Effect of sea lice chemotherapeutant hydrogen peroxide on the photosynthetic characteristics and bleaching of the coralline alga *Lithothamnion soriferum*

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The proliferation of sea lice (*Lepeophtheirus salmonis*) represents a major challenge for the salmonid aquaculture industry in Norway. Hydrogen peroxide (*H₂O₂*) is a chemotherapeutant frequently used on Norwegian farms, however, its toxicity to non-target benthic species and habitats remains poorly understood. Maerl beds are constructed by the accumulation of non-geniculate coralline algae and provide important ecological functions. Due to the rapid expansion of aquaculture in Norway and the continued use of *H₂O₂* as an anti-sea lice treatment, it is crucial to understand the impact of *H₂O₂* on the physiology of maerl-forming species. The effects of a 1 h exposure to *H₂O₂* on the photophysiology and bleaching of the coralline alga *Lithothamnion soriferum* were examined here through a controlled time-course experiment. PAM fluorimetry measurements showed that *H₂O₂* concentrations ≥ 200 mg l⁻¹ negatively affected photosystem II (PSII) in thalli immediately after exposure, which was observed through a significant decline in maximum photochemical efficiency (*Fₒ/Fm*) and relative electron transport rate (*rETR*). The negative effects on PSII induced by oxidative stress, however, appear to be reversible, and full recovery of photosynthetic characteristics was observed 48 h to 28 days after exposure to 200 mg *H₂O₂* l⁻¹ and 2000 mg *H₂O₂* l⁻¹, respectively. At 28 days after exposure, there was evidence of two- to four-times more bleaching in thalli treated with concentrations ≥ 200 mg *H₂O₂* l⁻¹ compared to those in the control. This indicates that despite the recovery of PSII, persistent damages can occur on the structural integrity of thalli, which may considerably increase the vulnerability of coralline algae to further exposure to *H₂O₂* and other chemical effluents from salmonid farms.

1. Introduction

Aquaculture is the fastest growing food-producing sector in the world. Atlantic salmon (*Salmo salar* L.) is one of the most important species counting for 4.5% of global seafood supply, with 2.4 million tonnes harvested in 2018 (FAO, 2020). Norway is the world’s largest producer of Atlantic salmon with 1.4 million tonnes in 2019, which represented around 50% of the total production (https://www.ssb.no/en/fiskeoppdrett) (MowI, 2020).

The proliferation of sea lice (*Lepeophtheirus salmonis*) represents a significant challenge for the salmon aquaculture industry in Norway (Costello, 2009; Liu and Bjelland, 2014; Grefsrud et al., 2021). These parasitic copepods thrive in fish-farming localities and infect surrounding ecosystems, with potentially detrimental consequences for wild salmon and trout populations (Torrissen et al., 2013; Kristoffersen et al., 2018). In order to regulate sea lice infestations, the industry relies on the use of chemotherapeutics – administrated as an in-feed drug (diflubenzuron, emamectin-benzoate, teflubenzuron) or dissolved in the water (hydrogen peroxide (*H₂O₂*), azamethiphos, deltamethrin) – or on other non-chemical approaches (e.g. mechanical and thermal treatments, use of cleaner fish) (Overty et al., 2019; Grefsrud et al., 2019; Directorate of Fisheries, 2020). Hydrogen peroxide was introduced in Norway in 1993 as an antiparasitic drug (Thomassen, 1993). From 2009, escalating doses of hydrogen peroxide were used to compensate for the increased resistance in sea lice to this treatment, reaching 43 246 tonnes in 2015 (Adams et al., 2012; Grontvedt et al., 2015; Hannisdal et al.,...
2020). Since 2016, a decline in the amount of hydrogen peroxide used was observed, explained by the development of resistance in sea lice and the implementation of non-chemical approaches (Overton et al., 2019; Helgesen et al., 2018). Despite this decrease, \( \text{H}_2\text{O}_2 \) remains the main chemotherapeutant used in Norway, with 5084 tonnes used in 2020 (Directorate of Fisheries, 2020). Furthermore, regional differences exist in the use of \( \text{H}_2\text{O}_2 \) treatments in Norway and the amounts of treatments applied are still high in many production areas (Remen and Sæther, 2018; Overton et al., 2019).

Hydrogen peroxide is generally administered as a 20 to 30 min bath treatment – either directly at the farm or using a well-boat – at concentrations of 1500 to 2100 mg l\(^{-1}\), depending on water temperature (www.felleskatologen.no). In order to administrate \( \text{H}_2\text{O}_2 \) at the farm, the sea cage is surrounded by a tarpaulin and its volume is temporarily reduced. After treatment, the tarpaulin is removed and the \( \text{H}_2\text{O}_2 \) is released to the surrounding seawater. Since \( \text{H}_2\text{O}_2 \) is slightly heavier than the surrounding seawater, the effluent plume can sink under unfavorable conditions and reach the seabed a few minutes after release (Refseth et al., 2019). Through a field investigation in the vicinity of a single farm, Andersen and Hagen (2016), measured up to 724 mg \( \text{H}_2\text{O}_2 \) l\(^{-1}\) (43% of the treatment concentration) on the sea floor at 60 m depth, 8 min after a discharge. Refseth et al. (2016) estimated that 50% of the initial treatment dose (800 mg l\(^{-1}\)) may reach the seafloor under fish cages and that these higher concentrations may persist 5 to 10 h due to the low horizontal transport compared to the surface layers. The half-life of \( \text{H}_2\text{O}_2 \) in seawater is approximately 7 at 15 °C but may reach up to 28 days, depending on seawater temperature, initial concentration and organic content of the seawater (Bruno and Raynard, 1994; Lyons et al., 2014).

