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Short-term exposure to hydrogen peroxide induces mortality and alters exploratory behaviour of European lobster (*Homarus gammarus*)

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ABSTRACT

Bath treatment chemotherapeutants, used to control sea lice infestations in the salmonid aquaculture industry, are released directly into the marine environment around fish farms and pose a serious risk to non-target species, particularly crustaceans. Hydrogen peroxide (H_2O_2) is the most frequently used bath treatment chemotherapeutant on Norwegian fish farms, however, limited information is available on its toxicity to European lobsters (*Homarus gammarus*), a commercially important species at risk of exposure due to its distribution overlapping with salmon farm locations. The aim of this study was to investigate the lethal effects of H_2O_2 on pelagic (stage I-IV) larvae/post-larvae and its sub-lethal effects on the benthic stage V *H. gammarus*. To assess the lethal effects of H_2O_2, we carried out a series of 1 h toxicity tests and assessed mortality after a 24 h post-exposure period. Exposure to H_2O_2 was toxic to all pelagic larval stages tested, with estimated median lethal concentrations (LC_{50}) of 177, 404, 665 and 737 mg/L for stage I, II, III and IV, respectively. These concentrations represent approximately 10, 23, 40 and 43%, of the recommended H_2O_2 concentrations used for delousing salmon on Norwegian fish farms, respectively. To assess the sub-lethal effects of H_2O_2 on *H. gammarus*, stage V juveniles were exposed to H_2O_2 at concentrations of 85, 170 and 510 mg/L for 1 h and shelter-seeking behaviour and mobility endpoints were assessed. Numerous behavioural parameters including distance travelled to shelter, time to locate shelter and the number of shelter inspections, were negatively affected in lobsters exposed to H_2O_2 when assessed immediately after the exposure period. However, no differences between control and exposed lobsters were detected after a 24 h post-exposure period. Our results demonstrate that short term exposures to H_2O_2 are lethal to pelagic *H. gammarus* life stages and can negatively affect the shelter seeking behaviour of benthic life stages, though these behavioural changes may be short-lived.

1. Introduction

Sea lice (*Lepeophtheirus salmonis*) infestations are a major challenge to the salmonid farming industry around the world (Costello, 2006; Torrisen et al., 2013; Vollset et al., 2016). The lice are naturally occurring parasitic copepods that affect both farmed and wild salmonid populations, causing skin damage and sub-epidermal hemorrhages that can lead to osmotic stress and secondary infections (Johnson et al., 2004; González et al., 2015). The high density of sea lice in the surrounding water of the salmon farms may lead to high mortality of the migrating post smolts of wild Atlantic salmon (*Salmo salar*) and the sea trout (*Salmo trutta*) (Costello, 2009; Vollset et al., 2016). In order to manage sea lice infestations on Norwegian fish farms, the Norwegian Salmon Lice Directive has limited the number of adult female lice per fish to 0.2 in spring and 0.5 for the rest of the year (FOR-2012-12-05-1140, 2012). To comply with these regulations, the industry relies on the use of chemotherapeutants, either dissolved in the water and applied as a bath treatment (hydrogen peroxide [H_2O_2], deltamethrin, azamethiphos) or applied as an in-feed drug (emamectin-benzoate, diflubenzuron, teflubenzuron) or on other non-medicated treatments e.g. mechanical removal or the use of warm or fresh water (Grefsrud et al., 2019).

Recently, Norway has seen a major decrease in the consumption of all chemotherapeutants (Folkehelseinstituttet, 2019), as a consequence of the development of resistance amongst the sea lice and the introduction of new delousing methods. Hydrogen peroxide is still, however, the predominate chemotherapeutant used in Norway, with 4523 tons used in 2019 (Folkehelseinstituttet, 2019). It acts on sea lice by inducing

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mechanical paralysis, inactivation of enzymes and DNA replication, and peroxidation of lipid and cellular organelle membranes by hydroxyl radicals (Cotran et al., 1989; Valenzuela-Muñoz et al., 2020). Studies have shown that the mechanical paralysis is caused by decomposition of H_2O_2 to water and O_2 gas/bubbles in the gut and hemolymph, resulting in the release of the pre-adult and adult lice from the fish which subsequently float to the surface (Thomassen, 1993; Bruno and Raynard, 1994; Aaen et al., 2014). On salmon farms, the target concentration of H_2O_2 for bath treatments is between 1500 and 2100 mg/L and treatment period can last for 20–40 min, depending on temperature (Treasure et al., 2000). Once the treatment is complete, the waste treatment water is released into the surrounding environment as the tarpaulin enclosing the net pens is removed or from wells boat release while in transport. As the plume of H_2O_2 disperses into the marine environment, pelagic non-target organisms may be exposed to the effluent (Burridge et al., 2014).

Several studies have found lethal effects of exposure to H_2O_2 on different marine crustacean species, including American lobster (*Homarus americanus*) larvae and adults, sand shrimp (*Crangon septemspinosa*), *Mysid* sp., amphipods (*Corophium volutator*), *Metacarcinus edwardsii*, brine shrimp (*Artenia salina*), northern shrimp (*Pandalus borealis*), and the copepods *Acartia hudsonica* and *Calanus* spp. (Smit et al., 2008; Burridge et al., 2014; Van Geest et al., 2014; Gebauer et al., 2017; Hansen et al., 2017; Bechmann et al., 2019; Escobar-Lux et al., 2019). A limited number of studies have also shown that exposure to H_2O_2 can have sub-lethal effects on crustaceans. For example, exposures to relatively low concentrations of H_2O_2 for short periods of time caused mechanical paralysis in copepod *Acartia hudsonica* ($\geq$10 mg/L), *Calanus* spp. ($\geq$17 mg/L) and *Pandalus borealis* (15 mg/L) (Van Geest et al., 2014; Bechmann et al., 2019; Escobar-Lux et al., 2019).

Acute toxicity tests often involve 24, 48 and 96 h exposure periods, which do not necessarily reflect acute exposures expected to occur in the marine environment (Ernst et al., 2001; Urbina et al., 2019). In recent years, there has been an increasing demand for toxicity tests to be performed under more environmentally relevant exposure conditions (Urbina et al., 2019). Shorter exposure times i.e. 1 h followed by a 24 h r post-exposure time (to assess delayed effects), would therefore provide a more accurate assessment of the impacts of bath treatment plumes on non-target species (Medina et al., 2004; Van Geest et al., 2014; Escobar-Lux et al., 2019).

The aim of this study was to investigate the toxicity of H_2O_2 to European lobster (*Homarus gammarus*), a non-target crustacean species native to the Norwegian marine environment. *H. gammarus* is an important commercial species and is at risk of exposure to bath treatment chemotherapeutants as its distribution overlaps with the location of salmon farms along the coast of Norway (Agnalt, 2008). The life history of *H. gammarus* includes a number of distinct developmental stages including a planktonic larval phase (stages I-III), a post-larva phase (stage IV) which marks the transition from planktonic to benthic living, followed by a fully benthic phase from stage V and onwards (Sars, 1874; Lawton and Lavalli, 1995). During the pelagic life stages, lobsters are most at risk of exposure to H_2O_2 when the pesticide disperses from the salmon cages into the surrounding marine environment following the operational release of bath treatment effluents. Our first objective, therefore, was to perform a series of 1 h toxicity tests to environmentally relevant concentrations of H_2O_2 with each of the *H. gammarus* pelagic larval stages (I-IV) in order to establish lethal concentrations. The benthic lobster life stages are also at risk of exposure to H_2O_2 under certain environmental concentrations. For example, when the water column is well mixed, H_2O_2 can potentially sink under salmon cages and undergo horizontal dispersion along the seafloor instead of in the surface layers (Refseth et al., 2017). Stage V lobsters naturally exhibit an exploratory and shelter-seeking behaviours when placed in new environments (Agnalt et al., 2017; van der Meeren, 2001) which can potentially be negatively affected by exposure to chemical pollutants. Therefore, our second objective was to examine the sub-lethal effects of H_2O_2 on *H. gammarus* stage V post-larvae following short (1 h) exposures, and specifically assess changes in their shelter seeking behaviour.

