



Mortality and reduced photosynthetic performance in sugar kelp *Saccharina latissima* caused by the salmon-lice therapeutant hydrogen peroxide

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ABSTRACT: Sugar kelp *Saccharina latissima* is one of the dominating kelp species on sheltered sublittoral sites along the Norwegian coastline, providing important habitats and ecosystem services. In finfish aquaculture in Norway, infections caused by salmon lice *Lepeophtheirus salmonis* are one of the biggest challenges the industry is currently facing, and presently the most-used therapeutant is hydrogen peroxide (H_2O_2). Commonly, this treatment involves bathing the fish in a solution of approximately $1700\text{ mg } H_2O_2\text{ l}^{-1}$ seawater before releasing the solution into the surrounding waters. The present study was conducted to increase the knowledge on how these H_2O_2 emissions from farm operations could impact juvenile *S. latissima*. This was achieved by determining the effects of a 1 h exposure to realistic H_2O_2 levels on *S. latissima* mortality and photosynthesis. Effects on photosynthesis were determined by incubating plants at 3 time intervals post-exposure. Toxicity potentials including lethal concentration for 50% of the population (LC_{50}) and effective concentration (EC_{50}) for photosynthetic capacity (P_{MAX}) and efficiency (α) were determined based on these data. Juvenile *S. latissima* was highly sensitive, having an LC_{50} of $80.7\text{ mg } H_2O_2\text{ l}^{-1}$, which is less than 5% of the dose commonly used at farms and emitted to the environment. A concentration of 85 mg l^{-1} caused an immediate 90% reduction in both P_{MAX} and α . The EC_{50} was found to be 27.8 and 35.4 mg l^{-1} for P_{MAX} and α , respectively. This may indicate that natural *S. latissima* populations in the vicinity of fish farms can be negatively affected by H_2O_2 bath treatments.

KEY WORDS: Kelp mortality · Kelp photosynthesis · Aquaculture effluents

1. INTRODUCTION

Production of salmonid fish is a major industry in Norway, with >1.3 million tons produced in 2016, a 3-fold increase over the last 15 yr (Directorate of Fisheries Norway 2018b). Fish in the high-density conditions of modern aquaculture are susceptible to bacterial, viral, and parasitic diseases. At present, infestation with salmon lice *Lepeophtheirus salmonis* is a major problem for the Norwegian fish-farming industry, increasing the requirement for effective antiparasitic therapeutants. Several antiparasitic thera-

peutants are currently in use to mitigate the effects of sea lice on cultured Atlantic salmon (Grefsrud et al. 2018). These are administered as a bath treatment or orally via feed and are ultimately released into the environment. Recent studies have shown that the release of these chemical therapeutants can have significant consequences for non-target organisms in marine ecosystems (e.g. Samuelsen et al. 2014, Van Geest et al. 2014).

Hydrogen peroxide (H_2O_2) is used in salmonid aquaculture in Norway, as well as in Chile, Canada, and Scotland (Burridge et al. 2010). H_2O_2 is adminis-

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tered as a 15–20 min bath treatment of approximately 1500–2100 mg H_2O_2 l^{-1} , depending on water temperature (www.felleskatalogen.no). This is conducted either at the farm by wrapping a tarpaulin around the net pen or using a well-boat (Andersen & Hagen 2016). After treatment, the tarpaulin is removed, and the H_2O_2 disperses into the surrounding water or is pumped out from the well-boat directly into the sea. H_2O_2 dissociates into oxygen and water, and is therefore seen as being 'environmentally friendly' (Burridge et al. 2010), and H_2O_2 emissions are currently not included in any environmental risk assessments in relation to fish-farm activities. However, the half-life is approximately 7 d at 15°C and longer at colder temperatures (Bruno & Raynard 1994), leaving a window of time where this highly reactive oxidizer may interact with non-target species. As the H_2O_2 solution has similar density to the surrounding seawater, the effluent plume is likely to be concentrated in the upper ~30 m of the water column (Andersen & Hagen 2016, Refseth et al. 2017), making subtidal zones the most likely to be affected. In Norway, H_2O_2 is currently the most-used antiparasitic therapeutic, with an annual consumption of 31 577, 43 246, and 26 597 t respectively for the years 2014–2016 (www.fhi.no). Nonetheless, more knowledge regarding potential environmental effects is required to decide if and how these H_2O_2 emissions should be included in future environmental risk assessments.

To be able to make inferences regarding the potential effects H_2O_2 emissions from fish farms may have on population levels of subtidal species, it is necessary to have knowledge of the toxicity potentials toward non-target species, as well as which doses these species may be exposed to in the natural environment. Two field studies (Andersen & Hagen 2016, Fagereng 2016) and 2 dispersion models (Refseth et al. 2017, O. Samuelsen unpubl. data) have looked at how the H_2O_2 plume may disperse post-emission under conditions found in Norwegian waters. Results from the field studies give a snapshot of the concentrations in very close vicinity (≤ 60 m) to the release point, whereas the dispersion models give indications of how the plume may move locally through time and space. For sheltered locations within 1 km of the emitting farm, it is predicted that remaining concentrations 24 h post-emission will range from 18% to <1% of the H_2O_2 bath-treatment dose used (Refseth et al. 2017, O. Samuelsen unpubl. data). The toxicity potential of these concentrations on subtidal species has not been assessed to date.

Along the Norwegian coastline, one of the most dominating macroalgal habitats are kelp (order Laminariales) forests, estimated to cover an area >10 000 km^2 , from the shore down to a depth of 20–30 m (Christie et al. 2009). Macroalgae are important primary producers on the continental shelf (Charpy-Roubaud & Sournia 1990) and key components in coastal ecosystems (Araújo et al. 2016). On sheltered and medium-exposed locations, the sublittoral vegetation is commonly dominated by the perennial sugar kelp *Saccharina latissima* (Linnaeus) C. E. Lane, C. Mayes, Druehl, & G. W. Saunders (Andersen et al. 2011, Moy & Christie 2012). Kelp forests are biodiversity hotspots, serving key functions in the ecosystem by providing refuge, habitat, nursery grounds, and feeding grounds for >100 marine faunal species (Christie et al. 2003, 2009, Fredriksen 2003), including economically important fish such as Atlantic cod *Gadus morhua* and saithe *Pollachius virens* (Norderhaug et al. 2005), and seabirds such as sea ducks, cormorants, and black guillemots *Cepphus grylle* (Bustnes et al. 1997).

Healthy photosynthetic activity is essential for algae both in terms of nutritional uptake and in maintaining a positive carbon budget. As in all living cells, H_2O_2 and other oxygen intermediates (superoxide radical, singlet oxygen, and hydroxyl radical) are produced in low rates in algae as metabolic by-products and commonly grouped as reactive oxygen species (ROS) (Bischof & Rautenberger 2012). Algae have antioxidative systems that scavenge ROS, but stress (e.g. suboptimal temperature or light conditions) may cause an imbalance between the accumulation of ROS and the system's antioxidative capacity, leading to oxidative stress (Bischof & Rautenberger 2012). This in turn may lead to oxidation of cellular components (lipids, nucleic acids, and proteins) and thus damage the photosynthetic apparatus and lead to photoinhibition (Asada & Takahashi 1987). Concentrations as low as 2 mg H_2O_2 l^{-1} within chloroplasts have shown to cause a 90% reduction of photosynthetic activity (Kaiser 1979). As H_2O_2 has the ability to readily diffuse across membranes (Apostol et al. 1989), exogenous exposure could therefore have detrimental effects on macroalgae.

High interspecific sensitivity of the photosynthetic system to H_2O_2 has been reported both for macroalgae (Collén & Pedersén 1996, Collén & Davison 1999) and within the Laminariaceae family (Dummermuth et al. 2003), but H_2O_2 sensitivity of *S. latissima* has not been assessed to date. The lethal effect-dose of H_2O_2 has been determined for one macroalgae (Collén & Pedersén 1996). For microalgae,

H₂O₂ has previously been evaluated as a growth inhibitor related to nuisance blooms (European Communities 2003 and references therein, Southard 2005). However, studies reporting on toxicity potentials required for environmental risk assessments—such as lethal concentration for 50% of the population (LC₅₀), no-effect concentration (NEC), or effective concentration affecting photosynthesis in 50% of the population (EC₅₀)—of H₂O₂ on macroalgae, in terms of concentrations and durations comparable to emissions from aquaculture, are lacking.

Reversible and irreversible damage to the photosynthetic apparatus caused by a stressor, such as photosynthetic toxicity potential of H₂O₂, can be observed by measuring different photosynthetic parameters. The algae's ability to utilize available light for carbon fixation (photosynthetic efficiency) and the maximum potential rate of carbon fixation (photosynthetic capacity) can be reduced during suboptimal temperature (Davison et al. 1991, Andersen et al. 2013) and light conditions (Borum et al. 2002). Use of fixated carbon (respiration) however, tends to increase, as shown for both temperature (Davison et al. 1991, Andersen et al. 2013) and light (Borum et al. 2002). A reduced photosynthetic efficiency and/or increased respiration will result in an increase in the minimum amount of light required to maintain a balanced carbon budget (compensation irradiance) and could thus reduce the amount of carbon available for growth (Davison et al. 1991). A lower photosynthetic capacity and/or efficiency will reduce the saturation irradiance of the algae (Davison et al. 1991, Andersen et al. 2013) and could consequently affect their ability to take advantage of high light conditions. Exogeneous exposure to H₂O₂ has been shown to reduce photosynthetic efficiency (Collén & Pedersén 1996, Collén & Davison 1999, Dummermuth et al. 2003, Vega et al. 2006), while respiration is less sensitive (Collén & Pedersén 1996, Vega et al. 2006). The effects of exogeneous exposure on photosynthetic capacity, saturation, or compensation depth have, however, not been assessed to date for macroalgae.