Hydrogen peroxide is a powerful oxidizing compound and produces free radicals, which can lead to oxidative damage to proteins and membrane lipids and can induce DNA damage (Valanavides et al., 2006; Torrisen et al., 2013; El-Bibany et al., 2014). Although \( \text{H}_2\text{O}_2 \) is an effective antiparasitic agent (Bruno and Raynard, 1994), it is also toxic for several non-target taxa (Munn et al., 2003; Urbina et al., 2019), especially crustaceans – such as European lobster (Homarus gammarus) – median lethal concentration after 1-h exposure (1-h LC\(_{50}\) = 177–737 mg l\(^{-1}\)) (Escobar-Lux et al., 2020), Northern krill (Meganyctiphanes norvegica; 1-h LC\(_{50}\) = 35.2 mg l\(^{-1}\)) (Escobar-Lux and Samuelsen, 2020), Northern shrimp (Pandalus borealis; 24-h LC\(_{50}\) = 2.7 mg l\(^{-1}\)) (Bechmann et al., 2019), copepods (Van Geest et al., 2014), Calanus spp.; 1-h LC\(_{50}\) = 421.4 mg l\(^{-1}\), Escobar-Lux et al. (2019) – molluscs (Potamopyrgus antipodarum; 24-h LC\(_{50}\) = 37.5 mg l\(^{-1}\)) (Oplinger and Wagner, 2015) and polychaetes (Capitella sp; 1-h LC\(_{50}\) = 1227 mg l\(^{-1}\), and Ophryotrocha spp.; 1-h LC\(_{50}\) = 296 mg l\(^{-1}\)) (Fang et al., 2018). In comparison, macroalgae exhibit a high interspecific variability in their response to \( \text{H}_2\text{O}_2 \) (Dummermuth et al., 2003). Although macroalgae are able to produce \( \text{H}_2\text{O}_2 \) at low concentrations as a normal part of their metabolism in order to dissipate energy at high light intensities (Collén and Pedersen, 1996; Rautenberger et al., 2013; Burdett et al., 2014) – the accumulation of \( \text{H}_2\text{O}_2 \) may negatively affect the photosynthetic apparatus due to the destruction of lipids, proteins and nucleic acids, leading to cell death (Asada and Takahashi, 1987; Karpinski et al., 1999; Dummermuth et al., 2003). In the green alga Ulva rigida, the photosynthetic process was affected by \( \text{H}_2\text{O}_2 \) concentrations of 102 mg l\(^{-1}\) and was totally inhibited at 3400 mg l\(^{-1}\) (Collén and Pedersen, 1996). Haugland et al. (2019) raised major concern about the potential negative effects of \( \text{H}_2\text{O}_2 \) treatment on the photosynthetic capacity and high sensitivity of sugar kelp Saccharina latissima. Using an exposure of 1 h, they reported a LC\(_{50}\) for \( \text{H}_2\text{O}_2 \) of 80.7 mg l\(^{-1}\) for juvenile S. latissima, which represents less than 5% of the recommended concentration for \( \text{H}_2\text{O}_2 \) bath treatments (Haugland et al., 2019).

Macroalgae have been widely used in ecological assessments (Stevenson, 2014; D’Archino and Piazz, 2021) due to their important ecological functions (Steneck et al., 2002) and their sensitivity to stress (Thibaut et al., 2014; Piazz and Ceccherelli, 2020). Among macroalgal habitats, maerl beds represent complex and productive coastal ecosystems constructed by the accumulation of non-geniculate coralline algae (Riosmena-Rodriguez et al., 2016). These coralline algae are important ecosystem engineers that provide three-dimensional structure for a diverse fauna and flora (Nelson, 2009; Sciberras et al., 2009; Peña et al., 2014; Schubert et al., 2020). Maerl beds are particularly threatened by a rising number of global and local anthropogenic pressures – such as climate change (e.g. ocean acidification and warming) (Martin and Gattuso, 2009; Cornwall et al., 2019), destructive fishing practices (Bernard et al., 2019; Hall-Spencer et al., 2008), sewage discharge (Grall and Glémarec, 1997) and aquaculture (Steller et al., 2003; Wilson et al., 2004; Hall-Spencer et al., 2006; Sanz-Lázaro et al., 2011; Aguado-Giménez and Ruiz-Fernández, 2012) – and are listed as declining habitat by the Oslo-Paris Convention (OSPAR; Hall-Spencer et al., 2010). The decline of this habitat may have drastic ecological and economic consequences, as many commercial species rely on maerl beds to fulfill their life cycle (Hall-Spencer, 1998; Kamenos et al., 2004). In Norway, aquaculture is projected to expand considerably in the near future, especially in the North, where most of the maerl beds are located.

To date, studies examining the effect of salmon fish farms on maerl beds are scarce and mainly concentrate on the impact of organic deposition on the associated diversity (Hall-Spencer et al., 2006) and on the physiological response of coralline algae (Legrand et al., 2021). Therefore, it is essential to provide new understanding about the impact of \( \text{H}_2\text{O}_2 \) treatment on the physiology of maerl-forming species in order to better inform environmental risk assessments.