2. Material and methods

2.1. Chemicals

Commercial H_2O_2 (Nemona, 49,50% H_2O_2 or 600 g L^{-1}) was purchased from Akzo Nobel, Pulp and Performance Chemicals, AB Sweden.

2.2. Animal collection and handling

This experiment was approved by the Norwegian Food Safety Authority (ID 15510) and was carried out according to The Code of Ethics of the World Medical Association for animal experiments (The Norwegian Ministry of Agriculture and Food, 2010, 2015). Six ovigerous *H. gammarus* females were purchased from a lobster dealer on May 22, 2018 and transferred to Austevoll Research Station, Institute of Marine Research (HI) (N60°05′15.36″, E5°15′54″).

The lobsters were subsequently kept in holding tanks (1.5 m × 1.5 m) containing sand filtrated seawater from 160 m depth (salinity of 34.7 ppt), with a flow rate of 30 L min^{-1} and a photoperiod of 16-h/8-h day/night. The water temperature was maintained at 8 °C to control hatching. In August 2018, the seawater temperature was gradually increased to 16 °C to stimulate hatching. Newly hatched larvae, staged according to Sars (1874), were collected and transferred to aerated 40 L incubators (Hughes et al., 1974) supplied with running seawater at 14 °C. In order to limit cannibalism, all larvae in an incubator had an age difference no greater than three days. Correspondingly, each incubator was stocked with a maximum of 1500–2000 larvae. The larvae were fed daily with frozen artemia and Otohime C2 (Marubeni Nisshin Feed Company, Japan). When the larvae reached stage IV, they were transferred to separate 170 ml^3 (7.0 cm × 3.5 cm x 7.0 cm) housing compartments made of white plastic PVC with 2.5 mm diameter holes in the bottom to allow water exchange. Coarse-grained sand was added to each compartment to induce normal claw development (Govind and Pearce, 1989; Agnalt et al., 2017). The compartments were held in holding tanks at 14 °C and the lobster juveniles were fed frozen shrimp once a day. The incubators were treated twice a week with chloramine T (0.02 g L^{-1}) for 1 h to control Leucatrix minor infections in the larvae (Dr. D. Boothroyd, pers. comm.).

2.3. Toxicity studies

Lethality studies were performed with the pelagic larvae (stages I-IV). Exposures were conducted for 1 h and were followed by a 24 h post-exposure period. The temperature in the water-system was set to 14 °C, and in order to keep the temperature in the exposure units and in the post-exposure period, the room temperature was regulated to keep the temperature accordingly. The water temperature ranged between 13 and 14 °C. As no previous studies have assessed the toxicity of H_2O_2 on *H. gammarus* larvae, the chosen concentrations were based on the recommended dose for treating salmon (1700 mg/L). All four larval stages were exposed to H_2O_2 at concentrations of 170, 510, 850, 1190, 1530 mg/L corresponding to 10%, 30%, 50%, 70% and 90% of the recommended treatment dose. The mean carapace length for stage I, II, III and IV was 2.3 mm ± 0.1, 3.3 mm ± 0.1, 3.8 mm ± 0.2 and 5.0 mm ± 0.5, respectively.

For larval stages I & II, exposures were carried out in glass tank containing five larvae with five replicates per concentration; for stages III & IV (due to increased cannibalism and the number of available animals) each tank had approximately four larvae with four replicates per concentration (Burridge et al., 2014; Parsons et al., 2020). The glass tanks used for exposure had a volume of 700 ml. Prior to the start of exposure (within 5 min), the tanks were filled with fresh sand filtered

seawater at 14 °C and mixed with the appropriate H_2O_2 volumes. Following the exposures, larvae were transferred to 1 L individual post-exposure tanks supplied with continuously aerated seawater at 14 °C. Mortality and general condition of the larvae were assessed at 0, 6 and 24 h (for larval stages I & II) post exposure. The lobsters were considered to be immobilised when normal swimming was absent, but there was movement of the pleopods and mouth parts after gentle prodding. Larvae were considered dead if they were discoloured, deformed (detached carapace), or if there was no movement of the pleopods after gentle stimuli. Mortality that occurred during the 1 h exposure was defined as acute mortality whereas total mortality was defined as the combined mortality of the 1 h exposure and the 24 h post-exposure period.

2.4. Behavioural studies

Sixty-four stage V lobsters were randomly divided into four groups, control and three exposure groups, which were exposed for 1 h to sub-lethal concentrations of H_2O_2 (85, 170 and 510 mg/L) in individual containers. The selected concentrations were based on the estimated LC_{50} values established for stage IV and represented 0, 5, 10 and 30% of the recommended treatment dose, respectively. Exposures were carried out in glass tanks containing 500 ml of the appropriate test solution at 13.5–14.0 °C, where solutions were made as described above for the toxicity tests. Thirty-two lobsters were randomly selected and photographed for length measurements. Carapace length (CL) was recorded as the distance from the posterior rim of the eye socket to the posterior edge of the carapace, using the open source software ImageJ (Image Processing and Analysis in Java, mean CL = 6.04 ± 0.06 mm).

Immediately after exposure, the lobsters were transferred to individual containers filled with fresh seawater and aeration, until the commencement of the behavioural studies (within approximately 2 min). To ensure that the lobsters had enough space to walk freely and explore the environment, four wide light acrylic diffusers (65 cm × 12 cm x 6 cm) were used as lanes for the behavioural studies (Fig. S1). The lanes were filled with 3.12 L of seawater and maintained at 13.5–14.0 °C. To observe and record the behaviour of the lobsters, two GoPro Hero5® cameras where position at a height of 53.5 cm above the lanes. White sand was used as a substrate to ensure a better contrast between the lobster and the bottom of the tank. Shelters (5.5 cm × 2 cm), made from white PVC pipes cut in half, were placed at one end of each lane. The four lanes were simultaneously recorded, with one lobster from each exposure group placed in each lane at the opposite end of the shelter. This set-up has been used previously to study the shelter-seeking behaviour and activity levels of *H. gammarus* juveniles (Taormina et al., 2020). The lobsters were recorded for 30 min, after which the following parameters were recorded: 1) total distance travelled (cm); 2) time to locate shelter (s); 3) total number of inspections of the shelter; 4) time to accept shelter (s)-defined as time of entering and remaining inside the shelter for the rest of the observation; 5) proportion of lobsters that accepted shelter by the end of the observation.

Once the recording period was over, the lobsters were returned to their individual holding tanks. This marked the beginning of the 24 h post-exposure period. During this period the lobsters were fed frozen deep-water shrimp (*Pandalus borealis*). After the 24 h post-exposure period, the behavioural assays were repeated, in order to assess if there was any improvement in their behaviour. Between each trial, the lanes were cleaned, and the water was changed.

2.5. Statistics

All statistical analyses were conducted in R (Version 3.4.3 (2018-07-02) Copyright © 2018 The R Foundation for Statistical Computing).

2.5.1. Toxicity studies

Median lethal concentrations (LC_{50} values), and their 95% confidence intervals (CI), were calculated for each stage using generalised linear models (GLM) with binomial error structures and probit links, according to Finney (1971). Concentrations were log10 transformed to linearize the data. The dose-response curves were plotted using the ggplot2 R package.

2.5.2. Behavioural studies

Behavioural data were firstly tested for normality using the Shapiro-Wilk Test. If the data met the requirement for normality, an unpaired two-sample *t*-test was performed to compare the measured endpoint between treatment groups. If the data did not meet the requirement for normality, a non-parametric Mann-Whitney *U* Test was performed. Multivariate repeated measures ANOVA was carried out to test if there was any difference between the data acquired after 1 h exposure and the data acquired after a 1 h plus a 24 h post-exposure period.