Aquaculture is projected to expand dramatically in the near future, and thorough risk assessments which include all potential impacts associated with the industry are essential to maintain healthy coastal ecosystems. Furthermore, *S. latissima* has been proposed as an excellent species for integrated multi-trophic aquaculture (IMTA) in cold-temperate waters (e.g. Petrell et al. 1993, Barrington et al. 2009, Handå et al. 2013), and the best practices for cultivation integrated with salmonid farming are currently being

intensely investigated in Norway (reviewed in Stévant et al. 2017). Mortality of *S. latissima* cultivated at fish farms has been observed, and coinciding H₂O₂ treatments may have been a possible factor (B. T. Haugland et al. unpubl. data).

Consequently, the aim of the present study was to evaluate the toxicity of H₂O₂ on *S. latissima*, mainly to (1) determine potential risks associated with emissions for natural *S. latissima* populations, and, additionally, to (2) determine if treatment with H₂O₂ at IMTA farms is innocuous to cultivated *S. latissima*. To achieve this, the toxicity potential of a 1 h exposure to ecologically relevant levels of H₂O₂ on mortality and photosynthetic activity of juvenile *S. latissima* was assessed at 3 time points post-exposure. Novel to effects of H₂O₂ exposure on macroalgae, photosynthetic capacity, compensation depth, and saturating irradiance were assessed, in addition to photosynthetic efficiency and dark respiration. LC₅₀, EC₅₀ and NEC values were also determined.

2. MATERIALS AND METHODS

2.1. Collection and experimental design

First-year sugar kelp *Saccharina latissima* was collected by free-diving in the upper subtidal zone (1–3 m depth) at Hjeltestad, SW of Bergen, Norway (60° 15' 40.4" N, 5° 12' 31.7" E) in March 2017 and transported to the Institute of Marine Research, Bergen, in dark plastic bags to avoid exposing the plants to high irradiances (after Dummermuth et al. 2003). Prior to initiation of the experiment, plants were kept at their collection temperature of 8°C in 15 l aquariums for minimum 24 h. Six fluorescent daylight lamps (OSRAM L 58W/954) provided irradiance of approximately 100 μmol photons m⁻² s⁻¹ (photosynthetically active radiation, PAR) at a natural seasonal regime of 12 h light: 12 h dark. A submersible micropump (flow rate: 150 l h⁻¹) maintained circulation of the water. Seawater in the aquarium was changed every other day, and no growth medium was added.

A preliminary study was conducted to define the dose–response relationship between H₂O₂ concentrations and lethal effects on *S. latissima*, ranging from 100% mortality to no effect (see Appendix). In addition to identifying the concentrations to be applied in the main experiment, the preliminary study also indicated that H₂O₂ affected the lamina tissue in patches, and not uniformly across the whole lamina. Whole-plant exposure and incubation (after

Andersen et al. 2013) was therefore chosen over lamina-disc incubation to better simulate how *S. latissima* plants would be impacted in nature in terms of exposure effects, post-exposure effects, and survival.

Consequently, following lab acclimatization, 5 healthy plants without wounds or fouling were chosen, numbered individually, measured by volume, and randomly assigned to 1 of 5 H₂O₂ exposure concentrations: 170 mg l⁻¹ (10% of the bath-treatment dose), 85 mg l⁻¹ (5%), 17 mg l⁻¹ (1%), 1.7 mg l⁻¹ (0.1%), or a seawater control (i.e. no H₂O₂). These concentrations were based on our preliminary dose–response study (see Appendix) and ranged from 10% to 0.1% of the bath-treatment dose of 1700 mg H₂O₂ l⁻¹ recommended by the producer (www.felleskataloge.no) for a temperature of 8°C. Plants were exposed in individual 2 l beakers for 1 h under low (50 PAR) light conditions. A total of 30 plants were included in the main study and divided equally between the 5 H₂O₂ concentrations (i.e. 6 replicate plants per concentration). A randomized schedule was prepared *a priori* to eliminate potential day effects and incubation chamber effects in the dataset. Following H₂O₂ exposure, plants were flushed with fresh seawater for a couple of minutes to remove any H₂O₂ on the surface before being transferred to individual incubation chambers per the randomized schedule. Exposures and incubations immediately after exposure were conducted over 9 consecutive days, as 5 plants were exposed at a time. As the preliminary study indicated that effects may not be apparent until several hours post-exposure, incubations to determine the effects on photosynthesis were conducted at 3 post-exposure time points: immediately after exposure (Day 0), 24 h post-exposure (Day 1), and 15 d post-exposure (Day 15), giving a total of 90 individual incubations.

2.2. Mortality

Determining time of mortality in macroalgae is challenging, and to date there are no standardized methods. From the preliminary study, it was found that it is essential to keep the plants in the laboratory for at least 7 d post-exposure to be able to determine mortality with certainty (see Appendix). The endpoint ‘mortality’ was determined to be when the lamina disintegrated and/or detached from the stipe when the plant was lifted out of the aquarium by the holdfast. In the main study, each individual was inspected daily and pictures were taken at the end of the 15 d post-exposure period or when the lamina showed visible signs of disintegration.

2.3. Effects on photosynthesis

To assess effects on photosynthetic activity (photosynthetic efficiency, α ; photosynthetic capacity, P_{MAX} ; dark respiration, R_D ; compensation irradiance, I_C ; and saturating light intensity, I_{SAT}), the methodology of Gerard (1988), Henley & Dunton (1997), and Andersen et al. (2013) was followed. Plants were incubated to construct a photosynthesis–irradiance (PI) curve based on measured oxygen (O₂) production or consumption over 11 different light intensities (0–350 PAR), starting with R_D (Table 1).

Incubations were conducted in 600 ml transparent, gas-tight, rectangular prisms (width: 5 cm, length: 15 cm, height: 15 cm) using magnetic stirring for water circulation. The magnetic stirrers were set to the same level (1250 rpm) to ensure the boundary layers around the thallus would be similar for all plants. Filtered seawater (glass-fibre filter, 1 μ m) was bubbled with N₂ gas to ~60% air saturation to prevent oversaturation of O₂ in the chambers during incubation. One thallus was hung within each incubation chamber from the lid. The incubation chambers were separated by partitions and illuminated directly from the front by one LED lamp (V-Light E14 dimmable HALED bulb; 230 V, 7 W). The rectangular shape of the chambers allowed for the thallus to be fixed in a perpendicular position to the light source

Table 1. Light levels used to generate photosynthesis–irradiance curves for juvenile *Saccharina latissima*. Values presented are the range (min. to max.) of measured light intensity, and time held, at each light level. α : photosynthetic efficiency, I_C : compensation irradiance, PAR: photosynthetically active radiation, P_{MAX} : photosynthetic capacity, R_D : dark respiration

Light level	Light intensity (PAR, μ mol photons m ⁻² s ⁻¹)	Time interval (min)
0 ^a (R_D)	0	120
1 ^a	10–12	30
2 ^a	19–22	30
3 ^a	29–32	30
4 ^a	42–45	30
5	64–66	20
6	84–88	20
7	125–128	10
8	146–150	10
9 ^b	169–174	10
10 ^b	230–235	8
11 ^b	347–350	8

^aBelow-saturating light levels used for mixed linear regression to determine α and I_C
^bAbove-saturating light levels used to determine P_{MAX}

throughout the incubation, independently of the water circulation.

The 11 different light levels (Table 1) were attained using a dimmer and density screens while measuring the PAR at every light level. Using separate light sources for each chamber increased control and allowed for adjustments to be made during incubations to ensure that all chambers were exposed to the same amount of irradiance at the different light levels for every incubation. Irradiance was measured as the maximum PAR hitting the front of the chamber at the centre point of the lamina. PAR was measured with a LI-190R quantum cosine-corrected sensor (up to 82° angle of incidence) coupled to a LI-250A light meter (LI-COR).

At each light level, changes in O₂ within the incubation chamber were determined using an optical non-invasive methodology (modified from Rastrick & Whiteley 2011). Each incubation chamber was internally equipped with an O₂ sensor spot and aligned with an exterior fibre optic sensor probe connected to a PreSens® OXY-10 mini sensor. OXY-10 software converted fluorescent readings to changes in seawater O₂ levels, and logged the values every 15 s. The plants were therefore held at each light level for a *priori* set time intervals (determined from test runs) until a ~5% change in O₂ was observed (Table 1), ensuring that all individuals were exposed to the same conditions. For all incubations, 1 control chamber was kept empty, from which the temperature was recorded (LabQuest® 2 multimeter, Vernier) and logged every 15 s. Relative humidity (RH %), barometric pressure (kPa), and salinity were also recorded for each incubation (LabQuest® 2 multimeter and Logger Lite software, Vernier). Immediately after incubation, the plants were blotted, weighed (fresh weight; FW), and transferred back to the aquarium.

