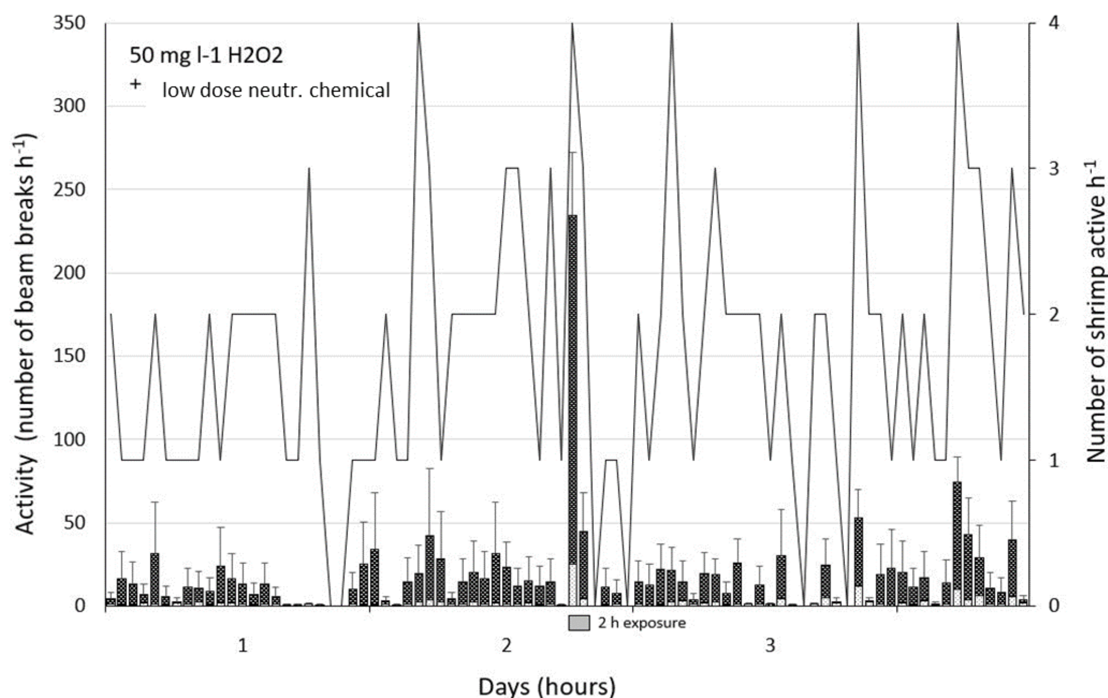

Figure 11. Shrimp activity during three days in response to exposure to H_2O_2 at 50 mg/L combined with low concentration of neutralisation chemical (+SEM). The 2 h exposure period for delivery of each treatment is indicated below the x-axis. The vertical bars show activity as beam breaks per hour and the dark sections of them show swimming activity while the lighter sections at the base of the columns represent walking behaviour. The solid continuous line indicates the number of shrimps recorded as active within each hour.