In this context, this study examined the toxicity of a 1 h exposure to environmentally relevant concentrations of \( \text{H}_2\text{O}_2 \) on the photosynthetic characteristics and the bleaching of the free-living coralline alga Lithothamnion soriferum Kjellman, 1883. Thalli were exposed to five concentrations of \( \text{H}_2\text{O}_2 \) in order to estimate the threshold concentration beyond which physiological processes are affected. The chlorophyll-a fluorescence induction pulse amplitude modulation (PAM) method was used to measure the photosynthetic response of \( L. \) soriferum at 6 time points after exposure – from 1 h to 28 days – and to examine the potential for physiological recovery of thalli. PAM method offers the advantage to be non-destructive for coralline algae (Burdett et al., 2012) and represents an effective way to measure oxidative stress affecting the photosynthetic process in macroalgae (Dummermuth et al., 2003). We hypothesize that the oxidative stress induced by exposure to high concentrations of \( \text{H}_2\text{O}_2 \) may negatively affect the photosynthetic capacity of coralline algae. Bleaching of thalli was also expected due to the degradation of photosynthetic pigments, as previously evidenced for the red alga Polysiphonia arctica (Dummermuth et al., 2003).

2. Material and methods

2.1. Sample collection

Healthy thalli (pink color on the whole thallus) of \( L. \) soriferum were collected in September 2020 from 11 m depth at Skårasund, Vestland County, Norway (60°8.30.2’N, 5°9.55.9’E) using a hand-held dredge (width: 0.5 m; height: 0.5 m; net: 1 m long). Samples were transported to the Institute of Marine Research (IMR) Austevoll Research Station, Norway, and epiphytes were removed. Thalli were randomly assigned to twenty 15 l flow-through tanks and acclimated for six weeks at ambient temperature (9.2 ± 0.3 °C), salinity (35.4 ± 0.2) and light (30 μmol photons m\(^{-2}\) s\(^{-1}\)). Five thalli were placed in each tank, which represents approximately 15 g of maerl per tank.

2.2. \( \text{H}_2\text{O}_2 \) treatment

Hydrogen peroxide stock solutions were prepared using commercial \( \text{H}_2\text{O}_2 \) (Nemona, 49.5% \( \text{H}_2\text{O}_2 \); Akzo Nobel, Pulp and Performance Chemicals, AB Sweden) diluted in filtered seawater. Following lab acclimation, \( L. \) soriferum thalli were exposed to five concentrations of
2.3. Pulse amplitude modulated fluorometry (PAM)

Chlorophyll a fluorescence measurement was made using a Diving-PAM II fluorometer and WinControl-3 software (Walz GmbH, Effeltrich, Germany). All measurements were made underwater in each experimental tank.

2.3.1. \( F_v/F_m \) measurements

Maximum quantum yield represents the maximum photochemical efficiency of energy transfer to the photosystem II (PSII) reaction centers and corresponds to the ratio of variable to maximal fluorescence (\( F_v/F_m \)) (Burdett et al., 2012). \( F_v/F_m \) was measured in maerl thalli dark-acclimated for 5 min prior to measurement (Burdett et al., 2012), and calculated as:

\[
F_v/F_m = (F_m - F_o)/F_m
\]

with \( F_v \), \( F_m \) and \( F_o \) the variable, minimum and maximum fluorescence yields, respectively. Fiber optic probe (standard PAM fiber optic probe; 5 mm) was placed approximately 1 mm away from the sample at an angle so the thalli were not shaded (Wilson et al., 2004). \( F_v/F_m \) was measured in each experimental tank just before exposure to \( \mathrm{H}_2\mathrm{O}_2 \) (T0) and 1 h, 24 h, 48 h, 7 days, 14 days and 28 days after completing the exposure (post-exposure). Measurements were carried out on five thalli per tank and three measurements were made on each thallus. Results were averaged per tank in order to avoid pseudo-replication.

2.3.2. Rapid light curves (RLC)

The RLCs were measured on 3 thalli per tank using the light curve function of the Diving-PAM II. Actinic light illumination was increased in eleven incremental 10 s intensities of photosynthetic active radiation (PAR 0, 26, 46, 66, 92, 128, 193, 290, 428, 641 and 838 \( \mu \text{mol photons m}^{-2} \text{s}^{-1} \)) each followed by a saturating light pulse. Results were averaged per tank in order to avoid pseudo-replication. The effective quantum yield \( (Y(\text{II})) \) and the relative electron transport rate \( (\text{reTR}; \mu \text{mol electrons m}^{-2} \text{s}^{-1}) \) of PSII were measured under each light intensity according to (Schreiber et al., 1995):

\[
Y(\text{II}) = (F_{m} - F_{i})/F_{m}
\]

with \( F_m \) the maximum chlorophyll fluorescence under actinic light, and \( F_i \) the steady-state fluorescence level under non-saturating illumination (Genty et al., 1989) and:

\[
\text{reTR} = Y(\text{II}) \times \text{PAR} \times 0.15
\]

with PAR the irradiance and 0.15 the fraction of chlorophyll \( \alpha \) (15%) associated with PSII in red algae (Goldstein et al., 1992; Figueroa et al., 2003).