2.4. Data processing

To obtain the rate of O₂ production or consumption for each light level, the following calculations were conducted. O₂ concentration (µmol O₂) over time in the incubation chambers was calculated from % O₂ values transformed to *p*O₂ using measured barometric pressure (adjusted for vapour pressure and relative humidity), multiplied by the solubility coefficients (after Benson & Krause 1980, 1984, adjusted for temperature and salinity) and the volume of seawater within each incubation chamber (adjusted for the volume of the sample). The values from incubation chambers containing a sample were then

corrected for changes in the control chamber. FW-standardized rate of O₂ consumption or production at each light level was calculated from the linear regression of O₂ concentration (µmol O₂) against time (h) divided by the FW of samples, giving rates expressed in µmol O₂ gFW⁻¹ h⁻¹. The first 2 min were excluded from each linear regression to allow the plants to adjust to the new light level, resulting in a minimum of 24 data points being used in all regressions (i.e. to determine net rate of photosynthesis for each light level). Rates of oxygen production or consumption plotted against light intensity yielded 1 PI curve for each individual plant for each of the 3 time points (Days 0, 1, and 15). Photosynthetic parameters (α , P_{MAX} , R_D , I_C , and I_{SAT}) could subsequently be calculated from the plotted curves.

α , defined as the initial slope of the PI curve at below-saturating light intensities, was obtained by fitting a linear mixed model (LMM) to a subset of the data including only the first light levels (see Table 1). I_C , defined as the light requirements for a net photosynthetic rate of zero, was obtained from the same LMM. P_{MAX} was calculated by averaging 3 values from above-saturating light levels (see Table 1). Subsequently, I_{SAT} was determined as the intercept between P_{MAX} and α . R_D was determined as O₂ consumption in darkness. Some of the plants exposed to 85 and 170 mg H₂O₂ l⁻¹ demonstrated negative P_{MAX} and/or negative α values. These were excluded (number of included plants is indicated in the figures) when calculating the light parameters (I_{SAT} and I_C), as inclusion would have produced unrealistic values.

2.5. Fitting dose–response curves to determine toxicity potentials

The extension package *drc* version 3.0-1 (Ritz et al. 2015) for R software (R Core Team 2017) was used to model the dose–response relationships. The statistical package provides a range of different regression models, a model-select function, and methods to extract model parameters such as EC₅₀, LC₅₀, and NEC using the delta method (Ritz et al. 2015). To determine the lethal toxicity potentials, mortality of *S. latissima* plants on Day 15 was regressed over H₂O₂ concentration using the model that gave the best fit: a 2-parameter log-logistic model with a binomial distribution and with the upper and lower asymptotes set to 1 and 0, respectively:

$$Y = \frac{1 - 0}{1 + \exp[b(\log(x) - \log(e))]} \quad (1)$$

where Y is the response, x is the dose, b is the slope of the line, and e is the inflection point of the curve (in this case also the LC_{50} value) (Ritz et al. 2015). To determine the photosynthetic toxicity potentials, the photosynthetic parameters that showed potential for being indicators for immediate effect (P_{MAX} and α) were regressed over H_2O_2 concentration. The models that gave the best fit were the 3-parameter log-logistic model (P_{MAX} , Eq. 2) and the 3-parameter Weibull 1 model (α , Eq. 3), both models with the lower asymptote set to 0:

$$Y = 0 + \frac{d-0}{1 + \exp[b(\log(x) - \log(e))]} \quad (2)$$

$$Y = 0 + (d-0)\exp\{-\exp[b(\log(x) - \log(e))]\} \quad (3)$$

where Y is the response, x is the dose, d is the upper asymptote, b is the slope of the line, and e is the inflection point of the curve (Ritz et al. 2015).

2.6. Statistical analysis

The effect of H_2O_2 concentration on photosynthesis (α , P_{MAX} , R_D) was tested by fitting generalized least squares (GLS) models using the package *nlme* (Pinheiro et al. 2017), where H_2O_2 concentration (5 levels: control, 1.7, 17, 85, and 170 $mg\ l^{-1}$) and time (2 levels: Day 0 and Day 1) were used as fixed effects. Loss of plants in the 85 and 170 $mg\ l^{-1}$ concentrations on Day 15 prevented the running of a full statistical model on all time points. As each plant was repeatedly measured over time, 'plant' was treated as a random factor and incorporated with a correlation term, allowing dependency between the response variables from the same plant (Zuur et al. 2009). Heteroscedasticity was obtained by allowing differing variances across H_2O_2 concentration for α and P_{MAX} and across time for R_D . The significance of the interaction H_2O_2 concentration \times Time and the 2 fixed effects were tested with the likelihood ratio test, and only significant terms were included in the final model (Zuur et al. 2009).

3. RESULTS

3.1. Mortality

Results from the preliminary study showed 100% mortality when exposing juvenile sugar kelp *Saccharina latissima* for 1 h to the commercially recommended bath-treatment dose of 1700 $mg\ l^{-1}$ and for

concentrations down to 10% (170 $mg\ l^{-1}$) of this dose (see Appendix).

In the main experiment, no mortality was observed on Day 0 or Day 1 in any of the concentrations ranging from no added H_2O_2 (seawater control) to 170 $mg\ H_2O_2\ l^{-1}$. At the end of the 15 d post-exposure period, mortality had increased to 100% in the 170 $mg\ l^{-1}$ concentration and to 67% in the 85 $mg\ l^{-1}$, while there was no mortality in plants exposed to 17 $mg\ l^{-1}$, 1.7 $mg\ l^{-1}$, or in the control (Fig. 1). On Day 15, the control plants showed no fragmentation or sign of pigment loss. Plants in the 170 $mg\ l^{-1}$ concentration, however, had lost pigments in larger parts of the lamina after 3 d (Fig. 2A). This was followed by continuous fragmentation until all plants in the 170 $mg\ l^{-1}$ concentration were completely disintegrated within 10 d. The same course of events was observed for the 4 plants that died in the 85 $mg\ l^{-1}$ concentration: loss of pigments followed by fragmentation and complete disintegration. The LC_{50} for juvenile *S. latissima* was determined to be $80.7 \pm 53.5\ mg\ l^{-1}$ and the NEC to be $72.9 \pm 0.4\ mg\ l^{-1}$ (Table 2).

3.2. Effects on photosynthesis

The PI relationship of control plants followed a typical pattern at all 3 time points: a linear increase in net photosynthetic rate for below-saturating light intensities (0 to 43.5 PAR) followed by a gradual decrease for above-saturating intensities (43.5 to 348 PAR) (Fig. 3). Exposure for 1 h to concentrations of 170 and 85 $mg\ H_2O_2\ l^{-1}$ resulted in immediate negative impacts on the net photosynthetic rate on Day 0, reducing the median (\pm SD) rate compared to

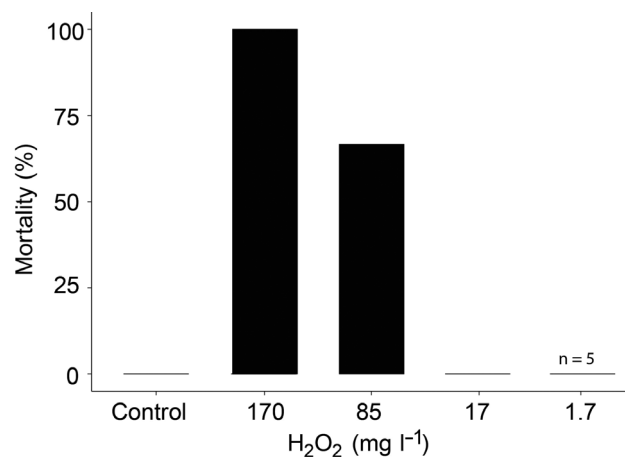


Fig. 1. Mortality of juvenile *Saccharina latissima* plants 15 d after 1 h exposure to 5 different H_2O_2 concentrations, including control ($n = 6$ unless otherwise stated)

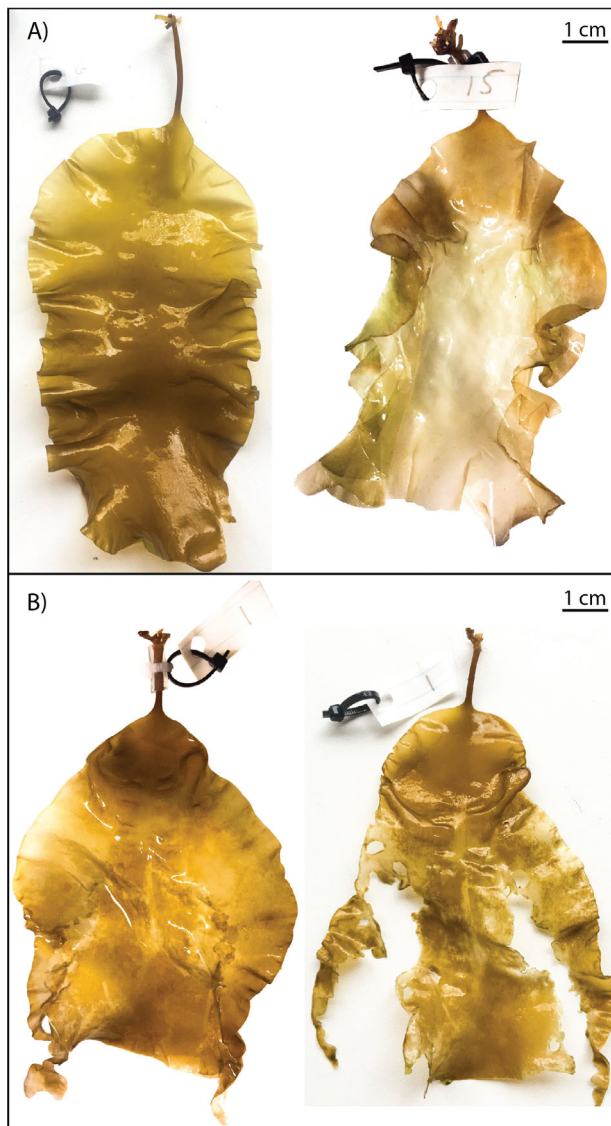


Fig. 2. Effects of different concentrations of H₂O₂ on lamina of juvenile *Saccharina latissima* plants at different time intervals. (A) Normal lamina with no visible damages 15 d post-exposure to concentrations ≤ 17 mg l⁻¹ (left), and discoloured and pale lamina 3 d post-exposure to 170 mg l⁻¹ (right); (B) discoloured lamina due to pigment loss 8 d post-exposure to 85 mg l⁻¹ (left), and lamina with loss of tissue due to cell death 15 d post-exposure to 85 mg l⁻¹ (right)