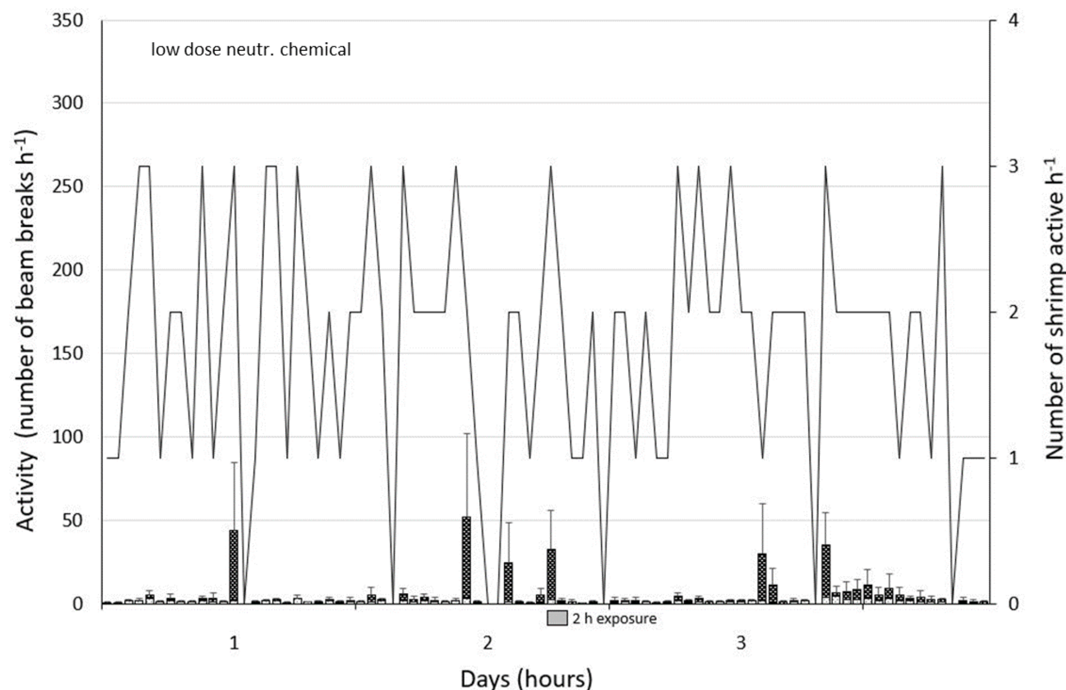

Figure 12. Shrimp activity during three days in response to exposure to low concentration of the neutralisation chemical (+SEM). The 2 h exposure period for delivery of each treatment is indicated below the x-axis. The vertical bars show activity as beam breaks per hour and the dark sections of them show swimming activity while the lighter sections at the base of the columns represent walking behaviour. The solid continuous line indicates the number of shrimps recorded as active within each hour.

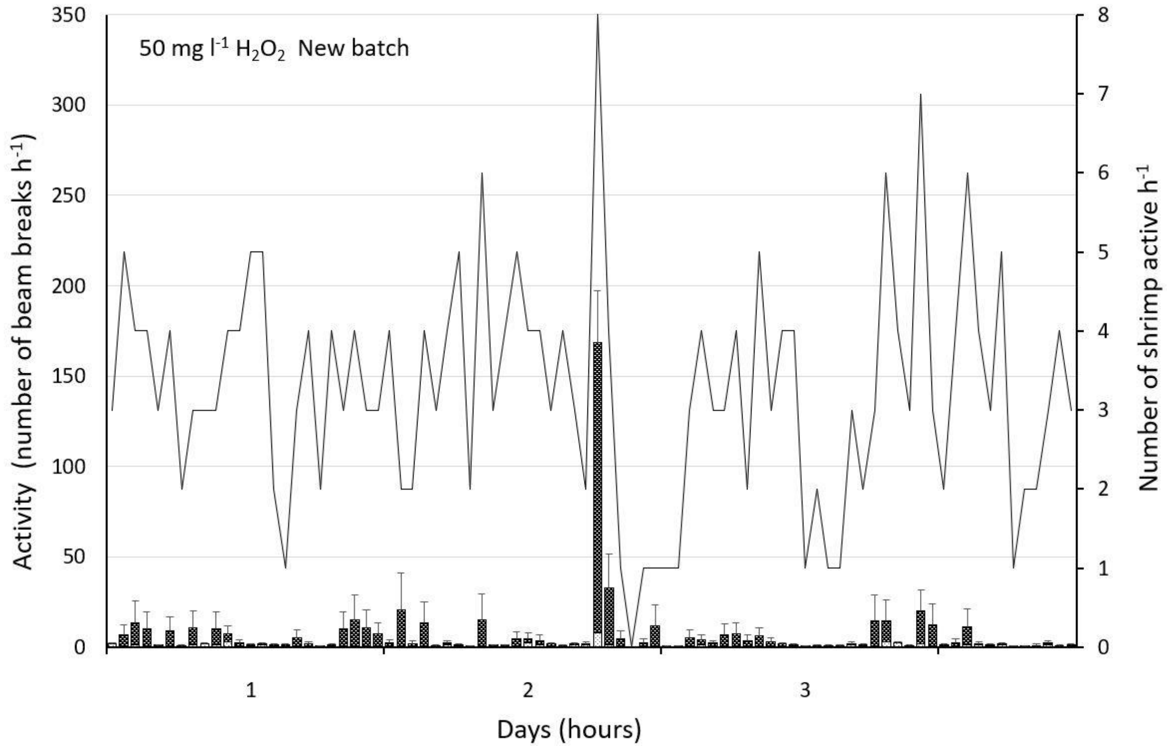

Figure 13. Responses of shrimp during three days to the new batch of hydrogen peroxide at 50 mg/L. The 2 h exposure period for delivery of each treatment is indicated below the x-axis. The vertical bars show activity as beam breaks per hour and the dark sections of them show swimming activity while the lighter sections at the base of the columns represent walking behaviour. The solid continuous line indicates the number of shrimps recorded as active within each hour.