3. Results

3.1. Toxicity studies

Acute mortality was low for all the treatment groups (Table 1), and the highest mortality of 15.4 ± 0.1% was obtained for stage I larvae exposed to the highest concentration of 1530 mg/L. No acute mortality was recorded for stage IV larvae, in any of the treatment groups. Immobilization and bubble formation on the inside of the carapace occurred in all larval stages but time-to-event was only recorded for stages I & II. In all of the H_2O_2 treatment groups, all stage I & II larvae developed air bubbles inside the carapace (Fig. 1), floated to the surface and subsequently became immobilised. This occurred within the first 5 min of the exposure period. Since many of the immobile and floating larvae did not recover, mortality increased during the 24 h post-exposure period. No acute mortality was recorded for any of the control groups immediately after the 1 h exposure.

Total mortality reached 100% for stage I larvae exposed to 1530 mg/L H_2O_2, and correspondingly, 92 ± 0.1% for stage II, 81 ± 0.2% for stage III and 75 ± 0.2% for stage IV (Table 1). In the groups exposed to 170 mg/L the total mortality observed after the 24 h post-exposure period was 44 ± 0.3%, 24 ± 0.1%, 25 ± 0.3% and 6.3 ± 0.1% for stages I, II, III and IV, respectively. Mortality was also observed in the control group for stage IV after the 24 h post-exposure period (12.5 ± 0.1). Estimated LC_{50} values and their CI for stage I, II, III and IV were 177 mg/L (142–212 mg/L), 404 mg/L (289–519 mg/L), 665 mg/L (423–906 mg/L) and 737 mg/L (507–967 mg/L), respectively (Fig. 2).

3.2. Behavioural studies

Independent of their treatment groups, the naïve stage V lobsters i.e. no previous encounter with shelter, started exploring their new environment as soon as they were released. The exploratory behaviour principally consisted of the lobsters freezing just as they were released in the lane, and then moving towards one of the lane borders. With the use of their antennae and claws, the lobsters maintained physical contact with the border, and then explored the long side of the lane in either direction. Once the lobsters made physical contact with the shelter, it was inspected multiple times occasionally followed by a second exploration of the lane before entering and accepting the shelter.

When examined immediately after the 1 h exposure period, the exposed lobsters (85, 170 and 510 mg/L H_2O_2) travelled significantly shorter distances compared to control (Mann-Whitney *U* Test, $p < 0.01$) (Fig. 3). The mean distances travelled were 569 ± 119 cm, 179 ± 40 cm, 242 ± 120 cm, and 130 ± 34 cm for lobsters in control, 85, 170 and 510 mg/L treatment groups, respectively.

Furthermore, the time spent by the lobsters to locate shelter for the first time was also greatly influenced by H_2O_2 exposure (Fig. 4). In particular, lobsters exposed to the two highest doses of H_2O_2 spent significantly longer times to locate the shelter compared to lobsters in

Table 1
Summary of the mean (±SD) acute mortality (1 h exposure) and total mortality (1 h exposure + 24 h post-exposure) of *H. gammarus* stage I (n = 150), stage II (n = 150), stage III (n = 109), and stage IV (n = 96) larvae after exposures to a range of H_2O_2 concentrations.

	Acute Mortality (%)				Total Mortality (%)			
	1-h exposure				1-h exposure + 24-h post-exposure			
H_2O_2 (mg/L)	Stage I	Stage II	Stage III	Stage IV	Stage I	Stage II	Stage III	Stage IV
1530	15.4 ± 0.1	0	4.8 ± 0.1	0	100	92 ± 0.1	81 ± 0.2	75 ± 0.2
1190	8.3 ± 0.1	4 ± 0.1	0	0	100	88 ± 0.1	45 ± 0.1	68.8 ± 0.3
850	8 ± 0.1	4 ± 0.1	0	0	100	80 ± 0.2	56.3 ± 0.2	62.5 ± 0.3
510	4 ± 0.1	0	0	0	100	44 ± 0.3	16.7 ± 0.1	37.5 ± 0.3
170	0	0	0	0	44 ± 0.3	24 ± 0.1	25 ± 0.3	6.3 ± 0.1
Control	0	0	0	0	0	5 ± 0.1	0	12.5 ± 0.1

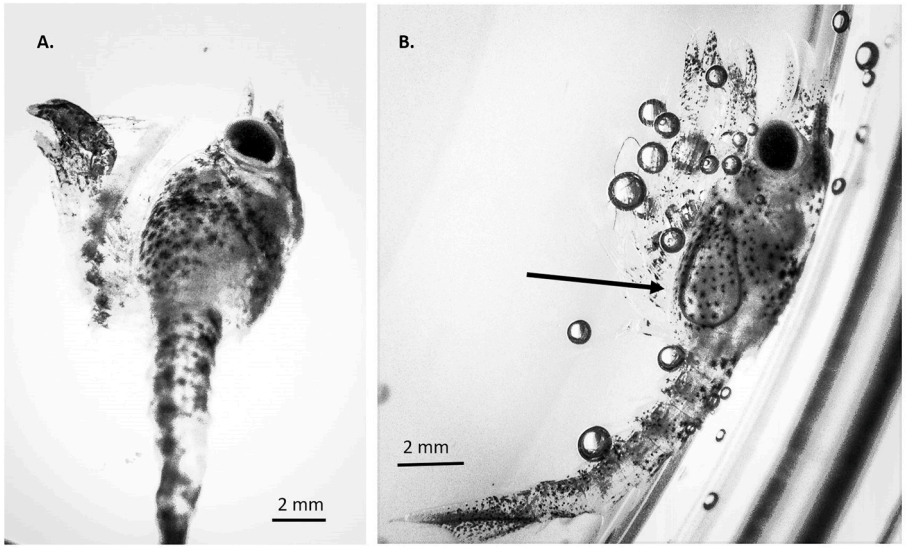

Fig. 1. Representative images of *H. gammarus* stage I larvae in the (A) control and (B) 850 mg/L H_2O_2 group. The black arrow indicates the presence of an air bubble inside the carapace.

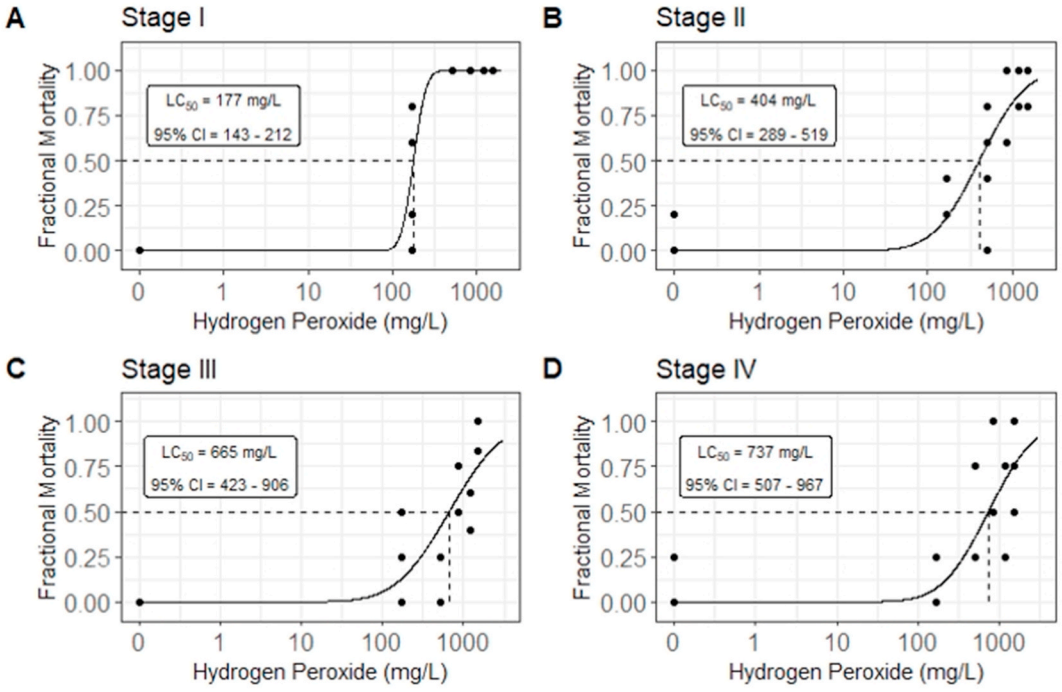

Fig. 2. The toxicity of H_2O_2 to *H. gammarus* larvae following a 1 h exposure and 24 h post-exposure period. Dose-response curves show mortality amongst pelagic *H. gammarus* (A) stage I, (B) stage II, (C) stage III and (D) stage IV larvae.