that of the control plants at both below-saturating irradiance (43.5 PAR; control: 13.99 ± 3.73 $\mu\text{mol O}_2$ gFW⁻¹ h⁻¹; 170 mg l⁻¹: -0.72 ± 1.01 $\mu\text{mol O}_2$ gFW⁻¹ h⁻¹; 85 mg l⁻¹: 0.17 ± 1.27 $\mu\text{mol O}_2$ gFW⁻¹ h⁻¹) and at the highest irradiance (348.5 PAR; control: 45.62 ± 8.77 $\mu\text{mol O}_2$ gFW⁻¹ h⁻¹; 170 mg l⁻¹: -0.77 ± 3.64 $\mu\text{mol O}_2$ gFW⁻¹ h⁻¹; 85 mg l⁻¹: 0.59 ± 3.37 $\mu\text{mol O}_2$ gFW⁻¹ h⁻¹). A slightly lower median (\pm SD) net photosynthesis was demonstrated by plants in the 17 mg H₂O₂ l⁻¹

Table 2. Toxicity potentials ($\pm 95\%$ CI, mg l⁻¹) of a 1 h exposure to H₂O₂ on juvenile *Saccharina latissima* for mortality and photosynthesis (α : photosynthetic capacity, P_{MAX} : photosynthetic efficiency). LC₅₀ and LC₉₀: median lethal concentrations and EC₅₀ and EC₉₀: median effective concentrations, for 50% and 90% of the population, respectively; NEC: no-effect concentration

	LC ₅₀	LC ₉₀	NEC
Mortality	80.7 \pm 53.5	95.1 \pm 134.1	72.9 \pm 0.4
	EC ₅₀	EC ₉₀	NEC
α	35.4 \pm 13.4	77.7 \pm 40.7	13.1 \pm 11.2
P_{MAX}	27.8 \pm 9.1	57.5 \pm 39.8	13.1 \pm 11.2

concentration immediately post-exposure at high light intensities (148 PAR: 31.92 ± 7.15 $\mu\text{mol O}_2$ gFW⁻¹ h⁻¹; 348.5 PAR: 33.47 ± 4.46 $\mu\text{mol O}_2$ gFW⁻¹ h⁻¹) compared to the control (148 PAR: 37.22 ± 5.85 $\mu\text{mol O}_2$ gFW⁻¹ h⁻¹), though not at the lower intensities. Plants exposed to 1.7 mg l⁻¹ concentration showed comparable median net photosynthesis across all irradiances to the control.

On Day 1, photosynthetic rates of *S. latissima* plants exposed to ≥ 85 mg H₂O₂ l⁻¹ were still heavily impacted at all light intensities, showing highly reduced maximum rates compared to the control plants (control: 41.87 ± 5.40 $\mu\text{mol O}_2$ gFW⁻¹ h⁻¹; 170 mg l⁻¹: 0.40 ± 3.02 $\mu\text{mol O}_2$ gFW⁻¹ h⁻¹; 85 mg l⁻¹: 8.15 ± 5.07 $\mu\text{mol O}_2$ gFW⁻¹ h⁻¹; median \pm SD). Net photosynthetic rates of plants in the 17 and 1.7 mg l⁻¹ concentrations were similar to that of the control for all light intensities.

On Day 15, control plants showed a lower median net photosynthesis compared to Day 0: 9.65 ± 1.77 $\mu\text{mol O}_2$ gFW⁻¹ h⁻¹ at 43.5 PAR, 18.14 ± 3.84 $\mu\text{mol O}_2$ gFW⁻¹ h⁻¹ at 148 PAR, and 24.87 ± 4.64 $\mu\text{mol O}_2$ gFW⁻¹ h⁻¹ at the highest light intensity (348.5 PAR). The 2 surviving plants in the 85 mg l⁻¹ concentration still showed signs of impact from the H₂O₂ exposure under light intensities up to 171.5 PAR, demonstrating a 50% reduction in net photosynthesis, while the response to high light >200 PAR appeared to have recovered more.

The reduced net photosynthetic rate found for plants exposed to concentrations ≥ 85 mg l⁻¹ resulted in a drop in α and P_{MAX} on Day 0 compared with the control plants (Fig. 4). Median (\pm SD) α -value for the 2 highest concentrations (170 mg l⁻¹: 0.00 ± 0.01 $\mu\text{mol O}_2$ gFW⁻¹ h⁻¹ / $\mu\text{mol m}^{-2} \text{s}^{-1}$; 85 mg l⁻¹: 0.02 ± 0.03 $\mu\text{mol O}_2$ gFW⁻¹ h⁻¹ / $\mu\text{mol m}^{-2} \text{s}^{-1}$) were significantly different ($p < 0.001$, Table 3) from the control

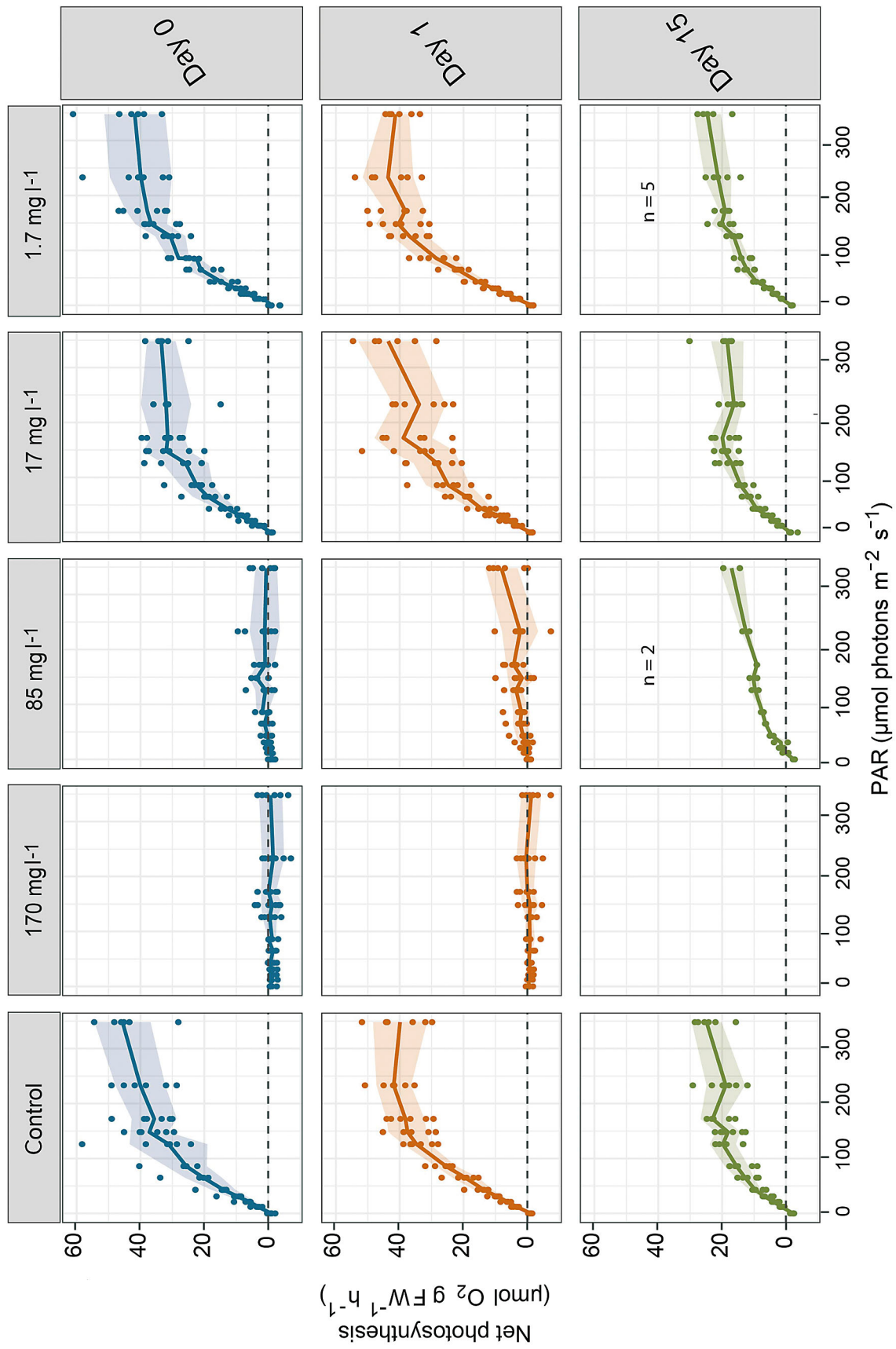


Fig. 3. Photosynthesis–irradiance curves for juvenile *Saccharina latissima* plants exposed for 1 h to 5 different concentrations of H_2O_2 , including control. Raw data and median (± 1 SD) for immediately post-exposure (Day 0), 24 h post-exposure (Day 1), and 15 d post-exposure (n = 6 unless otherwise stated). FW: fresh weight, PAR: photosynthetically active radiation