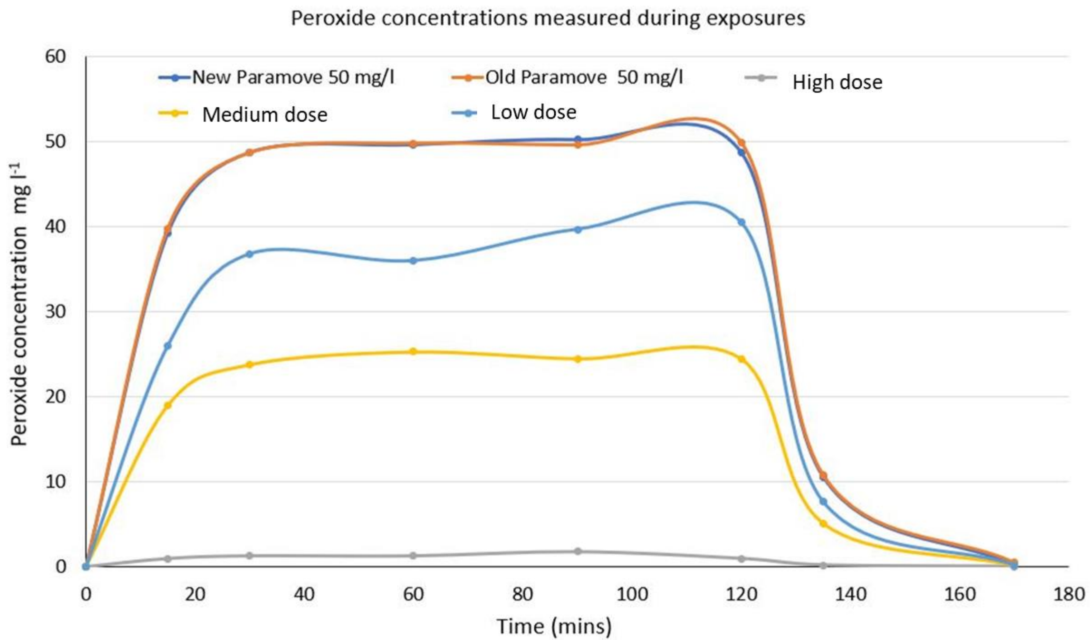

Figure 14. Plot of hydrogen peroxide concentrations measured in samples taken from test tanks during the exposure sequence, with low, medium and high dose of the neutralisation chemical.

3.3.1 Survival of adult male shrimps

All control shrimp and those exposed to low concentration of the neutralisation chemical were alive eight days after their 2 h exposure (Table 5). There were however mortalities in the two other exposures that included H_2O_2. One shrimp from each of these groups died during the actual exposure before being added to the observation tanks. The shrimp used for this procedure were adult males and the mortality recorded here was in contrast to that found in adult females, where no mortalities were recorded during the experiments or during several weeks post exposure.

Table 5. Survival of male adult shrimp exposed to H2O2 and neutralisation chemical and a combination of both.

Second exposure trial (n= 12 for each treatment at start)

Male adult shrimp – 2-hour exposure to treatment then observed for 8 days

Number of surviving shrimps each day

Day	Control	50 mg l^{-1} H_2O_2	50 mg l^{-1} H_2O_2 + low dose neutr. chemical	low dose neutr. chemical
0	12	11	11	12
1	12	10	11	12
2	12	9	10	12
3	12	8	10	12
4	12	8	10	12
5	12	8	10	12
6	12	8	10	12
7	12	8	9	12
8	12	8	9	12

3.3.2 Histology

Minor changes were observed in the gills of control shrimps (Figure 15). Analysed individuals showed uniform arrangement of lamellae with normal haemocytes and limited fusion of the lamellae. Significant and progressive changes in the histoarchitecture of the gills were observed in shrimps exposed to H_2O_2 alone and neutralisation chemical alone treatments. The highest ultrastructural changes were observed in the combined H_2O_2 + neutralisation chemical treatment with diffuse haemolytic infiltration, hyperplasia and swelling of gills as the most recurrent observations (Figure 16). A similar effect, but with less severe impairment was observed in the H_2O_2 alone and neutralisation chemical alone treatments. However, as shown in Figure 16, these changes are classified as mild histology. The occurrence of parasites was generally low, and there was no difference between treatments. Parasites were detected in at least two of the 10 shrimps from each treatment.