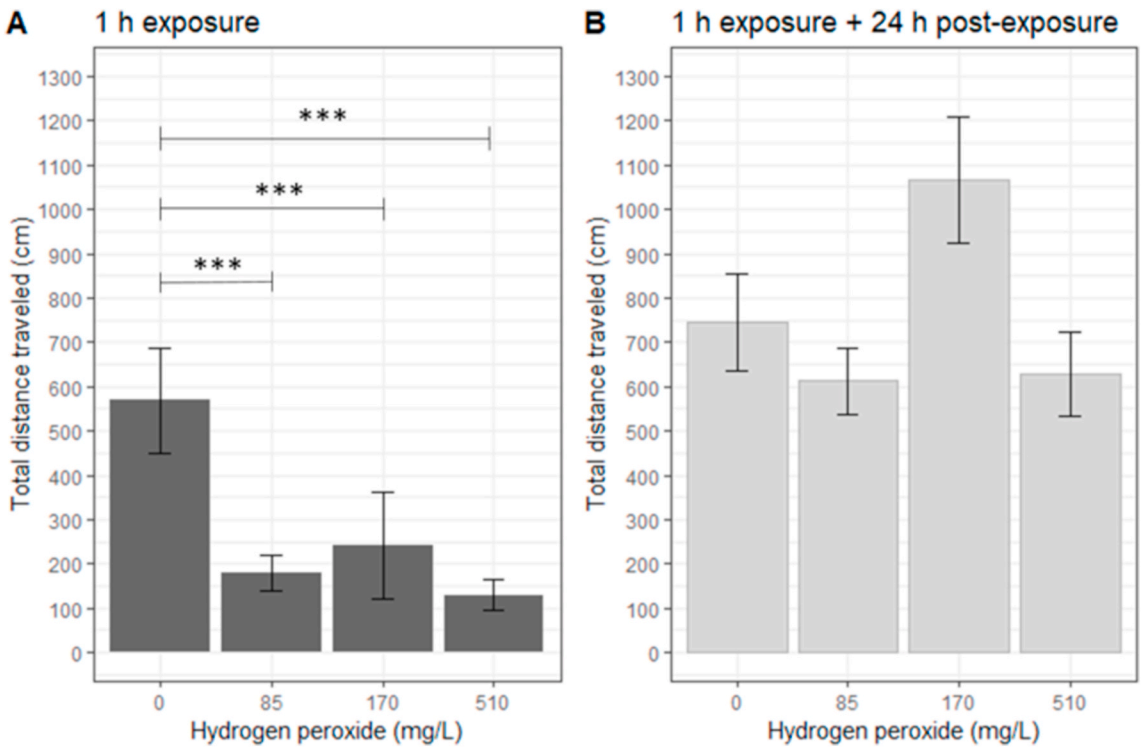

Fig. 3. The total distance travelled (cm) by *H. gammarus* stage V post-larvae in the 30 min behavioural assays performed following 1 h exposures to sub-lethal concentrations of H_2O_2. ***p < 0.001 treatment vs. control. Data is presented for behavioural assays performed A) immediately after the exposure period and B) after a 24 h post-exposure period. n = 16 per concentration.

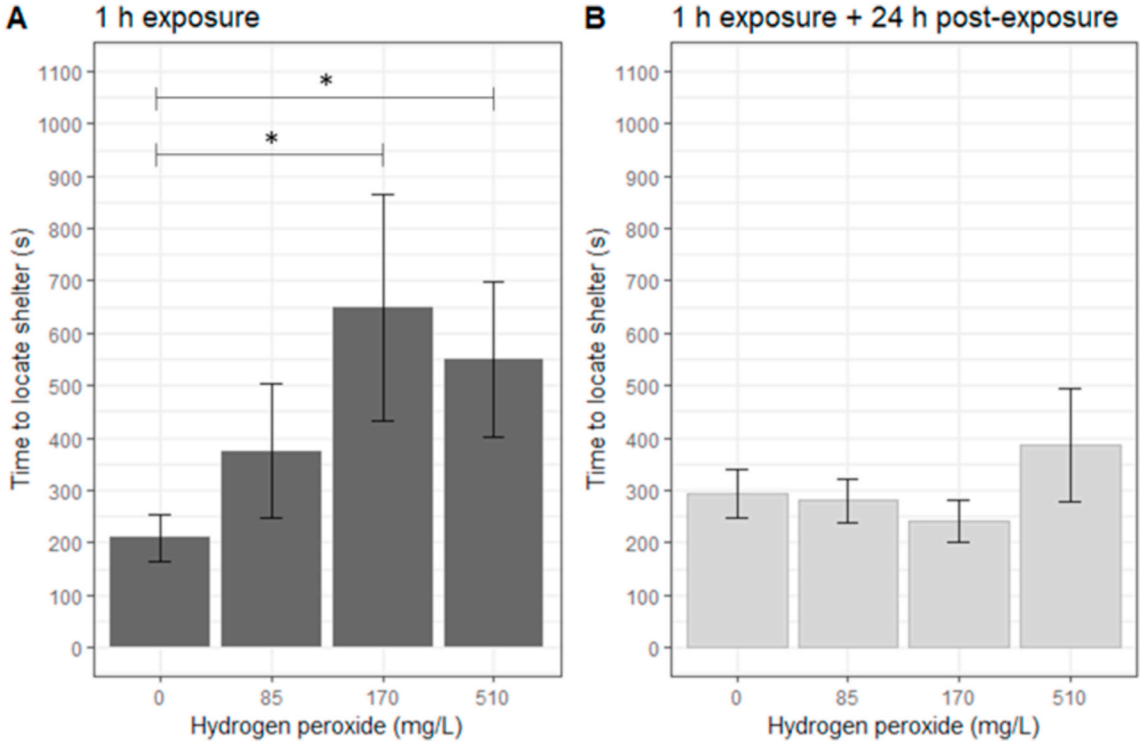

Fig. 4. Time (seconds) taken by *H. gammarus* stage V post-larvae to find and inspect the provided shelter for the first time during 30 min behavioural assays performed following 1 h exposures to sub-lethal concentrations of H_2O_2. *p < 0.05 treatment vs. control. Data is presented for behavioural assays performed A) immediately after the exposure period and B) after a 24 h post-exposure period. n = 16 per concentration.

the control group (Mann-Whitney *U* Test, $p < 0.05$). The mean time taken to locate the shelter for the first time were 210 ± 45 s, 375 ± 129 s, 649 ± 215 s, and 551 ± 148 s for lobsters in control, 85, 170 and 510 mg/L treatment groups, respectively.

Similarly, the total number of shelter inspections were affected by H_2O_2 exposure, where individuals in the control group inspected the shelter at a significant higher rate than the lobsters in all the treatment doses lesser (Mann-Whitney *U* Test, $p < 0.01$) (Fig. 5). There was, however, no significant effect of H_2O_2 exposure on the time (s) taken by the lobsters to accept the shelter (data not shown), though based on a limited data set since only one lobster in the 170 mg/L treatment group accepted the shelter. The proportion of lobsters that had accepted their shelters at the end of the experimental period (30 min) were 44, 12, 6 and 12% in the control, 85, 170 and 510 mg/L treatment groups, respectively, showing a significant influence by H_2O_2 exposure (Mann-Whitney *U* Test,$p < 0.01$) (Fig. 6).