The light-dependence of electron transport was determined for each RLC by fitting \( \text{reTR} \) values to PAR using the model of Hennige et al. (2008) modified from Jassby and Platt (1976), via least squares non-linear regression to derive the ascending slopes at limiting irradiances (\( \alpha \)) and the maximal electron transport rate \( (\text{reTR}_{\text{max}}) \):

\[
\text{reTR} = \text{reTR}_{\text{max}} \times [1 - \exp(-\alpha \times \text{PAR} / \text{reTR}_{\text{max}})]
\]

with \( \alpha \) the initial slope at limiting irradiances and \( \text{reTR}_{\text{max}} (\mu \text{mol electrons m}^{-2} \text{s}^{-1}) \) the maximum relative electron transport rate. The minimal saturating intensity \( (E_{0}; \mu \text{mol photons m}^{-2} \text{s}^{-1}) \) was calculated as the ratio of \( \text{reTR}_{\text{max}} \) to \( \alpha \) (Hill et al., 2004).

2.4. Bleaching measurements

At the end of the experiment, two branch tips of approximately 1 cm length were randomly selected from each of the five thalli present in each experimental tanks (total of ten fragments per tank; Supplementary...
Material, Fig. S.1). Samples were quickly dried on paper to remove excess water and then photographed. Bleached surface of corallines was measured from each picture using ImageJ software (Schneider et al., 2012). Percentage of bleaching was calculated as the ratio between bleached area and the total surface of collected branches. Results were averaged per tank.

2.5. Statistical analyses

Dose-response relationships were determined using the package drc (Ritz et al., 2015) for R software (R Core Team, 2020). Median effective concentrations (1-h EC50) for Fv/Fm alpha and rETRmax were calculated based on observations from 1 h post-exposure and were expressed with their 95% confidence intervals (CI). The models that gave the best fit were selected and 1-h EC50 was estimated for Fv/Fm using the three-parameter log-logistic model (LL3) and by model averaging LL3 and the 3-parameter Weibull 1 model (W1.3) (IC value < 10 difference) for alpha and rETRmax.

Assumptions of normality (Shapiro test) and homogeneity of variances (Bartlett test) were tested prior to statistical analyses and data was Box-Cox transformed when needed (Box and Cox, 1964). The effect of H2O2 treatment (5 levels: 0 (control), 2, 20, 200 and 2000 mg l−1), time (7 levels: T0, 1 h, 24 h, 48 h, 7 days, 14 days and 28 days) and their interaction on chlorophyll a fluorescence parameters (Fv/Fm, α, ETR, rETRmax) was analyzed using a two-way repeated measures ANOVA. Hydrogen peroxide treatment and time were considered as fixed factors and experimental tanks as a random factor (repeated measures over time). When significant, differences between H2O2 treatments were explored at each time interval using Tukey’s HSD post hoc comparisons (package emmeans) (Lenth et al., 2021). The effect of H2O2 treatment on bleaching was analyzed using one-way ANOVA. Differences among treatments were explored with Tukey’s HSD post hoc comparisons. For each parameter, the lowest observed effect concentration (LOEC) was determined as the lowest tested concentration that was significantly lower than control (Tukey’s HSD, p < 0.05).

3. Results

3.1. Chlorophyll fluorescence

The mean Fv/Fm ratio for L. soriferum in the control group (0 mg H2O2 l−1) was 0.62 ± 0.01 (± SE) and remained stable throughout the experiment (Fig. 2). No mortality (Fv/Fm of zero) occurred in the control group. The Fv/Fm ratio was significantly affected by H2O2 treatment, time and their interaction (Table 1). At 1 h post-exposure, the Fv/Fm ratio dropped by 20% and 73% in thalli exposed to 200 and 2000 mg H2O2 l−1, respectively, compared to thalli in the control. The Fv/Fm ratio measured in thalli in these treatment groups (200 and 2000 mg H2O2 l−1) then gradually increased throughout the experiment but remained significantly lower than for thalli in the control at 24 h post-exposure (200 and 2000 mg H2O2 l−1) and 14 days (2000 mg H2O2 l−1) (Tukey’s HSD, p < 0.05) (Fig. 2). There was no significant difference in the Fv/Fm ratio of thalli exposed to 2 and 20 mg H2O2 l−1 compared to the control (Tukey’s HSD, p > 0.05). The 1-h EC50 (with 95% CIs) for H2O2, based on the Fv/Fm ratio in L. soriferum thalli, was 881 mg l−1 (649–1113 mg l−1).

Hydrogen peroxide treatment altered the relative electron transport rates (rETR) in L. soriferum thalli, with the lowest rETR recorded in groups exposed to 2000 mg l−1 (Fig. 3). The α and rETRmax values were significantly affected by H2O2 treatment, time and their interaction, while ETR values were only affected by H2O2 treatment (Table 1). At 1 h post-exposure, the rETRmax and α values for thalli exposed to 2000 mg H2O2 l−1 dropped significantly compared to the control (Table 2, Fig. 3) whereas the ETR values significantly increased. The rETRmax and α values for thalli in this treatment group remained significantly lower than the control for 48 h and 7 days post-exposure, respectively, while the ETR values were affected by H2O2 treatment at 2000 mg l−1 up to 14 days. The α, ETR and rETRmax values did not vary significantly after exposure to H2O2 concentrations of 2, 20 and 200 mg l−1. The 1-h EC50 (with 95% CIs) calculated for H2O2 based on α and rETRmax measurements in L. soriferum thalli, were 268 mg l−1 (0-618 mg l−1) and 532 mg l−1 (11-1053 mg l−1), respectively.