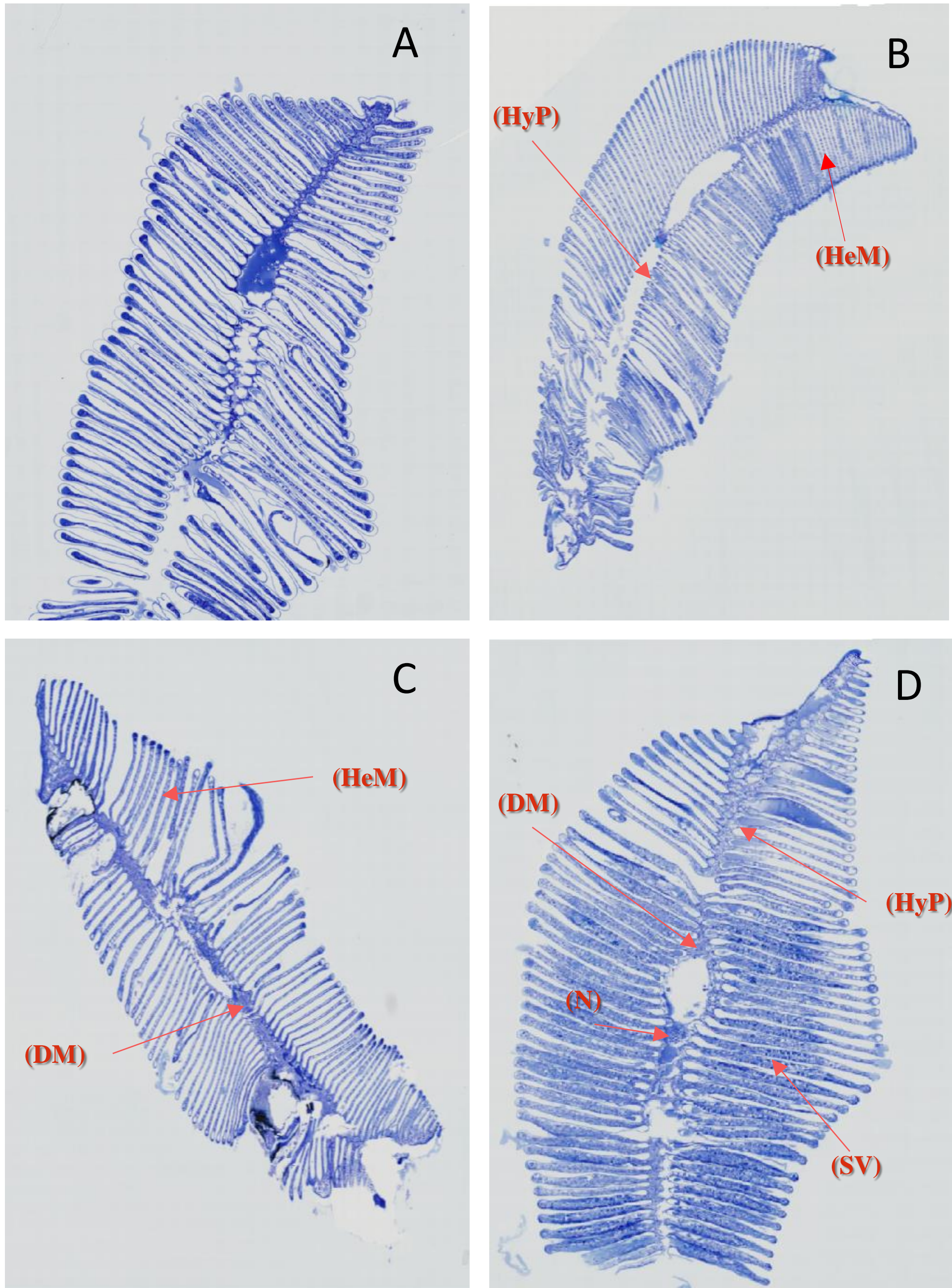

Figure 15. Gills from control shrimp P. borealis (A), shrimp exposed to H_2O_2 solution alone, B) neutralisation chemical alone, C) combination of H_2O_2 + neutralisation chemical, D). Accumulation of haemocytes (HeM) in the haemocoelic space, swelling (SV) and marked hypertrophy and hyperplasia (HyP) in the gill epithelium. Furthermore, necrotic (N) and formation of a disorganized mass (DM) of disrupted gill lamellae are symptoms of necrosis and tissue de-organisation phenomena.