Twenty-four hours after the exposure, there were no significant differences between control and H_2O_2-exposed larvae for any of the behavioural parameters assessed (Figs. 3–5) (Mann-Witney *U* Test, $p > 0.05$).

4. Discussion

4.1. Mortality

Exposure to H_2O_2 was lethal to *H. gammarus* larval stages (I-IV). In this study, we have shown that a 1 h exposure to H_2O_2, at environmentally relevant concentrations, was lethal to each of the pelagic *H. gammarus* larval stages (I-IV). The stage I larvae were the most sensitive life stage tested with an LC_{50} value for H_2O_2 of 177 mg/L, followed by stage II (LC_{50} = 404 mg/L), stage III (LC_{50} = 676 mg/L) and stage IV (LC_{50} = 738 mg/L). Consistent with our results, stage-specific differences in sensitivity to H_2O_2 were also observed in toxicity studies with sea lice (*L. salmonis*), *Calanus* spp. and *Acartia* sp. (Aaen et al., 2014; Van Geest et al., 2014; Bravo et al., 2015; Escobar-Lux et al., 2019).

In line with our findings, previous studies have also reported that short term (1 h) exposures to H_2O_2 were toxic to non-target marine crustaceans, and where species-specific differences in sensitivity are apparent. For example, while Burridge et al. (2014) observed that a short-term exposure (1 h + 96 h post-exposure period) to H_2O_2 was lethal to *Mysid* spp., C. septemspinosa and *H. americanus* larvae, the estimated LC_{50} values (973, 3182 and 1637 mg/L, respectively) were much higher than those reported here, especially when compared to *H. gammarus* stage I. Furthermore, a number of other studies have reported that H_2O_2 was not acutely toxic to crustacean species like *P. flexuosus*, *P. elegans* and adult *H. americanus* (Brokke, 2015; Burridge et al., 2014) following a 1 h exposure.

In comparison, a recently published paper reported that 1 h exposures (followed by a 24 h post-exposure period) to H_2O_2 were acutely toxic to copepodite V and adult *Calanus* spp., and both life stages were more sensitive than *H. gammarus* larvae (as examined here), with LC_{50} values of 77.1 mg/L and 30.6 mg/L calculated, respectively (Escobar Lux et al., 2019). Taken together these studies demonstrate that there are species- and life-stage specific differences in sensitivity to H_2O_2 exposure amongst crustaceans, and especially *H. gammarus* stage I larvae appears to be one of the most sensitive species tested to date.

While H_2O_2 exposures were lethal to all of the *H. gammarus* larval stages tested, the deleterious effect of H_2O_2 appeared to be delayed, with larval mortalities mostly occurring during the 24 h post-exposure period. For example, the acute mortality amongst stage I larvae ranged between 0 and 15%, but the total mortality reached 44–100% at 24 h post-exposure. Delayed effects following a post-exposure period was also observed in H_2O_2 toxicity studies with *P. borealis* (Bechmann et al., 2019), *Calanus* spp. (Escobar-Lux et al., 2019) and zoea *M. edwarsii* (Gebauer et al., 2017). These studies combined demonstrates the importance of including a post-exposure period in the experimental design to prevent an underestimation of the toxic effects of H_2O_2 on non-target crustaceans.

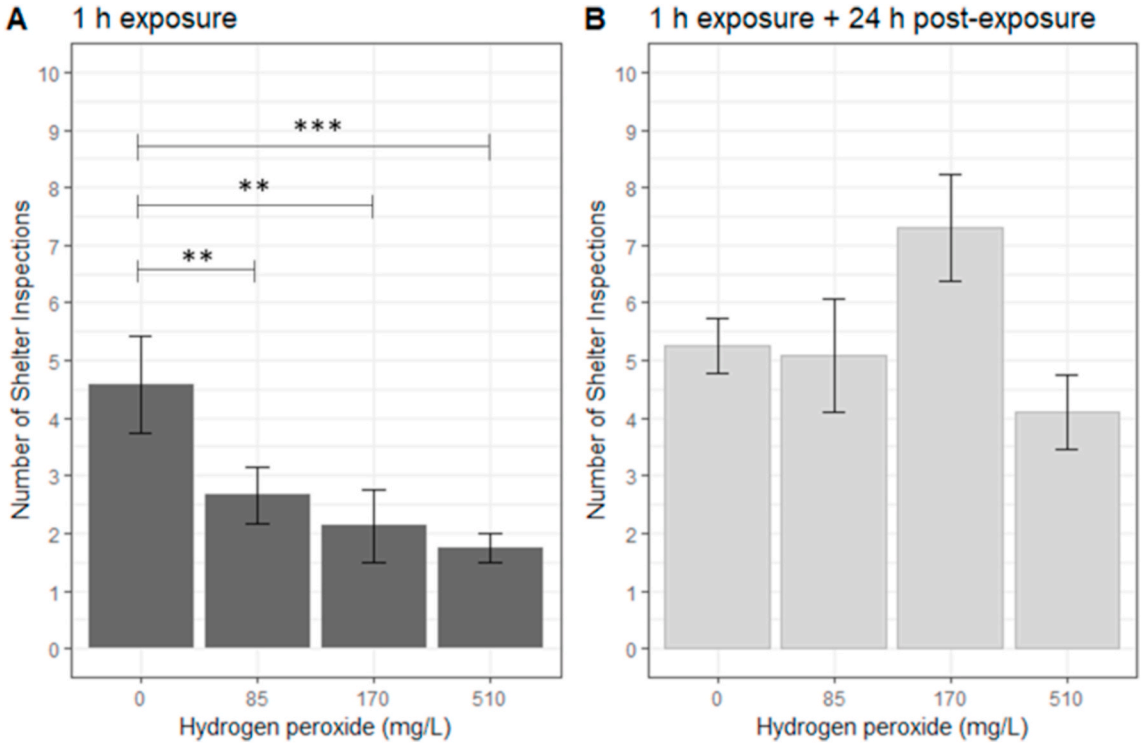

Fig. 5. Total number of shelter inspections by *H. gammarus* stage V post-larvae during 30 min behavioural assays performed following 1 h exposures to sub-lethal concentrations of H_2O_2. **$p < 0.01$; ***$p < 0.001$ treatment vs. control. Data is presented for behavioural assays performed A) immediately after the exposure period and B) after a 24 h post-exposure period. n = 16 per concentration.