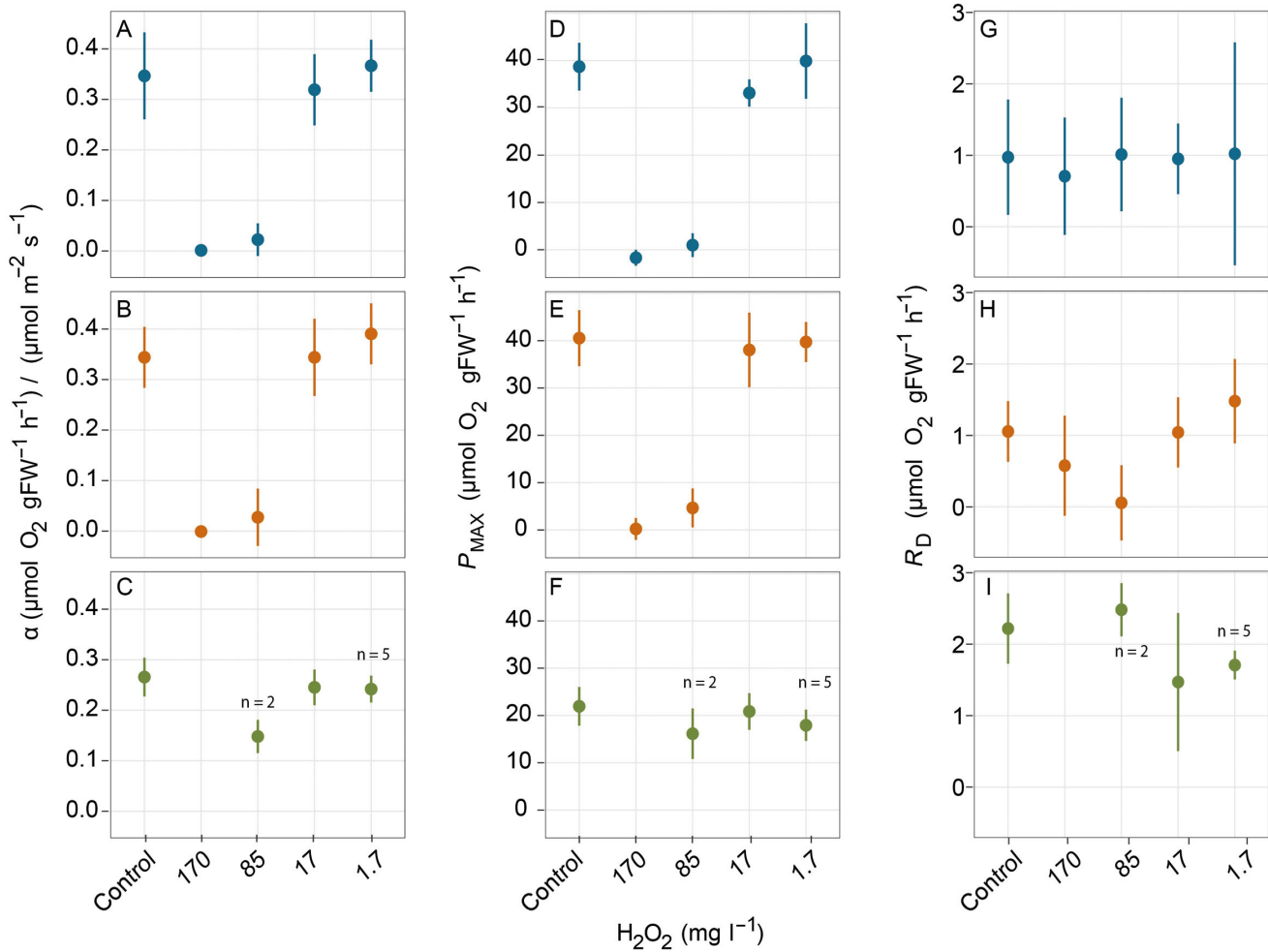


Fig. 4. Effect after 1 h exposure to 5 different concentrations of H₂O₂, including control, on photosynthetic parameters (A–C: α : photosynthetic efficiency, D–F: P_{MAX} : maximum photosynthetic rate at high light, G–I: R_{D} : respiration rate in the dark) of juvenile *Saccharina latissima* plants. Median (± 1 SD) response (A,D,G) immediately post-exposure (Day 0), (B,E,H) 24 h post-exposure (Day 1), and (C,F,I) 15 d post-exposure ($n = 6$ unless otherwise stated). FW: fresh weight

($0.35 \pm 0.09 \mu\text{mol O}_2 \text{ gFW}^{-1} \text{ h}^{-1} / \mu\text{mol m}^{-2} \text{ s}^{-1}$). For concentrations $\leq 17 \text{ mg l}^{-1}$, however, there were no significant differences compared to the control (17 mg l^{-1} : $0.32 \pm 0.07 \mu\text{mol O}_2 \text{ gFW}^{-1} \text{ h}^{-1} / \mu\text{mol m}^{-2} \text{ s}^{-1}$, $p = 0.429$; 1.7 mg l^{-1} : $0.37 \pm 0.05 \mu\text{mol O}_2 \text{ gFW}^{-1} \text{ h}^{-1} / \mu\text{mol m}^{-2} \text{ s}^{-1}$, $p = 0.543$; median \pm SD). No significant H₂O₂ concentration \times time interaction ($p = 0.078$, $\text{df} = 2$) or significant differences between Day 0 and Day 1 ($p = 0.615$, $\text{df} = 11$) were found; hence α was equally unaffected by time in all concentrations.

The median (\pm SD) P_{MAX} for plants in the 170 and 85 mg l^{-1} concentrations (-1.70 ± 1.69 and $0.97 \pm 2.50 \mu\text{mol O}_2 \text{ gFW}^{-1} \text{ h}^{-1}$, respectively) were significantly different to that of the control ($38.71 \pm 5.04 \mu\text{mol O}_2 \text{ gFW}^{-1} \text{ h}^{-1}$) on Day 0 ($p < 0.001$, Table 3). For plants exposed to 17 mg l^{-1} , the median P_{MAX} (\pm SD) was lower ($33.15 \pm 2.88 \mu\text{mol O}_2 \text{ gFW}^{-1} \text{ h}^{-1}$)

than that of the control plants, but neither this nor the P_{MAX} of plants exposed to 1.7 mg l^{-1} were significantly different to the control (17 mg l^{-1} : $p = 0.077$; 1.7 mg l^{-1} : $p = 0.594$). No significant H₂O₂ concentration \times time interaction ($p = 0.531$, $\text{df} = 2$) or significant differences between Day 0 and Day 1 ($p = 0.125$, $\text{df} = 11$) were found; hence P_{MAX} was equally unaffected by time in all concentrations.

Based on these results, the EC₅₀ for α for juvenile *S. latissima* plants to H₂O₂ exposure was determined to $35.4 \pm 13.4 \text{ mg l}^{-1}$, while the EC₅₀ for P_{MAX} was determined to $27.8 \pm 9.1 \text{ mg l}^{-1}$ (Table 2). The determined NEC value for both photosynthetic parameters was $13.1 \pm 11.2 \text{ mg l}^{-1}$.

Median (\pm SD) respiration rate (R_{D}) was not affected in any concentration on Day 0 compared to the control ($0.97 \pm 0.81 \mu\text{mol O}_2 \text{ gFW}^{-1} \text{ h}^{-1}$) (Fig. 4). On Day 1, me-

Table 3. Estimated regression parameters (H_2O_2 concentration, and time), standard error (SE), t -values, and p -values from generalized least squares (GLS) models fitted to the photosynthetic response variables. Only significant terms were included in the final models

Source of variation	Estimate	SE	t	p
α (photosynthetic efficiency)				
Intercept	0.36808	0.024391	15.09055	<0.001
1.7 mg l ⁻¹	0.021754	0.035521	0.612437	0.543
17 mg l ⁻¹	-0.02804	0.035201	-0.79665	0.429
85 mg l ⁻¹	-0.33031	0.029399	-11.2355	<0.001
170 mg l ⁻¹	-0.3708	0.024876	-14.906	<0.001
P_{MAX} (photosynthetic rate at high light)				
Intercept	39.88723	1.625547	24.53773	<0.001
1.7 mg l ⁻¹	1.36651	2.545536	0.536824	0.594
17 mg l ⁻¹	-4.48864	2.49097	-1.80197	0.077
85 mg l ⁻¹	-36.8761	1.956721	-18.8459	<0.001
170 mg l ⁻¹	-40.7071	1.746224	-23.3115	<0.001
R_{D} (respiration rate at low light)				
Intercept	1.2415940	0.2714216	4.574411	<0.001
1.7 mg l ⁻¹	0.4078867	0.3196865	1.275896	0.208
17 mg l ⁻¹	0.1167600	0.3196865	0.365233	0.716
85 mg l ⁻¹	-0.7006096	0.3196865	-2.191552	0.033
170 mg l ⁻¹	-0.3700247	0.3196865	-1.157461	0.252
Day 1	-0.2961013	0.1466637	-2.018913	0.049

dian (\pm SD) R_{D} was significantly reduced to $0.06 \pm 0.53 \mu\text{mol O}_2 \text{ gFW}^{-1} \text{ h}^{-1}$ for plants exposed to 85 mg l^{-1} ($p = 0.033$, Table 3). The R_{D} of plants in concentrations 170 and $\leq 17 \text{ mg l}^{-1}$ were not significantly different to that of the control (170 mg l^{-1} : $p = 0.252$; 17 mg l^{-1} : $p = 0.716$; 1.7 mg l^{-1} : $p = 0.208$; Table 3). Unlike P_{MAX} and α , there was a significant difference over time from Day 0 to Day 1 ($p = 0.049$, Table 3), most likely related to the change in the 85 mg l^{-1} concentration. The interaction H_2O_2 concentration \times time was non-significant ($p = 0.130$, $\text{df} = 2$).