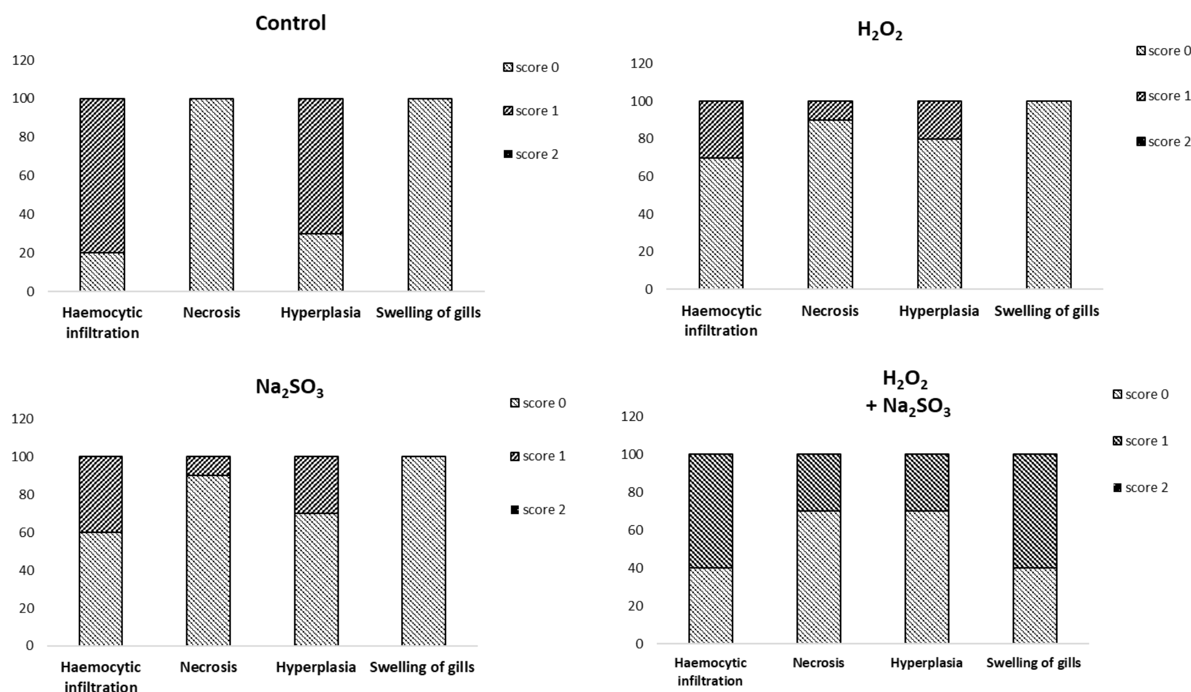

Figure 16. Gill histopathology in control shrimp (a) and shrimp exposed to H_2O_2, neutralisation chemical and H_2O_2 + neutralisation chemical. Score 0: no histopathology in any field, score 1: mild histopathology present in < 25% of the fields, class 2: moderate histopathology present in 25%–75% of the fields, and score 3: severe histopathology present in > 75% of the field.

3.4 Modelling

Illustrations showing H_2O_2 concentrations after a release from a 4-cage delousing treatment without neutralisation (Figure 17) and with 90% neutralisation (Figure 18) are presented. Please note that the concentration scale on those Figures differ, to be able to visually illustrate the distribution of H_2O_2 with and without neutralisation measurements. Concentrations after release from a well boat are shown in Figure 19 (without neutralisation), and in Figure 20 (with 98% neutralisation). Note the different scale on the y-axes in the Figures. The areas with different blue shades are affected by H_2O_2 concentrations above threshold value (0.14 mg/L, Refseth et al. (2019)). As seen in Figure 17 vs 18 and 19 vs 20, the area where H_2O_2 might be a potential risk for the ecosystem is largely reduced when the neutralisation process is implemented. Neutralisation, in combination with well boat seems to be a very efficient risk reducing measurement. It is reason to believe that also a 50% neutralisation of H_2O_2 onboard a well boat will be beneficial for the ecosystem. Hence, if time, energy and equipment onboard a well boat turns out to be limiting factors for achieving 90-100% removal, also lower removal efficiencies might be “good enough” after evaluation of dispersion and vulnerability of the area where H_2O_2 are released.

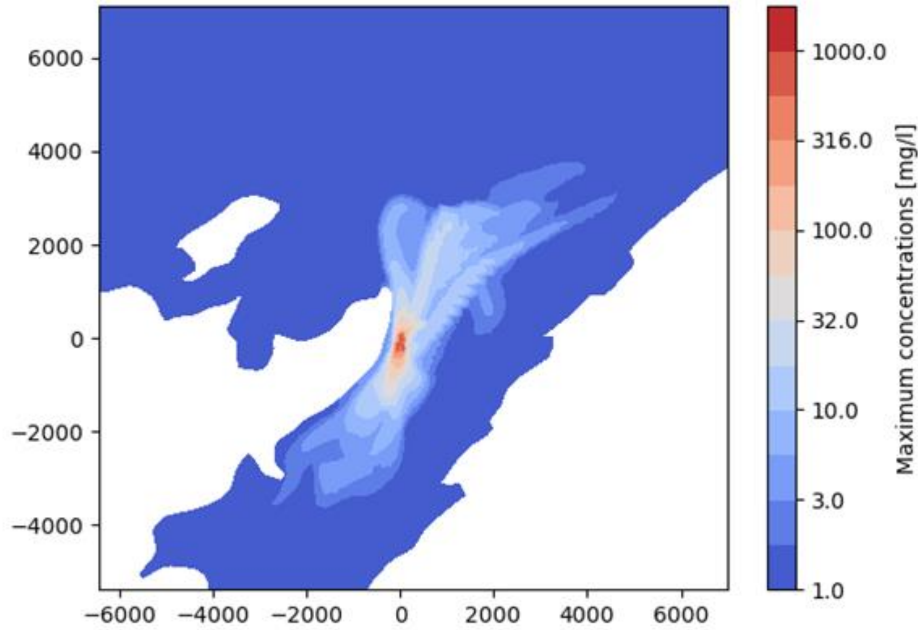

Figure 17. Maximum concentrations of H_2O_2 at Jacobsteinsvika during 12 simulated farm delousing operation (4 consecutive releases from different cages for each operation). Simulations without treatment of H_2O_2. X- and Y-axes are in metres relative to the point of emission.