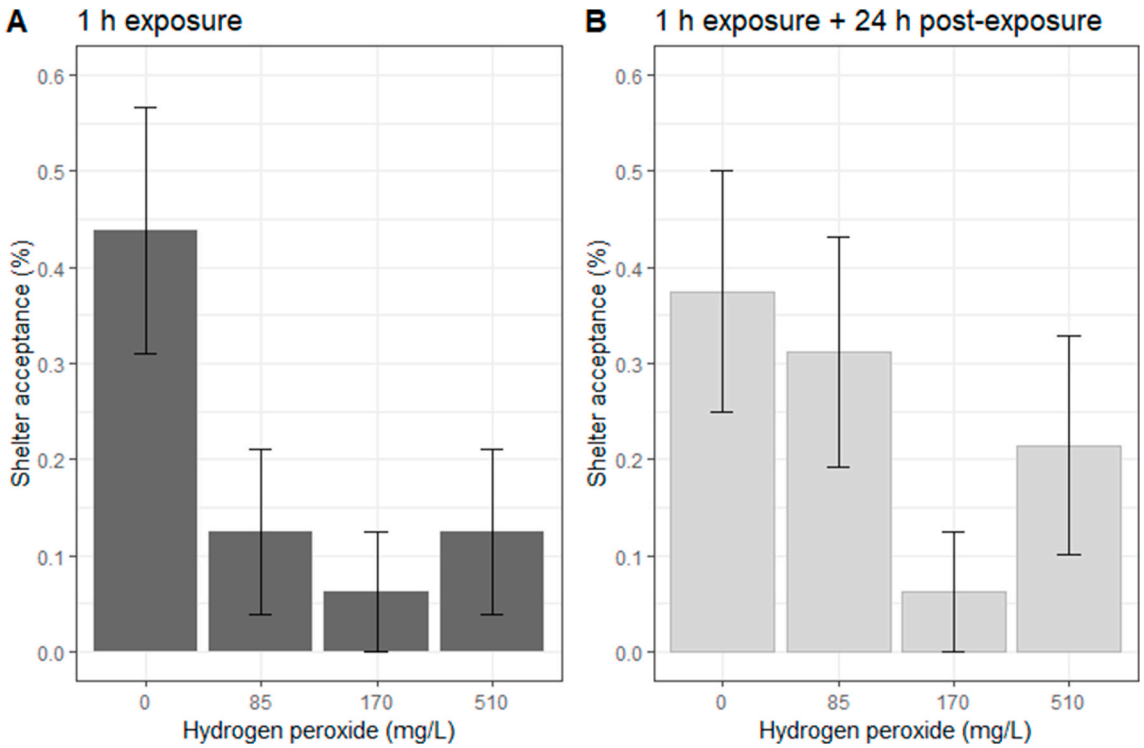

Fig. 6. Proportion (%) (±SD) of *H. gammarus* stage V post-larvae that accepted the shelter during 30 min behavioural assays performed following 1 h exposures to sub-lethal concentrations of H_2O_2. Data is presented for behavioural assays performed A) immediately after the exposure period and B) after a 24 h post-exposure period. n = 16 per concentration.

4.2. Immobilization and bubble formation

We observed that exposure to H_2O_2, even at the lowest dose tested, resulted in the formation of bubbles almost immediately (within 5 min of the exposure commencing) inside the carapace of all *H. gammarus* stage I and II larvae. Bubbles were also observed inside the carapace of stages III and IV though time to event was not monitored. As these larvae were subsequently paralysed at the surface of the water and only a limited number of individuals recovered after 24 h post-exposure period, our results suggest that the negative effects of H_2O_2 exposure on these larval stages may be substantial and rapid. In the wild, paralysed larvae would be unable to feed and unable to maintain their position in the water column and negatively impact their predator avoidance behaviour. These larvae may, therefore, be considered as ecologically dead and the effect in the wild may be larger than what is indicated by the LC_{50} value alone. While mechanical paralysis and the formation of O_2 bubbles in the hemolymph has previously been observed amongst H_2O_2-exposed adult sea lice (Thomassen, 1993; Bruno and Raynard, 1994; Aaen et al., 2014), this was not reported for *H. americanus* larvae in H_2O_2 acute toxicity tests (Burridge et al., 2014). Interestingly, while *M. edwardsii* larvae and copepods (*A. hudsonica* and *Calanus* spp.) were paralysed following short-term exposures to H_2O_2, the formation of bubbles was not reported/observed (Van Geest et al., 2014; Gerbauer et al., 2017; Escobar-Lux et al., 2019), suggesting differences in mechanistic pathways of toxicity amongst crustacean species.

It is interesting to note, that although we observed that a single 1 h H_2O_2 exposure had lethal and sub-lethal effects on *H. gammarus* larva, delousing operations can involve the concurrent and sequential pesticide applications in many cages within a single fjord. Consequently, multiple discharges and cumulative loading of the pesticides can occur and non-target crustaceans are likely to be exposed to multiple H_2O_2 plumes over a longer period (Grefsrud et al., 2018). Lower LC_{50} and EC_{50} values have been reported as a result of longer exposure times or pulse-like exposures for both *H. americanus* and *P. borealis* (Burridge et al., 2000, 2008; Bechmann et al., 2019). Therefore, the impact of H_2O_2 on wild lobster larvae may be more pronounced under these conditions than the effects observed here for single exposures.

4.3. Effects of H_2O_2 on the shelter-seeking behaviour

Here we have shown that short (1 h) exposures to sub-lethal concentrations of H_2O_2 negatively affected several behavioural parameters associated with shelter-seeking in stage V *H. gammarus* lobsters when examined immediately after the exposure period. In all H_2O_2 treatment groups (85–510 mg/L), the lobster juveniles moved significantly less (total distance travelled) and inspected the shelter fewer times compared with control juveniles. Such negative impacts on locomotion observed in short-term sub-lethal exposures to pesticides have previously been linked to a failure in predator avoidance for other crustacean species (Farr, 1977; Rasmussen et al., 2013). Furthermore, juveniles exposed to the two higher H_2O_2 concentrations (170 and 510 mg/L) spent a longer period of time exploring their surroundings and to locate and recognise the shelter. As far as we are aware, no published studies to date have examined the effect of H_2O_2 on the shelter seeking behaviour of *H. gammarus* or any other lobster species, though exposure to H_2O_2 did have measurable effects on the escape behaviour of *Calanus* spp. (Escobar-Lux et al., 2019). Interestingly, a recent study reported reduced exploratory behaviour amongst *H. gammarus* juveniles exposed to sub-lethal concentrations of the in-feed anti-sea lice drug teflubenzuron (Cresci et al., 2018). Specifically, and in line with our findings, the study found that teflubenzuron exposed juveniles took significantly more time to find and recognise shelter (Cresci et al., 2018). Furthermore, sub-lethal concentrations of the organophosphate pesticide azamethiphos negatively affected the use of shelters by juvenile *H. americanus*, with an increase in the lobsters' latency to re-enter the shelter observed with increasing azamethiphos concentrations (Abgrall et al., 2000). Taken together, these studies demonstrate that shelter seeking

behaviour of juvenile lobsters is negatively affected following exposure to a range of anti-sea lice pesticides, including H_2O_2, and this may have negative consequences on the lobster's ability to avoid predators. Post-larvae or early benthic juvenile lobsters are more dependent on the rapid attainability of their shelters than adults (Mehrtens et al., 2005), and multiple studies have shown that the vulnerability of newly settled juveniles due to lack of protective shelters is high, and therefore important for survival (Hudon, 1987; Lawton and Lavalli, 1995; van der Meeren, 2001). Juveniles that reside in the vicinity of salmon farms treating with H_2O_2, may therefore be at a higher risk of predation if they cannot rapidly attain a shelter. Interestingly, however, all of the behavioural endpoints affected immediately after the exposure period returned to baseline levels at 24 h post-exposure, with no significant differences between exposed and control lobsters. This suggests that the effects of H_2O_2 on the shelter seeking behaviour of *H. gammarus* larvae may only be short lived, with the risk of predation in the wild likely to be highest in the immediate aftermath of an exposure scenario.

4.4. Potential effects of H_2O_2 to wild populations

Hydrogen peroxide has previously been described as the most environmental friendly bath treatment chemotherapeutant on the market and it is estimated that it poses little threat in terms of lethality to non-target crustaceans, such as lobster and shrimp, after short term exposures (Burridge et al., 2014). Here, however, we have shown that the 1 h-LC_{50} values calculated for stage I, II, III and IV *H. gammarus* larvae represent approximately 10, 23, 40 and 43%, respectively, of the recommended H_2O_2 concentrations used for treating sea lice infestations on Norwegian fish farms. Furthermore, we have also shown that lobster juvenile behavioural parameters associated with shelter seeking were also affected following short-term exposure to H_2O_2 at concentrations as low as 85 mg/L (or 5% of the recommended treatment dose). It is important, however, to assess whether these concentrations, calculated from laboratory based toxicity tests, are likely to pose a risk to lobster larvae living in the wild near aquaculture facilities. While it has previously been reported that H_2O_2 breaks down into water and oxygen, the speed of this process is influenced by several parameters including temperature and the amount of organic matter in the water. Degradation studies have estimated that the half-life of H_2O_2 ranged between 1 and 56 days (Bruno and Raynard, 1994; Lyons et al., 2014; Fagereng, 2016; Parsons and Samuelsen unpubl. data), and even the shortest of these estimated degradation times is considerably longer than the 1 h needed to induce mortalities, paralysis and altered exploratory behaviours amongst the pelagic and benthic larval stages of *H. gammarus*. Since H_2O_2 is expected to rapidly dilute in receiving waters, it is, however, reasonable to assume that the degradation rate will have limited impact on the environmental concentrations and dispersal dynamics instead will greatly influence the impact of H_2O_2 on non-target species. Considering that H_2O_2 is extensively used as an anti-sea lice pesticide around the world, relatively few field studies have, however, measured the concentration of H_2O_2 in the waters surrounding fish farms after the discharge of bath treatment effluents. One such study from the west coast of Norway, found that concentrations of H_2O_2 were either below the limit of detection or relatively low in water sampled 20–60 m from the edge of a salmon cage after the bath treatment water was discharged (Fagereng, 2016). In contrast, a later Norwegian study measured relatively high concentration of H_2O_2 (up to 778 mg/L), similar to or greater than the LC_{50} values observed here for *H. gammarus* larvae (177–738 mg/L) in the water directly under (at depths up to 60 m) and surrounding (within 15 m) a salmon cage post treatment. These higher H_2O_2 concentrations did, however, decrease with time (Andersen and Hagen, 2016).