Median (\pm SD) I_{SAT} was reduced to $57 \pm 36 \text{ PAR}$ for plants exposed to 85 mg l^{-1} on Day 0 compared to the control ($106 \pm 16 \text{ PAR}$) (Fig. 5). This reduction was caused by the observed drop in P_{MAX} and α . In the 170 mg l^{-1} concentration, only 1 plant met the criteria (see 'Materials and methods') to calculate the I_{SAT} (9861 PAR). On Day 1, the variation (SD) around the median was highly inflated for plants exposed to these 2 concentrations (170 mg l^{-1} : $511 \pm 459 \text{ PAR}$; 85 mg l^{-1} : $89 \pm 305 \text{ PAR}$) compared to the control ($108 \pm 13 \text{ PAR}$). Due to the high variation, and in 1 case, lack of replicates, further interpretation of this response parameter for plants exposed to $\geq 85 \text{ mg l}^{-1}$ on Day 0 and Day 1 may be misleading and is thus avoided (Fig. 5). For concentrations $\leq 17 \text{ mg l}^{-1}$, the median (\pm SD) I_{SAT} values were similar to the control both on Day 0 (17 mg l^{-1} : $102 \pm 13 \text{ PAR}$; 1.7 mg l^{-1} :

$107 \pm 17 \text{ PAR}$) and Day 1 (control: $108 \pm 14 \text{ PAR}$; 17 mg l^{-1} : $102 \pm 19 \text{ PAR}$; 1.7 mg l^{-1} : $101 \pm 13 \text{ PAR}$) (Fig. 5).

Median I_{C} (\pm SD) was 98 and 9 times higher on Day 0 for plants in concentrations 170 mg l^{-1} ($297 \pm 2034 \text{ PAR}$) and 85 mg l^{-1} ($29 \pm 141 \text{ PAR}$), respectively, compared to the control ($3 \pm 2 \text{ PAR}$) (Fig. 6). The higher I_{C} was attributed to the drop in α values, as there was no acute effect on R_{D} (Fig. 4). Due to the high variation (SD), the values were deemed uncertain, as was the I_{C} for the same concentrations on Day 1 (170 mg l^{-1} : $231 \pm 245 \text{ PAR}$; 85 mg l^{-1} : $14 \pm 14 \text{ PAR}$), and further interpretation was not conducted. The median (\pm SD) I_{C} for the 17 mg l^{-1} ($3 \pm 1 \text{ PAR}$) and 1.7 mg l^{-1} ($3 \pm 3 \text{ PAR}$) concentrations were comparable to the control. On Day 1, the I_{C} showed no change for the $\leq 17 \text{ mg l}^{-1}$ concentrations, including for the control (control: $3 \pm 1 \text{ PAR}$; 17 mg l^{-1} : $3 \pm 1 \text{ PAR}$; 1.7 mg l^{-1} : $3 \pm 1 \text{ PAR}$).

3.3. Prolonged effects of H_2O_2

On Day 15, median (\pm SD) α of control plants was $0.27 \pm 0.04 \mu\text{mol O}_2 \text{ gFW}^{-1} \text{ h}^{-1} / \mu\text{mol m}^{-2} \text{ s}^{-1}$ (Fig. 4). The 2 plants that survived the 85 mg l^{-1} concentration demonstrated a 50% reduction in α ($0.15 \pm 0.03 \mu\text{mol O}_2 \text{ gFW}^{-1} \text{ h}^{-1} / \mu\text{mol m}^{-2} \text{ s}^{-1}$) compared to the control (Fig. 4), causing a 50% higher I_{C} (85 mg l^{-1} : $15 \pm 6 \text{ PAR}$, control: $7 \pm 2 \text{ PAR}$) (Fig. 6). The other parameters (P_{MAX} , R_{D} , and I_{SAT}) were comparable to the control for all concentrations. The parameters of the control plants had changed over time; the P_{MAX} and α showed a reduction of 24% and 20%, respectively, while R_{D} had increased from 0.97 ± 0.81 to $2.22 \pm 0.49 \mu\text{mol O}_2 \text{ gFW h}^{-1}$, leading to a higher I_{C} of $7 \pm 1 \text{ PAR}$ and a lower I_{SAT} of $81 \pm 30 \text{ PAR}$.

The 2 plants that survived the 85 mg l^{-1} concentration had the highest initial biomass compared to other plants in the same concentration. Some loss of pigments was observed after 8 d, shown as paler patches on the lamina (Fig. 2B). Reduced structural integrity of the lamina was observed in the same areas as the pigment loss, leading to tearing. After 15 d, these patches had disintegrated, leaving a degraded lamina (Fig. 2B). A 20% reduction in biomass from Day 0 to Day 15 was demonstrated by these 2 plants, whereas plants in the control showed an average increase of 105% (Fig. 7). Plants in the 17 and 1.7 mg l^{-1} concentrations showed respectively 88% and 110% increase in biomass in the same period (Fig. 7).

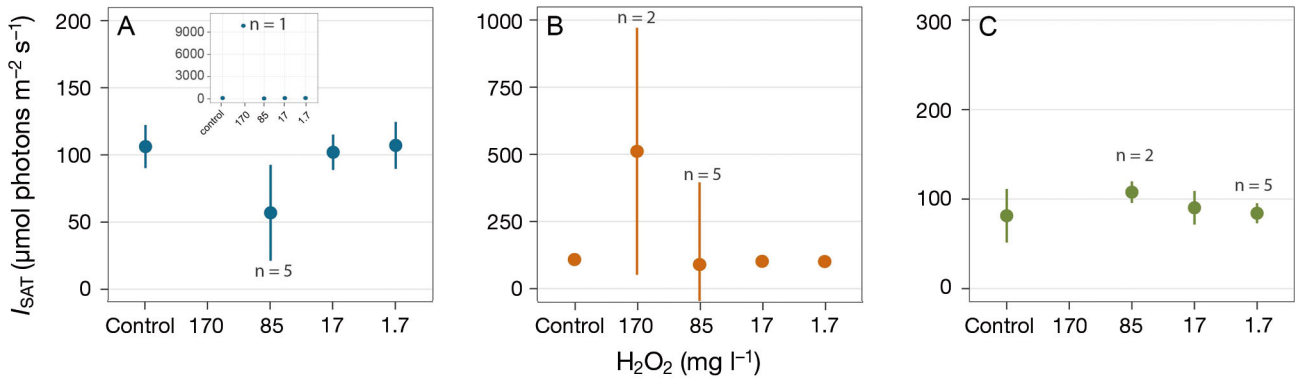


Fig. 5. Effect of 1 h exposure to 5 different concentrations of H₂O₂, including control, on the saturating irradiance (I_{SAT}) of juvenile *Saccharina latissima* plants. Median (± 1 SD) response (A) immediately post-exposure (Day 0), (B) 24 h post-exposure (Day 1), and (C) 15 d post-exposure (n = 6 unless otherwise stated). Note different y-axis scales. Inset: zoomed-out view of plant I_{SAT}

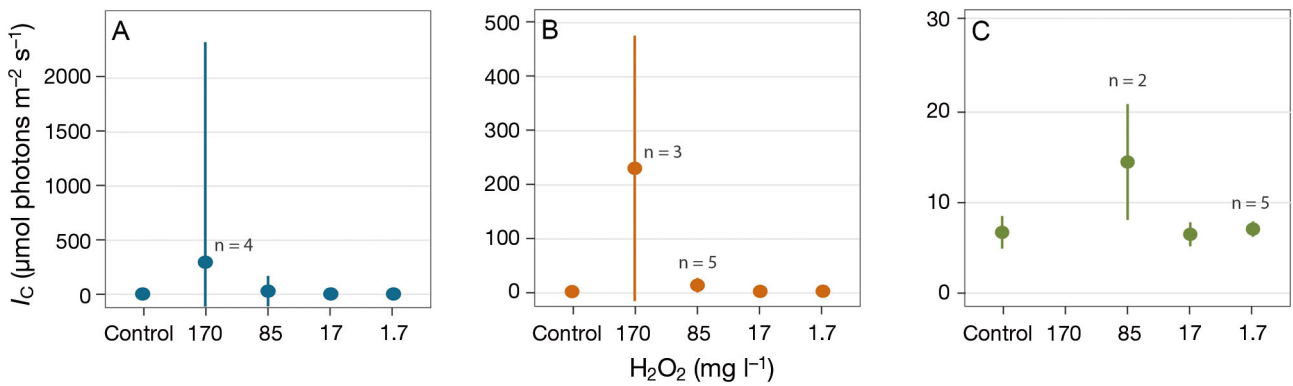


Fig. 6. Effect of 1 h exposure to 5 different concentrations of H₂O₂, including control, on the light compensation irradiance (I_C) of juvenile *Saccharina latissima* plants. Median (± 1 SD) response (A) immediately post-exposure (Day 0), (B) 24 h post-exposure (Day 1), and (C) 15 d post-exposure (n = 6 unless otherwise stated). Note different y-axis scales