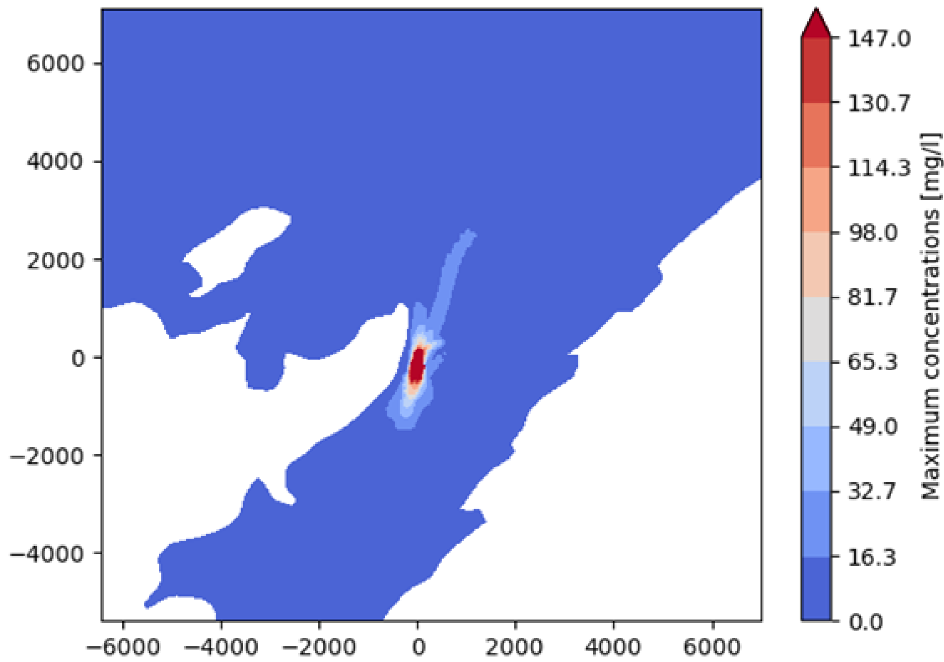

Figure 18. Maximum concentrations of H_2O_2 at Jacobsteinsvika during 12 simulated farm delousing operation (4 consecutive releases from different cages for each operation). Simulations with approx. 90% removal of H_2O_2. X- and Y-axes are in metres relative to the point of emission.

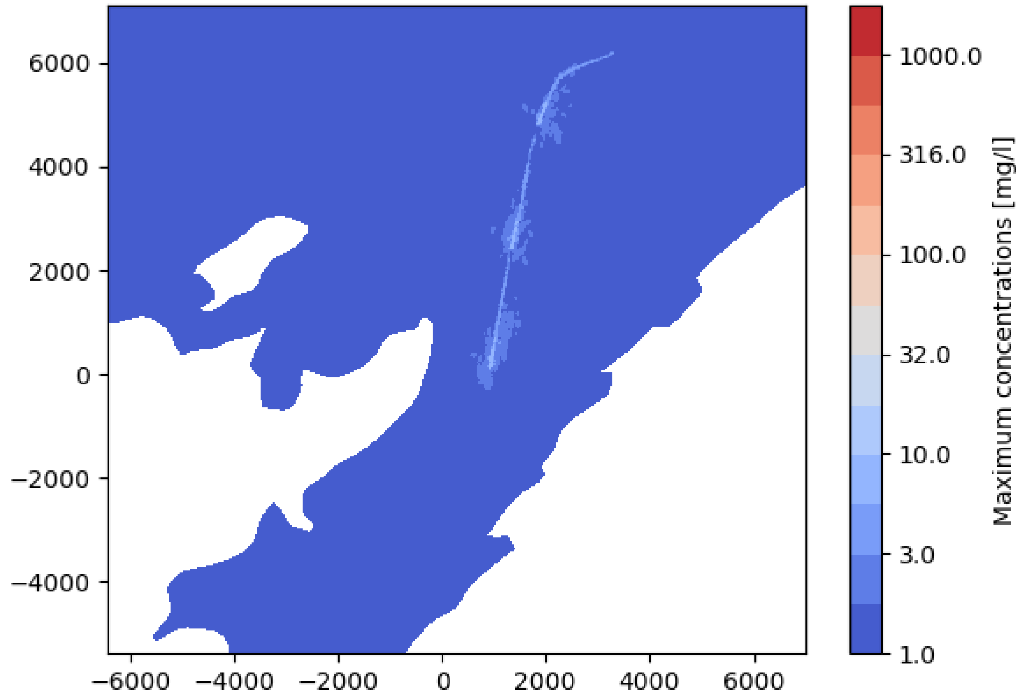

Figure 19. Maximum concentration of H_2O_2 from all simulations from 4 cage releases at Jakobsteinsvika using wellboat. Simulation without treatment of H_2O_2.