Recently, studies have started to use mathematical models to predict the dispersal of bath treatment pesticides from Norwegian farms and indicate that the spread of H_2O_2 in the marine environment may be more substantial than field studies imply (Refseth et al., 2017; Parsons et al., 2020). Model simulations, performed by Refseth et al. (2017), found that low concentrations of H_2O_2 (<100 mg/L) should be detected in surface waters (0–3 m depth) at large distances from Norwegian farms, up to several hours after the discharge. This study also reports that areas closer to the farm (within 1 km) may experience higher H_2O_2 concentrations (>300 mg/L) for the first hour after discharge, while areas within a 2 km radius may be exposed to concentrations of 100 mg/L (Refseth et al., 2017). These simulations suggest that pelagic life stages of *H. gammarus*, in particular stage I and II larvae, that are living within 1–2 km of a salmon farm may be exposed to lethal concentrations of H_2O_2.

It is interesting to note that both field measurement and model simulation studies report that when environmental conditions result in a well-mixed water column, H_2O_2 plumes can sink to the seafloor within minutes of discharge. These findings have serious implications for benthic non-target species and life stages, such as juvenile and adult lobsters, living in the vicinity of fish farms. For example, Andersen and Hagen (2016), measured H_2O_2 concentrations that were 43% of the treatment concentration on the sea floor (at 70 m depth) 8 min after a discharge. Similarly, Refseth et al. (2017), predicted that 50% of the initial treatment doses (800 mg/L) could sink to the seafloor under fish cages and horizontal transport along the bottom would be reduced compared to the surface layers, meaning that these higher concentrations would persist for longer periods of time (up to 5–10 h). Considering that we observed behavioural changes in newly settled stage V *H. gammarus* juveniles, at 5% of the recommended treatment concentration, these studies suggest that H_2O_2 poses a risk to bottom-dwelling lobster life stages as well as the pelagic life stages.

In summary, the results presented here clearly demonstrate that short-term exposures to H_2O_2, at and below recommended industry concentrations, have lethal and sub-lethal effects on multiple life stages of the commercially important European lobster. In order to better understand the potential effects of H_2O_2 in the Norwegian marine environment, further studies which assess the impact of acute and chronic exposures to H_2O_2 on a wide variety of native non-target species are required.

Credit author statement

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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References

Aaen, S.M., Aunsmo, A., Horsberg, T.E., 2014. Impact of hydrogen peroxide on hatching ability of egg strings from salmon lice (Lepeophtheirus salmonis) in a field treatment and in a laboratory study with ascending concentrations. Aquaculture 422, 167–171.

Abgrall, P., Rangeley, R.W., Burridge, L.E., Lawton, P., 2000. Sublethal effects of azamethiphos on shelter use by juvenile lobsters (Homarus americanus). Aquaculture 181 (1–2), 1–10.

Agnalt, A.L., 2008. Stock Enhancement of European Lobster (Homarus gammarus) in Norway; Comparisons of Reproduction, Growth and Movement between Wild and Cultured Lobster. Dr. Scient. Thesis. Department of Biology, University of Bergen, Norway, p. 56.

Agnalt, A.L., Grefsrud, E.S., Farestveit, E., Jørstad, K.E., 2017. Training camp—a way to improve survival in European lobster juveniles? Fish. Res. 186, 531–537.

Andersen, P.A., Hagen, L., 2016. Fortynningsstudier - Hydrogenperoksid, September 2016. Aqua Kompetanse A7S, Flatanger, p. 30. https://docplayer.me/34408749 Fortynningsstudier-hydrogenperoksid-september-2016.html.

Bechmann, R.K., Arnberg, M., Gomiero, A., Westerlund, S., Lyng, E., Berry, M., Agustsson, T., Jager, T., Burridge, L.E., 2019. Gill damage and delayed mortality of Northern shrimp (Pandalus borealis) after short time exposure to anti-parasitic veterinary medicine containing hydrogen peroxide. Ecotoxicol. Environ. Saf. 180, 473–482.

Bravo, S., Silva, M.T., Agusti, C., Sambra, K., Horsberg, T.E., 2015. The effect of chemotherapeutic drugs used to control sea lice on the hatching viability of egg strings from Caligus rogercresseyi. Aquaculture 443, 77–83.

Brokke, K.E., 2015. Mortality caused by de-licing agents on the non-target organisms chameleon shrimp (Praunus flexuosus) and grass prawns (Palaemon elegans). In: Department of Biology. University of Bergen Norway.

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. Aquacult. Int. 2 (1), 10–18.

Burridge, L.E., Haya, K., Waddy, S.L., Wade, J., 2000. The lethality of anti-sea lice formulations Salmosan®(Azamethiphos) and Excis®(Cypermethrin) to stage IV and adult lobsters (Homarus americanus) during repeated short-term exposures. Aquaculture 182 (1–2), 27–35.

Burridge, L.E., Haya, K., Waddy, S.L., 2008. The effect of repeated exposure to azamethiphos on survival and spawning in the American lobster (Homarus americanus). Ecotoxicol. Environ. Saf. 69 (3), 411–415.

Burridge, L.E., Lyons, M.C., Wong, D.K.H., MacKeigan, K., VanGeest, J.L., 2014. The acute lethality of three anti-sea lice formulations: AlphaMax®, Salmosan®, and Interox® Paramove™ 50 to lobster and shrimp. Aquaculture 420, 180–186.

Cotran, R.S., Kumar, V., Robbins, S.L., 1989. Pathological Basis of Disease, fourth ed. Saunders, Toronto.

Costello, M.J., 2006. Ecology of sea lice parasitic on farmed and wild fish. Trends Parasitol. 22 (10), 475–483.

Costello, M.J., 2009. The global economic cost of sea lice to the salmonid farming industry. J. Fish. Dis. 32 (1), 115.

Cresci, A., Samuelsen, O.B., Durif, C.M., Bjelland, R.M., Skiftesvik, A.B., Browman, H.I., Agnalt, A.L., 2018. Exposure to teflubenzuron negatively impacts exploratory behavior, learning and activity of juvenile European lobster (Homarus gammarus). Ecotoxicol. Environ. Saf. 160, 216–221.

Ernst, W., Jackman, P., Doe, K., Page, F., Julien, G., Mackay, K., Sutherland, T., 2001. Dispersion and toxicity to non-target aquatic organisms of pesticides used to treat sea lice on salmon in net pen enclosures. Mar. Pollut. Bull. 42 (6), 432–443.

Escobar-Lux, R.H., Fields, D.M., Browman, H.I., Shema, S.D., Bjelland, R.M., Agnalt, A.L., Skiftesvik, A.B., Samuelsen, O.B., Durif, C.M., 2019. The effects of hydrogen peroxide on mortality, escape response, and oxygen consumption of Calanus spp. FACETS 4 (1), 626–637.