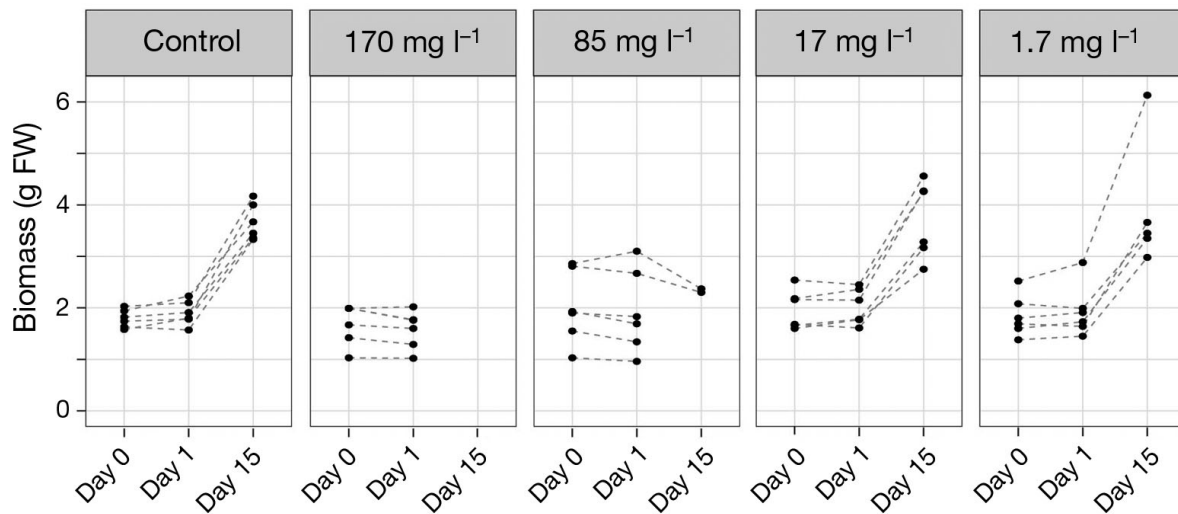


Fig. 7. Effect of 1 h exposure to 5 different concentrations of H₂O₂, including control, on biomass of individual juvenile *Saccharina latissima* plants over time at intervals immediately post-exposure (Day 0), 24 h post-exposure (Day 1), and 15 d post-exposure. FW: fresh weight

4. DISCUSSION

This study demonstrates for the first time the potential consequences of emissions of H_2O_2 , a common sea-lice therapeutant in salmon farming, on the survival and photosynthetic performance of the habitat-building sugar kelp *Saccharina latissima* in marine ecosystems. Mortality of juvenile *S. latissima* was observed for plants exposed to concentrations as low as 85 mg l^{-1} , equivalent to only 5% of the treatment dose commonly used in net pens (1700 mg l^{-1}). The impact of an H_2O_2 concentration of 85 mg l^{-1} and higher was immediate, reducing both P_{MAX} and α by >90%. Furthermore, we saw prolonged effects 15 d post-exposure for individuals that survived the 85 mg l^{-1} concentration, both in terms of decreased biomass and reduced α and I_C . The LC_{50} and EC_{50} values indicate that *S. latissima* is highly sensitive to H_2O_2 levels that natural local populations could be exposed to from aquaculture emissions.

4.1. Lethal effects of H_2O_2

The highly reduced photosynthetic performance and subsequent mortality of 83% of the plants exposed to concentrations $\geq 85 \text{ mg l}^{-1}$ strongly indicates that the antioxidative scavenging system of juvenile *S. latissima* was not able to cope with H_2O_2 concentrations this high, and that oxidative stress occurred. Oxidative stress caused by H_2O_2 may lead to irreversible damages through the auto-destruction of cells (e.g. membrane leakage, oxidation of lipids) and inhibition of photosynthesis (Davison 1991, Collén & Pedersén 1996, Dummermuth et al. 2003). The observed pigment loss and more flaccid lamina that preceded mortality suggest that oxidation of pigments, membrane leakage, and cell death are possible main causes for the reduced photosynthesis and ultimate plant mortality seen in this study. Inhibition and destruction of photosystem II may also have played a role, as has been suggested as a cause for cell death post- H_2O_2 exposure of the macroalgae sea lettuce *Ulva rigida* (Collén & Pedersén 1996), though this was not tested in this study.

A much lower H_2O_2 concentration caused mortality for *U. rigida* (10.2 mg l^{-1} ; Collén & Pedersén 1996) than for *S. latissima* (85 mg l^{-1} ; present study). The difference could be related to plant structure, as a higher stress tolerance has been linked to the thickness of the thallus (Roleda et al. 2007). *S. latissima* has a thicker, corticated, and thus more complex thallus structure than *U. rigida*, which has distro-

matic (2 cell-layers thick) thallus (Bold & Wynne 1978). Furthermore, the Laminariales contain the carbohydrate mannitol that can act as an antioxidant and stabilize proteins (Bartsch et al. 2008). The use of whole plants in the present study compared to tissue discs (2 cm in diameter) in the *U. rigida* study might have also influenced mortality; H_2O_2 always affected the *S. latissima* lamina in patches, and one could infer that the larger the lamina, the larger the chance that healthy, unaffected tissue is left post-exposure that could aid in lamina regeneration. Older *S. latissima* sporophytes have been shown to be more tolerant than juveniles to high light stress, and to recover faster after damage to the photosystem (Hanelt et al. 1997). It is therefore likely that older sporophytes are also more tolerant to H_2O_2 stress than juveniles, both due to physiology and lamina size. The position of the patch, i.e. a non-affected meristem, will most likely also increase chance of survival, as has been observed in relation to bryozoan cover on the lamina of *S. latissima* (pers. obs.). Both higher initial biomasses and unaffected meristems might have increased the endurance and led to survival of 2 of the plants in the 85 mg l^{-1} concentration.

4.2. Effects of H_2O_2 on photosynthesis and carbon budget

Our results demonstrate that the antioxidative system of *S. latissima* plants can cope with exogenous exposure to low ($\leq 17 \text{ mg l}^{-1}$) concentrations of H_2O_2 , most likely through scavenging before any significant oxidative stress and irreversible damage are caused. Exposure to high ($\geq 85 \text{ mg l}^{-1}$) concentrations, however, caused an immediate and dramatically reduced P_{MAX} and α , indicating that the oxidative stress was too high for the photosynthetic system to function. This can prevent plants from maintaining a positive carbon budget and consequently have no surplus carbon available for cell repair and growth. The response is comparable to that of *U. rigida* (Collén & Pedersén 1996) post-exposure to a lower concentration ($10.2 \text{ mg H}_2\text{O}_2 \text{ l}^{-1}$), and to *S. latissima* plants exposed to extreme temperature stress (Andersen et al. 2013). Dummermuth et al. (2003) studied antioxidative potentials of several species of seaweed by exposing them to different concentrations of H_2O_2 . For an H_2O_2 concentration of 68 mg l^{-1} , they found a 90 to 95% reduction in photosynthetic efficiency (in F_v/F_m) for the kelp species bladderlocks *Alaria esculenta* and arctic kelp *Laminaria solidungula*, equivalent to the α -reduction we observed for *S. latissima*.

After 15 d, only 2 plants were left in the 85 mg l⁻¹ concentration, and the following discussion on the prolonged impact should therefore be interpreted with care. Paler patches on the lamina were still visible on the plants at this time, indicating non-recovery in pigment concentration. Under low light, photosynthetic rate is limited by the rate of photochemical reactions (Steeemann Nielsen 1975), which depends on light-harvesting pigments. Correlations between lower pigment concentration and lower α have previously been reported for *S. latissima* (Gerard 1988, Davison et al. 1991, Hanelt et al. 1997) as well as for higher plants (Pastori & Trippi 1993). The still reduced α found for these plants may therefore have been caused by a prolonged effect on their pigment concentration. P_{MAX} of these 2 plants was similar to that of the control plants, indicating recovered response to high light conditions.