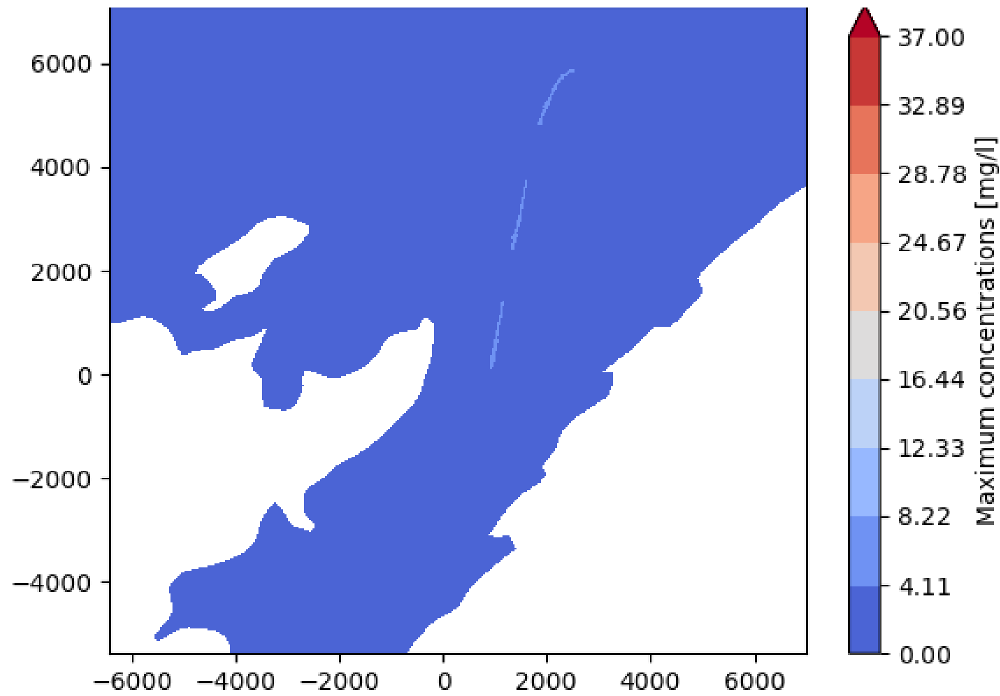

Figure 20. Maximum concentration of H_2O_2 from all simulations from 4 cage releases at Jakobsteinsvika using wellboat. Simulation with treatment of H_2O_2 (approx. 98 % reduction).

3.5 Risk assessment

Female egg carrying shrimp exposed to H_2O_2 at 50 mg/L both alone and in combination with neutralisation chemical at high, medium and low dose showed a significant increase in swimming activity during a 2 h pulsed exposure, though there was no mortality seen at least 14 days post exposure. This suggests the shrimp can detect the chemical change and attempt to swim away from the source, and that a 2-hour exposure at this concentration is non-lethal to these intermoult female adult shrimp. It also suggests that regular exposure to H_2O_2 in this concentration range in the field may not kill the shrimp but could move them away from their typical habitat ranges. Shrimp in moulting phase (males used in the present study) appear to be more vulnerable to H_2O_2 exposure. Based on this finding, seasonal impacts from H_2O_2 discharges could occur and should be taken into account in both management and further research. Exposure of shrimp to the neutralisation chemical alone at the lowest concentration used in the H_2O_2 exposures did not induce a significant increase in activity nor did it lead to mortality in any of the tested shrimps.

The conclusion from Refseth et al. (2019), is that there are negative environmental impacts on local communities at distances up to several kilometres away from release points of H_2O_2 after a delousing event, and usage of well boat is a risk reduction measurement. However, concentrations associated to mortality of e.g. shrimps and concentrations above the PNEC (0.14 mg/L; Refseth et al., 2019) will still be present, although in smaller areas. By applying the H_2O_2 neutralisation process, the risk is reduced substantially as the affected area is drastically reduced when neutralisation and removal procedures are applied both from 4-cage release and when using well boats (Figure 17-20).

3.6 Industrial application

The suggested solution for neutralising H_2O_2 has proven to be efficient in small scale (laboratory and with pilot equipment). The solution is patent pending, although it will need technological optimisation before ship tests will be possible. With a growing aquaculture in Norway and increasing focus and research on environmental impacts, there are obvious benefits by implementing this solution. Treatment solutions of wastewater in general is needed for a sustainable growth. Due to the lice' ability of developing resistance against pharmaceutical treatments and a rising awareness regarding e.g. fish welfare related to some of the non-pharmaceutical treatments, it is important to have several tools for the aquaculture industry to combat lice. Our solution is focused mainly on delousing onboard well boats due to the practical handling of the water afterwards. Also, for a further, industrial adaptation of this tool, there are no need to test the treatment solution on the salmon itself since they will not be in contact with the chemicals nor technology applied. This makes the implementation process more straight-forward and less costly.