3.2. Bleaching

Exposure to H2O2 caused bleaching of the L. soriferum thalli (Supplementary Material, Fig. S.1). Twenty-eight days after H2O2 treatment, bleaching was significantly higher in thalli exposed to concentrations of 200 and 2000 mg H2O2 l−1 (28% and 63%, respectively), compared to thalli in the control group (Fig. 4; ANOVA, p < 0.001, Tukey test; p < 0.01). However, no significant difference in bleaching of thalli was evident between the control (14%) and exposure to 2 mg H2O2 l−1 (14%) and 20 mg H2O2 l−1 (19%) treatment groups.

4. Discussion

The present study provides new and important information about the effect of H2O2 treatment, commonly used as a sea lice chemothertapent in salmon farming, on the photophysiology of the coralline alga L. soriferum. A clear negative impact of H2O2 was observed on several photosynthetic characteristics of coralline algae immediately after exposure to concentrations of 200 mg l−1 and above. Despite this
Table 1
Results of two-way repeated measures ANOVA performed on \( \frac{F_v}{F_m} \) and \( \alpha \), \( E_k \) and \( E_{\text{TR}}^{\text{max}} \) values calculated from rapid light curves (RLCs) before (T0) and 1 h, 24 h, 48 h, 7 days, 14 days and 28 days after exposure to different concentrations of \( \text{H}_2\text{O}_2 \) (0, 2, 20, 200 and 2000 mg l\(^{-1}\)). \( n = 4 \). df: degree of freedom. Significant values (\( p < 0.05 \)) are in bold.

<table>
<thead>
<tr>
<th></th>
<th>df</th>
<th>( \frac{F_v}{F_m} )</th>
<th>( p )</th>
<th>( \alpha )</th>
<th>( p )</th>
<th>( E_k )</th>
<th>( p )</th>
<th>( E_{\text{TR}}^{\text{max}} )</th>
<th>( p )</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \text{H}_2\text{O}_2 ) treatment</td>
<td>6</td>
<td>130.2</td>
<td>&lt; 0.001</td>
<td>31.3</td>
<td>&lt; 0.001</td>
<td>6.6</td>
<td>0.003</td>
<td>5.4</td>
<td>0.007</td>
</tr>
<tr>
<td>Time</td>
<td>4</td>
<td>118.9</td>
<td>&lt; 0.001</td>
<td>7.6</td>
<td>&lt; 0.001</td>
<td>1.7</td>
<td>0.136</td>
<td>12.2</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>( \text{H}_2\text{O}_2 \times \text{Time} )</td>
<td>24</td>
<td>44.6</td>
<td>&lt; 0.001</td>
<td>2.4</td>
<td>0.001</td>
<td>0.8</td>
<td>0.781</td>
<td>5.9</td>
<td>&lt; 0.001</td>
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</table>

Fig. 3. Rapid light curves (RLCs) generated from \( \text{L. soriferum} \) thalli exposed for 1 h to concentrations of \( \text{H}_2\text{O}_2 \) of 0, 2, 20, 200 and 2000 mg l\(^{-1}\). RLCs were obtained before exposure (T0) and 1 h, 24 h, 48 h, 7 days, 14 days and 28 days after exposure. Mean rETR are plotted with standard errors. (\( n = 4 \)).

Fig. 3. Rapid light curves (RLCs) generated from \( \text{L. soriferum} \) thalli exposed for 1 h to concentrations of \( \text{H}_2\text{O}_2 \) of 0, 2, 20, 200 and 2000 mg l\(^{-1}\). RLCs were obtained before exposure (T0) and 1 h, 24 h, 48 h, 7 days, 14 days and 28 days after exposure. Mean rETR are plotted with standard errors. (\( n = 4 \)).
α, E and ETR calculated from rapid light curves (RLCs) of *L. soriferum* before (T0) and 1 h, 24 h, 48 h, 7 days, 14 days and 28 days after exposure to different concentrations of H$_2$O$_2$ (0, 2, 20, 200 and 2000 mg l$^{-1}$). Mean values ± SE. Letters indicate statistical differences between treatments (Tukey test; p < 0.05). (* = 0.05; ** = 0.01; *** = 0.001).

Table 2

<table>
<thead>
<tr>
<th>Time (days)</th>
<th>Control (0 mg H$_2$O$_2$)</th>
<th>2 mg H$_2$O$_2$</th>
<th>20 mg H$_2$O$_2$</th>
<th>200 mg H$_2$O$_2$</th>
<th>2000 mg H$_2$O$_2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>T0</td>
<td>0.032 ± 0.002</td>
<td>0.048 ± 0.002</td>
<td>0.055 ± 0.003</td>
<td>0.065 ± 0.003</td>
<td>0.074 ± 0.003</td>
</tr>
<tr>
<td>1h</td>
<td>0.036 ± 0.002</td>
<td>0.043 ± 0.002</td>
<td>0.063 ± 0.003</td>
<td>0.077 ± 0.003</td>
<td>0.086 ± 0.003</td>
</tr>
<tr>
<td>24h</td>
<td>0.045 ± 0.003</td>
<td>0.054 ± 0.003</td>
<td>0.076 ± 0.003</td>
<td>0.091 ± 0.003</td>
<td>0.101 ± 0.003</td>
</tr>
<tr>
<td>48h</td>
<td>0.061 ± 0.004</td>
<td>0.070 ± 0.004</td>
<td>0.094 ± 0.004</td>
<td>0.109 ± 0.004</td>
<td>0.124 ± 0.004</td>
</tr>
<tr>
<td>7 days</td>
<td>0.091 ± 0.002</td>
<td>0.100 ± 0.002</td>
<td>0.124 ± 0.002</td>
<td>0.140 ± 0.002</td>
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</tr>
<tr>
<td>14 days</td>
<td>0.133 ± 0.003</td>
<td>0.142 ± 0.003</td>
<td>0.167 ± 0.003</td>
<td>0.183 ± 0.003</td>
<td>0.200 ± 0.003</td>
</tr>
<tr>
<td>28 days</td>
<td>0.179 ± 0.003</td>
<td>0.189 ± 0.003</td>
<td>0.214 ± 0.003</td>
<td>0.231 ± 0.003</td>
<td>0.248 ± 0.003</td>
</tr>
</tbody>
</table>