The slow recovery of α prolongs the negative impact on both I_C and I_{SAT} , and under natural light regimes, this could result in an overall reduced daily carbon fixation for *S. latissima* (Bruhn & Gerard 1996). Firstly, by having a higher I_C , plants may be more susceptible to light limitation and more vulnerable to additional factors, such as shading by epiphytic growth, which could result in an imbalance of the plants' carbon budget (Andersen et al. 2013). Secondly, having a reduced I_{SAT} may prevent plants from taking advantage of occasional high light conditions. *S. latissima* inhabits the sublittoral, where the light conditions are often sub-saturating (Davison et al. 1991), and to be able to take advantage of any available light, it is important to maintain a viable carbon budget to support growth and reproduction. A reduced daily carbon fixation, in addition to a continuous loss of tissue, are possible causes for the lack of growth observed 15 d post-exposure for these 2 plants. Lack of growth may also have been affected by energy being diverted from growth due to the demand for repair (Roleda et al. 2007). The rate of carbon fixation might have also been too low for the plants to replace the lost pigments, preventing α and consequently I_C and I_{SAT} to recover. Based on the above-mentioned results, we propose that exposure to 85 mg l⁻¹ is either lethal to juvenile *S. latissima*, or may have a prolonged negative effect on their physiology and carbon budget.

An increase in R_D is typically observed for algae during stress (Davison et al. 1991, Andersen et al. 2013), although impacted plants demonstrated a reduction in the present study, as was observed by Vega et al. (2006) for microalgae. A reduced R_D would be an advantage for the plant, as it would

reduce carbon loss during dark periods (Davison et al. 1991). However, as P_{MAX} and α at this time indicate that the photosynthetic system was not only reduced, but barely functioning; it may be the same for respiration. The delayed effect on R_D compared to photosynthesis indicates that respiration is less sensitive to H₂O₂ and oxidative stress, as shown for *U. rigida* (Collén & Pedersén 1996) and microalgae (Vega et al. 2006). H₂O₂ primarily targets and oxidizes enzymes of the Calvin cycle, such as fructose-1,6-bisphosphatase (Asada 1992), enzymes that are essential for photosynthesis to take place, but not respiration. In addition, photosynthesis is reliant on chlorophyll as a catalyst, which has been shown to decrease with H₂O₂ exposure, whereas no catalyst is required for respiration (Campbell & Reece 2002). It may be that R_D is not negatively affected until the photosystem is severely inhibited, hence no response is seen immediately post-exposure.

For the control plants, the values for P_{MAX} , α , and R_D were within the natural variation found for *S. latissima* in other studies (Davison et al. 1991, Borum et al. 2002, Andersen et al. 2013), indicating the method used to measure and calculate the parameters was suitable. Using photosynthetic performance as an indicator of physiological stress is a well-established method in the field of phycology (Dummermuth et al. 2003), and has been shown in this study to be suitable for studying the toxicity potentials of a chemical. The change in P_{MAX} and α observed for the control plants after 15 d may have been due to different levels of environmental conditions in the laboratory compared to what the plants were adapted to in nature. Possible explanations include: lower water flow which could reduce the boundary layer and thus lead to a decreased gas exchange and accessibility to nutrients, lower nutrient levels as no medium was added to the seawater, and/or lower light conditions. However, this does not affect the reliability of the results in this study, as interpretative caution of the results has already been taken due to the low number of surviving plants in the high (85 mg H₂O₂ l⁻¹) concentration.

4.3. Potential effects on natural populations

The dispersion models (Refseth et al. 2017, O. Samuelsen unpubl. data) offer a more complete picture of the spatial and temporal movements of the H₂O₂ plume compared to the field studies (i.e. by Andersen & Hagen 2016, Fagereng 2016) and are hence considered more suitable for discussing the

potential concentrations that *S. latissima* may be exposed to and the subsequent impacts. Simulations done by Refseth et al. (2017) on the dilution and spread of H₂O₂ in surface waters (0–3 m depth) predicted that areas within 1000 m of the emitting fish farm can experience concentrations almost twice as high as the concentration found in this study to cause 100 % mortality. Within 2000 m, simulated concentrations were higher than the herein-determined LC₉₀. The second dispersion model (O. Samuelsen unpubl. data) predicted a higher dilution rate of H₂O₂ and that the highest concentration of H₂O₂ within 5600 m post-emission would be lower than the current study's determined LC₅₀, but still higher than the EC₅₀ values for both α and P_{MAX} . Both models thus indicate that exposure to H₂O₂ levels, that in the current study were found to negatively affect the photosynthesis of *S. latissima*, near farms are possible, while lethal exposures to *S. latissima* juveniles (within 2000 m) are only predicted in the Refseth et al. (2017) model. Delousing operations usually involve multiple bath-treatments (1 net pen at a time), hence non-target species will most likely experience several H₂O₂ exposures over several days (Grefsrud et al. 2018), which may further decrease the LC₅₀ and EC₅₀ values, as reported for American lobster *Homarus americanus* (Burrige et al. 2000, 2008). Fish farms are generally located close to land or skerries, thus often overlapping with the distribution of *S. latissima* beds. Furthermore, the number of H₂O₂ prescriptions given to fish farms in 2016 was highest for the months January to March (Grefsrud et al. 2018), which overlaps with the recruitment period (October to March) for sugar kelp (Andersen et al. 2011, Andersen 2013). A high mortality or reduced photosynthesis of the local juvenile *S. latissima* population during the recruitment period could lead to reduced recruitment and affect the development of the demography of the adult *S. latissima* population. Furthermore, a reduced carbon budget may lead to lower fitness and make them more susceptible to other stressors (Wernberg et al. 2010), such as grazing, storm surges, or elevated temperatures, and in that way indirectly lead to mortality of local *S. latissima* beds.

Monitoring programmes are regularly executed at all aquaculture sites in Norway (MOM system: Ervik et al. 1997), to ensure environmental impacts are not exceeding set thresholds (Grefsrud et al. 2018). However, these do not include monitoring of marine flora, including kelp beds. The total proportion of local *S. latissima* beds that would experience lethal or sub-lethal concentrations of H₂O₂ from 1 emission will be

highly dependent on the specific location of the farm, farm practice, treatment dose used, stratification of the water body, and current velocity and directions at the time of release (Andersen & Hagen 2016, Refseth et al. 2017). There is thus a need for further field studies on the distribution of H₂O₂ in the vicinity of fish farms and well-boats during treatment operations to improve the models. Juvenile *S. latissima* may be a good indicator organism for future field studies of H₂O₂ distribution around farms, as they have demonstrated a high sensitivity, are easy to check for lethal effects (i.e. detachment of lamina), and are cost-effective to use as no maintenance is required after deployment. Nonetheless, based on the high sensitivity of *S. latissima*, the available dispersion models (Refseth et al. 2017, O. Samuelsen unpubl. data) and the importance of kelp forests in coastal ecosystems (Araújo et al. 2016), it is recommended that H₂O₂ use and emissions are taken under consideration in future risk assessments and monitoring programmes to ensure that these habitats remain healthy.

4.4. Implications for H₂O₂ treatments at IMTA farms

The increased interest for developing an industry in Norway based on *S. latissima* cultivation in integration with salmonid fish is evident from the rapid increase in permits awarded to the private sector over the last couple of years (Stévant et al. 2017). In 2017, a total amount of 135 000 tons was harvested, with a value of 355 000 NOK (Directorate of Fisheries Norway 2018a). Development of the cultivation technology to optimize yield and make the industry as cost-efficient as possible is currently the main focus (MACROSEA: www.sintef.no/projectweb/macrosea/, Stévant et al. 2017). Our results may have implications for the production yield of cultivated *S. latissima* next to fish farms, as on-site H₂O₂ emissions will most likely harm the cultured seaweeds, and it is therefore recommended that these emissions are considered. Sporophytes are commonly deployed in the sea when they are smaller than the ones exposed in this study. Emissions should therefore especially be avoided in the beginning of the sea-cultivation cycle to minimize chances of negative impacts on the yield.

This study clearly demonstrates that *S. latissima* is highly sensitive, with a high mortality found down to a concentration of 85 mg H₂O₂ l⁻¹ seawater. Moreover, a prolonged negative effect of the H₂O₂ exposure was demonstrated in surviving plants in the form of reduced photosynthetic efficiency and lack of new

growth. The current extensive use and emission of H₂O₂ from fish farms in shallow areas is therefore likely to have negative impacts on the kelp community.

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APPENDIX. Preliminary H₂O₂ dose–response study in juvenile *Saccharina latissima* plants (n = 6). Plants were exposed for 1 h to different dilutions of the common bath-treatment dose used in net pens for treating salmon for sea-lice infestations. Visible effects were recorded over time, from 0 h to 7 d post-exposure: discoloured lamina (Dis), lamina covered in mucus (Muc), soft and flaccid lamina (Fla), no visible effects (NVE), lamina had disintegrated and detached from stipe (Dead)

Concentration (mg H ₂ O ₂ l ⁻¹)	Dilution of bath treatment (%)	0 h	8 h	22 h	2 d	3 d	6 d	7 d
1700	100	NVE	Lamina white, Dead	–	–	–	–	–
1250	75	NVE	NVE	Dis, Muc	Dis, Muc, Fla	Dis, Muc, Fla	Dis, Muc, Fla	Dead
850	50	NVE	NVE	Dis, Muc	Dis, Muc, Fla	Dis, Muc, Fla	Dis, Muc, Fla	Dead
475	25	NVE	NVE	Dis, Muc	Dis, Muc, Fla	Dis, Muc, Fla	Dis, Muc, Fla	Dead
170	10	NVE	NVE	Dis, Muc	Dis, Muc, Fla	Dis, Muc, Fla	Dis, Muc, Fla	Dead
Control	Control	NVE	NVE	NVE	NVE	NVE	NVE	NVE

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