Taking the cost of a modern well boat into consideration (prices around 250 000 NOK/24h have been estimated from the industry, although with variations depending on size, contracts, equipment etc), the main focus further are on minimalizing the time spent on neutralisation onboard. The chemical process is rather fast (minute-scale in laboratory and pilot experiments), while the time needed for technological treatment is longer. However, the technological treatment process can be shortened with increased technological treatment dose (which will require more energy) combined with optimised water flow and technological treatment interaction.

4 Conclusion

The aim of the project was to find a treatment solution for removal of hydrogen peroxide (H_2O_2) after de-lousing in salmon aquaculture to prevent releases of large amounts of H_2O_2 which has proven to pose a risk for the environment around release point, on a kilometre scale.
The initial experiments revealed benefits and drawbacks for a suit of chemicals and we have identified a promising neutralisation chemical for large-scale experiments and industrial scale. However, due to loss of oxygen during that treatment, new technologies and ideas were applied and technological treatment was found to provide promising initial results in combination with a neutralisation chemical. Hence, those experiments led to a patent application (prior art, July 2020) and were later scaled up to m^3 scale. Also those experiments seemed promising and efficient enough for a further, larger upscaling.
Shrimps were exposed to the proposed mixture with a 30x dilution of the hydrogen peroxide treatment concentration. No additional impact caused by the neutralisation chemical on swimming behaviour was observed and hence, there is reason to believe that the suggested treatment will be beneficial for the environment and at the same time allow efficient medical de-lousing treatments.

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6 Appendix

6.1 Sodium ascorbate experiments

Table A1. The 45 L experiments with 135g of a 49,5% H_2O_2 treatment mixture with A) 197 g sodium ascorbate in a molar ratio of 1:2 (H_2O_2: sodium ascorbate) and B) a 1:1 molar ratio (393 g g sodium ascorbate).

Time (minutes)	g/L H_2O_2	Temperature (oC)	pH	Oxygen (% saturation)	Oxygen (mg/L)
1:2 molar ratio					
Before H_2O_2	<LOD	12.7	8.12	98.5	10.44
H_2O_2 added (t=0)	1.66	12.6	7.89	100.2	
1	0.98		7.88	110.3	
1.5	0.76		7.84	108	
2	0.95		7.78		
3	0.85	12.6	7.75	114.8	12.22
5	1	12.6	7.67	117.3	12.4
10	1.15	12.6	7.47	111.5	11.83
20	0.63	12.7	7.04	121.3	12.85
34		12.5	6.8	103.5	11.03
60	0.83	12.6	6.44	103.7	10.88
102			6.1	90	
132	0.82	14	5.81	85.2	8.87
1:1 molar ratio					
Before H_2O_2	<LOD	11.5	8.09	101.5	11.07
H_2O_2 added (t=0)	1.56	11.5	7.91	103.1	11.34
1	0.89	11.3	7.82	106.4	
1.5	0.81		7.79		
2	0.51	11.5	7.74		
3	0.7		7.68		
5	0.92	11.4	7.62	105	11.37
10	0.43	11.5	7.42		11.5
13		11.5	7.18	107	11.63
20	0.16	11.6	6.9	97.5	10.61
55			6.4	87	
85	<LOD	12.5	6.04	68	7.3

Table A2. The 400 L experiments with 1751g sodium ascorbate and 1200g of a 49,5% H_2O_2 treatment mixture in a molar ratio of 1:2.

Time (minutes)	g/L H_2O_2	Temperature (°C)	pH	Oxygen (% saturation)	Oxygen (mg/L)
#1					
Before H_2O_2	<LOD	8	7.99	108	12.55
H_2O_2 added (t=0)	1.38		7.94		
1	1.49				
1.5	1.07				
2	0.97	7.9	7.94	107	12.6
3	1.05				
5	0.99	7.8	7.83		12.47
10	1.03	8	7.71		12.2
		7.9	7.6	102	12.11
20	0.97	7.9	7.38	102	12.02
40	0.99	8.5	7.12		11.12
45	0.96	8.3	6.74		11.12
70	1.04				
#2					
Before H_2O_2		7.9	7.95	105	12.44
H_2O_2 added (t=0)	1.43		7.94	105	12.44
1	1.12		7.94		12.64
1.5	0.81				
2	0.72				
3	0.96	7.7	7.87	105	12.48
5.3	1.06	7.8	7.8		12.36
10	0.9	7.8	7.65		11.95
20	0.8		7.55		12.08
35	1.09	7.8	7.2		11.8
45	0.87	7.8	7.06		11.49
24h	0.44	10.2	4.46	4.7	0.52

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