Fig. 4. Percentage of bleaching measured on *L. soriferum* thalli 28 days after 1 h exposure to different concentrations of H$_2$O$_2$ (0, 2, 20, 200 and 2000 mg l$^{-1}$). Mean values ± SE. Letters indicate statistical differences between treatments (Tukey test; p < 0.05). (* = 0.05; ** = 0.01; *** = 0.001).

impact, the photosynthetic apparatus of *L. soriferum* gradually recovered throughout the experiment, suggesting efficient repairing mechanisms of PSII. At the 28 days post-exposure period, thalli that had been exposed to 200 and 2000 mg l$^{-1}$ showed significant bleaching, which indicates that H$_2$O$_2$ may have impacted other metabolic functions not revealed by PAM fluorometry. If these laboratory experiments are indicative of processes in the vicinity of salmon fish farms, coralline algae exposed to H$_2$O$_2$ could indeed be harmed, and be particularly vulnerable to further exposure to this compound and to other chemical effluents from salmon fish farms.

4.1. Photosynthetic characteristics in *L. soriferum* using PAM

Chlorophyll fluorescence analysis has become a powerful non-invasive, non-destructive technique, widely used to investigate the photosynthetic characteristics in coralline algae, particularly under stress conditions (Häder et al., 1996; Wilson et al., 2004; Harrington et al., 2005; Burdett et al., 2015; Sordo et al., 2020). Maximum quantum yield (F$_{V}$/F$_{M}$) in red algae varies between taxa but is generally considered to be approximately 0.5–0.6 (Dring et al., 1996). In the temperate coralline alga *Lithothamnion glaciale*, Burdett et al. (2012) reported F$_{V}$/F$_{M}$ of around 0.6, which is consistent with the value of 0.62 obtained in our study for *L. soriferum* maintained under control conditions (0 mg H$_2$O$_2$ l$^{-1}$). Photosynthetic parameters α and rETR$\text{max}$, calculated from RLCs in the control were 0.037 and 1.37 μmol electrons m$^{-2}$ s$^{-1}$, respectively, and appeared to be lower than those measured in the lab in *L. glaciale* (0.12 and 3.23 μmol electrons m$^{-2}$ s$^{-1}$) (Burdett et al., 2012). This may be explained by the experimental set-up, as thalli in our study were maintained at light intensities of 30 μmol photons m$^{-2}$ s$^{-1}$ (natural conditions in October at Austevoll, Norway), which were 3-times lower than the light intensities used for *L. glaciale* (90 μmol photons m$^{-2}$ s$^{-1}$, average light intensities in Loch Swean, Scotland) (Burdett et al., 2012). The E$_{0}$ of 39.2 μmol photons m$^{-2}$ s$^{-1}$ measured in the control is consistent with the value of 35.5 μmol photons m$^{-2}$ s$^{-1}$ obtained for *L. glaciale* in the lab (Burdett et al., 2012). Coralline algae generally exhibit lower E$_{0}$ values than other photosynthetic organisms, which indicates that these species are well adapted to low irradiance (Kühl et al., 2001; Payri et al., 2001; Egilsdottir et al., 2016). Low-light adaptation of coralline algae may play a key role in the distribution of *L. soriferum* to high latitudes. In the eastern North Atlantic, *L. soriferum* has been described in shallow coastal waters from ca. 70°N to ca. 53°N.
4.2. Short-term effects of H$_2$O$_2$ on the photosynthetic characteristics of L. soriferum