Fagereng, M.B., 2016. Use of hydrogen peroxide in fish farms; dilution studies and effects on flower shrimp (Pandalus montagui). In: Department of Biology. University of Bergen Norway.

Farr, J.A., 1977. Impairment of antipredator behavior in Palaemonetes pugio by exposure to sublethal doses of parathion. Trans. Am. Fish. Soc. 106 (3), 287–290.

Finney, D.J., 1971. Probit Analysis. Cambridge University Press, Cambridge, UK.

Folkehelseinstituttet, 2019. Legemidler i fiskeoppdrett. In: Stor Nedgang I Bruken Av Legemidler Mot Lakselus 2018. March 2020. https://www.fhi.no/hn/legemiddelbruk/fisk/2019-bruk-av-legemidler-i-fiskeoppdrett/.

FOR-2012-12-05-1140, 2012. Regulations on the Control of Salmon Lice in Aquaculture Facilities. Ministry of Trade, Industry and Fisheries, Oslo, Norway. www.lovdata.no/dokument/SF/forskrift/2012-12-05-1140.

Gebauer, P., Paschke, K., Vera, C., Toro, J.E., Pardo, M., Urbina, M., 2017. Lethal and sub-lethal effects of commonly used anti-sea lice formulations on non-target crab Metacarcinus edwardsii larvae. Chemosphere 185, 1019–1029.

González, M.P., Marín, S.L., Vargas-Chacoff, L., 2015. Effects of Caligus rogercresseyi (Boxshall and Bravo, 2000) infestation on physiological response of host Salmo salar (Linnaeus 1758): establishing physiological thresholds. Aquaculture 438, 47–54.

Govind, C.K., Pearce, J., 1989. Growth of inhibitory innervation in a lobster muscle. J. Morphol. 199 (2), 197–205.

Grefsrud, E.S., Glover, K., Grøsvik, B.E., Husa, V., Karlsen, Ø., Kristiansen, T.S., Kvamme, B.O., Mortensen, S., Samuelsen, O.B., Stien, L.H., Svåsand, T., 2018. Risk Report Norwegian Fish Farming 2018.

Grefsrud, E.S., Svåsand, T., Glover, K., Husa, V., others (Eds.), 2019. Risikorapport Norsk Fiskeoppdrett 2019. Fisken Og Havet, Særnr. 1-2019. Havforskningsinstituttet, Bergen. https://www.hi.no/hi/nettrapporter/fisken-og-havet-2019-5.

Hansen, B.H., Hallmann, A., Altin, D., Jenssen, B.M., Ciesielski, T.M., 2017. Acute hydrogen peroxide (H2O2) exposure does not cause oxidative stress in late-copepodite stage of Calanus finmarchicus. J. Toxicol. Environ. Health, Part A 80 (16–18), 820–829.

Hudon, C., 1987. Ecology and growth of postlarval and juvenile lobster, Homarus americanus, off Iles de la Madeleine (Quebec). Can. J. Fish. Aquat. Sci. 44 (11), 1855–1869.

Hughes, J.T., Shleser, R.A., Tchobanoglous, G., 1974. A rearing tank for lobster larvae and other aquatic species. Progress. Fish Cult. 36 (3), 129–132.

Johnson, S.C., Bravo, S., Nagasawa, K., Kabata, Z., Hwang, J., Ho, J., Shih, C.T., 2004. A review of the impact of parasitic copepods on marine aquaculture. Zool. Stud. 43 (2), 229–243.

Lawton, P., Lavalli, K.L., 1995. Postlarval, juvenile, adolescent, and adult ecology. In: The Biology of the Lobster, Homarus Americanus. Academic Press Inc., New York, NY.

Lyons, M.C., Wong, D.K.H., Page, F.H., 2014. Degradation of hydrogen peroxide in seawater using the anti-sea louse formulation Interox® Paramove™50. Can. Tech. Rep. Fish. Aquat. Sci. 3080, 15.

Medina, M., Barata, C., Telfer, T., Baird, D.J., 2004. Assessing the risks to zooplankton grazers of continuous versus pulsed cypermethrin exposures from marine cage aquaculture. Arch. Environ. Contam. Toxicol. 47 (1), 67–73.

Mehrtens, F., Stolpmann, M., Buchholz, F., Hagen, W., Saborowski, R., 2005. Locomotory activity and exploration behaviour of juvenile European lobsters (Homarus gammarus) in the laboratory. Mar. Freshw. Behav. Physiol. 38 (2), 105–116.

Parsons, A.E., Escobar-Lux, R.H., Sævik, P.N., Samuelsen, O.B., Agnalt, A.L., 2020. The Impact of Anti-sea Lice Pesticides, Azamethiphos and Deltamethrin, on European Lobster (Homarus gammarus) Larvae in the Norwegian Marine Environment. Environmental Pollution, p. 114725.

Rasmussen, J.J., Nørum, U., Jerris, M.R., Wiberg-Larsen, P., Kristensen, E.A., Friberg, N., 2013. Pesticide impacts on predator–prey interactions across two levels of organisation. Aquat. Toxicol. 140, 340–345.

Refseth, G.H., Sæther, K., Drivdal, M., Nøst, O.A., Augustine, S., Camus, L., Tassara, L., Agnalt, A.-L., Samuelsen, O.B., 2017. Miljørisiko Ved Bruk Av Hydrogenperoksid. Økotoksikologisk Vurdering Og Grenseverdi for Effekt. CRC Press, Boca Raton, FL, ISBN 1-56032-091-5. https://www.fhf.no/prosjekter/prosjektbasen/901249/.Assessment.

Sars, G.O., 1874. Om Hummerens Postembryonale Udvikling.

Smit, M.G., Ebbens, E., Jak, R.G., Huijbregts, M.A., 2008. Time and concentration dependency in the potentially affected fraction of species: the case of hydrogen peroxide treatment of ballast water. Environ. Toxicol. Chem.: Int. J. 27 (3), 746–753.

Taormina, B., Di Poi, C., Agnalt, A.L., Carlier, A., Desroy, N., Escobar-Lux, R.H., D'eu, J. F., Freytet, F., Durif, C.M., 2020. Impact of magnetic fields generated by AC/DC submarine power cables on the behavior of juvenile European lobster (Homarus gammarus). Aquat. Toxicol. 220, 105401.

Thomassen, J.M., 1993. A new method for control of salmon lice. Fish Farming Technology 74, 281–289.

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 (3), 171–194.

Treasure, J.W., Gran, A., Davi, P.J., 2000. Physical constraints of bath treatments of Atlantic salmon (Salmo salar) with a sea lice burden (Copepoda: caligidae). Contrib. Zool. 69 (1–2), 129–136.

Urbina, M.A., Cumillaf, J.P., Paschke, K., Gebauer, P., 2019. Effects of pharmaceuticals used to treat salmon lice on non-target species: evidence from a systematic review. Sci. Total Environ. 649, 1124–1136.

Valenzuela-Muñoz, V., Gallardo-Escárate, A., Sáez-Vera, C., Garcés, F., Bonfatti, J., Gallardo-Escárate, C., 2020. More than Bubbles: in Vivo Assessment and Transcriptome Modulation of Caligus Rogercresseyi and Atlantic Salmon Exposed to Hydrogen Peroxide. PARAMOVE®), Aquaculture, p. 735170.

van der Meeren, G.I., 2001. Effects of experience with shelter in hatchery-reared juvenile European lobsters Homarus gammarus. Mar. Freshw. Res. 52 (8), 1487–1493.

Van Geest, J.L., Burridge, L.E., Fife, F.J., Kidd, K.A., 2014. Feeding response in marine copepods as a measure of acute toxicity of four anti-sea lice pesticides. Mar. Environ. Res. 101, 145–152.

Vollset, K.W., Barlaup, B.T., Mahlum, S., Bjørn, P.A., Skilbrei, O.T., 2016. Estimating the temporal overlap between post-smolt migration of Atlantic salmon and salmon lice infestation pressure from fish farms. Aquaculture Environment Interactions 8, 511–525.