The effect of H$_2$O$_2$ stress on macroalgae maximum quantum yield of photosynthesis has been investigated in several studies, suggesting a wide range of responses among taxa, mainly explained by differences in their antioxidative defense systems (Collen and Pedersen, 1996; Aguilara et al., 2002; Dummermuth et al., 2003). In the green alga Ulva rigida, F$_{v}$/F$_{m}$ dropped significantly at H$_2$O$_2$ concentrations above 34 mg l$^{-1}$ (Collen and Pedersen, 1996). Within Rhodophyta, Palmatia palmata also exhibited a high sensitivity to H$_2$O$_2$, with F$_{v}$/F$_{m}$ reduced by 50% directly after a 30-min bath treatment at 34 mg H$_2$O$_2$ l$^{-1}$ (Dummermuth et al., 2003). In the coralline alga L. soriferum, the LOEC of 200 mg l$^{-1}$ and the 1-h EC$_{50}$ of 881 mg l$^{-1}$ measured on F$_{v}$/F$_{m}$ indicate that this species had a greater tolerance towards H$_2$O$_2$ exposure than most of macroalgae studied to date (Collen and Pedersen, 1996; Dummermuth et al., 2003). Examination of RLGS confirmed the higher tolerance of L. soriferum photosynthetic efficiency to H$_2$O$_2$ exposure, as no significant effect was evidenced on α, E$_2$ and RETR$_{max}$ for concentrations of 2, 20 and 200 mg H$_2$O$_2$ l$^{-1}$. In juvenile sugar kelp S. latissima, an immediate effect was observed after exposure to H$_2$O$_2$ with a drop in photosynthetic capacity (α) and efficiency (P$_{max}$) for thalli exposed to concentrations of 85 mg H$_2$O$_2$ l$^{-1}$ and above (Haugland et al., 2019). The 1-h EC$_{50}$ values estimated for H$_2$O$_2$ based on α and P$_{max}$ measured in S. latissima, were 35.4 mg l$^{-1}$ and 27.8 mg l$^{-1}$, respectively (Haugland et al., 2019), while the 1-h EC$_{50}$ values estimated here for L. soriferum, based on α and RETR$_{max}$, were 268 mg l$^{-1}$ and 352 mg l$^{-1}$, respectively. As most of the effects of H$_2$O$_2$ were observed at concentrations between 200 and 2000 mg l$^{-1}$, including an intermediate concentration (e.g. 800 mg l$^{-1}$) would have helped to pinpoint more accurately the concentration at which effects occur. Despite this, higher tolerance to H$_2$O$_2$ stress was observed here in L. soriferum, compared to other macroalgae species, which may be related to differences in enzyme and non-enzymatic mechanisms governing the antioxidative potential (Aguilara et al., 2002; Dummermuth et al., 2003). Plant structure may also play an important role, as it is possible that the uptake of H$_2$O$_2$ is reduced through heavily calcified layers of coralline algae, with a lower impact on chloroplasts and PSII (Harrington et al., 2005).

The present results showed that exposure of thalli to H$_2$O$_2$ concentrations of 200 and 2000 mg l$^{-1}$ caused a reduction in F$_{v}$/F$_{m}$ of 20% and 73%, respectively, measured 1 h after bath treatment. Moreover, 1-h EC$_{50}$ values estimated for H$_2$O$_2$ based on α and RETR$_{max}$ underlined the sensitivity of L. soriferum to environmentally realistic H$_2$O$_2$ concentrations. The LOEC of 200 mg H$_2$O$_2$ l$^{-1}$ determined in this study represents 10% of the recommended dose for bath treatments. In the vicinity of salmon fish farms, H$_2$O$_2$ concentration on the sea floor may reach about 50% of the initial treatment dose few minutes after a discharge and may persist up to 10 h (Andersen and Hagen, 2016; Refseth et al., 2016). Therefore, exposure to H$_2$O$_2$ levels of 200 mg l$^{-1}$ and above may lead to immediate drastic consequences for the ability of L. soriferum to maintain photosynthesis. Impaired photosynthetic characteristics induced by H$_2$O$_2$ is the result of a significant oxidative stress in L. soriferum, damaging PSII, probably due to destruction of lipids, proteins and nucleic acids (Collen and Pedersen, 1996; Dummermuth et al., 2003). Maintaining photosynthesis is essential for macroalgae in order to preserve dissolved inorganic carbon uptake (Collen and Pedersen, 1996). It is commonly suggested that photosynthesis is a controller of calcification in coralline algae by providing substrate (Chisholm, 2003). Photosynthesis also plays an active role in calcification process by locally elevating internal pH, which facilitates precipitation of calcium carbonate (Borowitzka, 1984; Lee and Carpenter, 2001; Comeau et al., 2012). Therefore, reduced photosynthetic performances induced by H$_2$O$_2$ treatment is likely to affect negatively other metabolic processes, such as calcification and growth.

4.3. Prolonged effects of H$_2$O$_2$ on L. soriferum photosynthetic characteristics and bleaching

The evaluation of the effects of H$_2$O$_2$ on L. soriferum suggests that despite of impaired photosynthetic functions induced by concentrations ≥ 200 mg H$_2$O$_2$ l$^{-1}$ after a 1 h exposure period, thalli can gradually restore their photosynthetic apparatus. Recovery of L. soriferum photosynthetic ability was different depending on H$_2$O$_2$ concentration and photosynthetic parameters (e.g. F$_{v}$/F$_{m}$ fully recovered 48 h and 28 days after exposure to 200 and 2000 mg l$^{-1}$, respectively, while RETR$_{max}$ and E$_{k}$ recovered 7 days and 28 days after exposure to 2000 mg l$^{-1}$). Therefore, damages to PSII induced by oxidative stress appear to be reversible, which implies the presence of an elaborate repair system to restore PSII function. PSII repair system have been well documented in plants and algae and involves targeted reaction-center protein proteolysis and replacement of damaged core proteins, in order to reassemble new functional PSII (Nickelsen and Rengstl, 2013; Liu et al., 2019).

Active repairing of PSII is critical to restore photosynthetic activity – necessary for carbon fixation and growth – but may act as an energy-demanding process (Miyata et al., 2012). Although photosynthetic characteristics of L. soriferum thalli returned to control levels 48 h to 28 days after exposure, it is possible that H$_2$O$_2$ may have impacted other metabolic functions not revealed by PAM fluorometry.

At the 28 days post-exposure period, L. soriferum thalli exposed to H$_2$O$_2$ concentrations of 200 and 2000 mg l$^{-1}$ exhibited bleaching values two- to four-times higher than thalli from the control. In coralline algae, bleaching has been widely described as a response to environmental stressors – such as exposure to high light intensities, high temperature, low pH, desiccation and pathogens (Littler and Littler, 1998; Irving et al., 2004; Anthony et al., 2008; Martin and Gattuso, 2009; Martone et al., 2010) – and is associated with a decline in productivity (Figueredo et al., 2000). Bleaching has been commonly used as an indicator of death (Littler, 1973; Hawkins and Hartnoll, 1985; Martin and Gattuso, 2009), although it can be reversible in some situations, depending on the duration of environmental stress and the severity of damages (Figueredo et al., 2000; McCoy and Kamenos, 2015). Bleaching occurs due to the loss or degradation of photosynthetic pigments in surface tissue (McCoy and Kamenos, 2015). In the red alga Polysiphonia arctica, bleaching of thalli has also been observed after exposure to H$_2$O$_2$ and was associated with the drastic decline in protein content and most likely to the degradation of phycobiliproteins (Dummermuth et al., 2003). The phycobiliproteins are major light-harvesting pigments in marine red algae (Beer and Esbel, 1985) and their degradation in L. soriferum under high H$_2$O$_2$ stress may explain observed high bleaching levels.

Coralline algal bleaching induced by H$_2$O$_2$ (≥ 200 mg l$^{-1}$), may have drastic consequences on thalli, weakening their structural integrity (McCoy and Kamenos, 2015). In the present study, L. soriferum thalli were exposed to H$_2$O$_2$ for a single 1 h exposure period. It is likely that the persistent damages observed on the structural integrity of thalli may exacerbate their vulnerability in case of further exposure to this compound, but also to other effluents released by salmon fish farms (e.g. dissolved nutrients, organically rich waste feed and feces, other anti-parasitic therapeutics and antifouling compounds) (Carroll et al., 2003; Burridge et al., 2010). Within salmon fish farms, delousing operations generally involve simultaneous and sequential applications of pesticides in many cages and non-target species are likely to face multiple exposure to H$_2$O$_2$ over several (Grefczuk et al., 2019). Multiple exposure may lead to cumulative impacts on non-target species with even more pronounced effects on their physiology than a single exposure (Bechmann et al., 2019). Exposure of thalli to oxidative stress over repeated and longer periods may drastically increase damages on PSII, potentially inhibiting repair systems through irreversible damages on cells. The loss of structural integrity in coralline algae may also lead to increased vulnerability to natural physical disturbances (e.g. wave action, bioerosion and grazing activity) (Ragazzola et al., 2012) and other
anthropogenic pressures (e.g. ocean acidification and warming, destructive fishing practices, sewage discharge).

5. Conclusion

Hydrogen peroxide has long been described as the most environmentally friendly sea lice chemotherapeutant. Our results demonstrate, however, serious impacts of H₂O₂ treatment on the photophysiology of *L. soriferum*, which suggests that the effects of H₂O₂ require more attention in research and risk assessments than previously assumed. In the present study, the sensitivity of *L. soriferum* to H₂O₂ occurred at concentrations well below the treatment dose of 1500 to 2100 mg l⁻¹, commonly used at farms and emitted to the environment. However, the sensitivity of organisms is not only determined by the concentration of H₂O₂, but also by the exposure time (Refseth et al., 2019). Hydrogen peroxide negatively affected the photosynthetic characteristics and bleaching of *L. soriferum* after a 1 h exposure, while several studies estimated that the half-life of H₂O₂ may vary between 1 and 28 days (Beno and Raynard, 1994; Lyons et al., 2014). It is likely that prolonged and multiple exposure to high H₂O₂ concentrations will have even more adverse impacts on coralline algae than those observed in the present study. The distance up to which coralline algae may be affected depends on the dispersal of H₂O₂ in the environment after delousing operations, which relies on several factors, such as the farm size and local environmental conditions (water depth, currents, stratification etc.) (Refseth et al. 2019). General observation from different model locations is that concentrations up to about 300 mg l⁻¹ can occur up to approximately 1 km from the release site (Refseth et al., 2019), which represents a potential risk for coralline algae living in impacted zones. Since coralline algae provide major ecological functions for many faunal and floral species, their decline, induced by H₂O₂ treatment, may lead to a drastic loss in associated diversity, altering the community structure and functioning (Nelson, 2009; Schubert et al., 2020).

To date, there is limited available knowledge about the ecological significance of maerl beds in Norwegian waters and more information appears essential to understand how the overlap with salmonid farms affects this ecosystem (Taranger et al., 2015). Moreover, no commonly agreed indicators exist to estimate the quality for maerl beds, and there is little information on maerl bed species that may be used as biological indicators. The proportion (%) of live coralline coverage is generally significant of maerl beds in Norwegian waters and more information to be taken into consideration due to their potential detrimental effects on faunal communities associated with maerl beds. Therefore, it appears critical to develop other quality indicators – describing potential community shifts and the dominance of opportunistic species – in order to better understand the impact of fish farms on maerl beds and limit environmental degradation of this biogenic habitat.

CRediT authorship contribution statement


Declaration of Competing Interest

None.

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Supplementary materials


References


