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# Assessment of hydrogen peroxide as a bioindicator of stress in seaweed aquaculture

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The rapid expansion in commercial seaweed farming has highlighted the need for more effective monitoring methods, and health diagnostics. The production of the reactive oxygen species (ROS) hydrogen peroxide ( $\text{H}_2\text{O}_2$ ) is a trait that is tied to all major macroalgal groups and holds significance both for its involvement in the oxidative stress response and in the production of climatically relevant gases such as halocarbons. Observations of increased production of  $\text{H}_2\text{O}_2$  by plants as a stress response, along with its comparative stability and ease of quantification in seawater in comparison to other ROS, suggest that  $\text{H}_2\text{O}_2$  could be used as an indicator of health. In this study we characterized aqueous  $\text{H}_2\text{O}_2$  dynamics across a diel cycle, in response to small shifts in light and temperature, as well as when exposed to acute stress. Our results reveal that exposure to acute stressors leads to rapid and sustained concentrations of  $\text{H}_2\text{O}_2$  that are orders of magnitude higher than changes in  $\text{H}_2\text{O}_2$  concentrations observed throughout the day. These findings provide tantalizing evidence that monitoring  $\text{H}_2\text{O}_2$  could be used as a health indicator in seaweed aquaculture and serve as an early warning sign of stress.

By 2050, the United Nations projects that the human population will reach 9.7 billion people<sup>1</sup> generating an unprecedented demand for food and clean energy. The discovery and development of new farming practices, and food resources, lie at the crux of sustainably managing this challenge. Seaweed aquaculture could provide a multi-faceted solution to the problems of hunger, nutrient deficiency, and alternative energy supplies<sup>2–5</sup>. The global seaweed aquaculture industry has rapidly expanded in the last decades at a growth rate of 6.2%  $\text{yr}^{-1}$  (long-term average 2000–2018), demonstrating promise as part of a sustainable solution to impending challenges<sup>2</sup>. However, recent years have also witnessed an increased occurrence of crop loss. For instance, in the Philippines a 15% decline in biomass of *Kappaphycus alvarezii* due to ice-ice disease alone amounted to a loss of around 310 million US dollars between 2011 and 2013<sup>6</sup>.

Seaweeds grown on aquaculture farms are subject to a variety of possible environmental stressors, including changes in seawater levels, temperature, salinity, metals, and nutrients<sup>7,8</sup>. Apart from environmental stressors, intensive macroalgal farming has been accompanied with more frequent disease outbreaks, and grazing from copepods and amphipods<sup>9,10</sup>. In fact, changes in abiotic factors such as rising ocean temperatures may be linked to an increased risk of grazing and incidence of disease. For example, grazing by a marine isopod (*Cymodocea japonica*) on seaweed (*Undaria pinnatifida*) is linked to an increase in ocean temperature of 3°C<sup>11</sup>. Although the increased prevalence of environmental stressors and disease outbreak have led to considerable economic losses, very little is known about successful mitigation strategies<sup>12</sup>. Current methods tend to be time- and labor-intensive such as washing algal blades in acid solution, and visually monitoring stocks for epiphytes that are then removed by hand<sup>13</sup>.

Reactive oxygen species (ROS) are a group of short-lived oxygen-containing molecules, that are formed by all aerobic organisms<sup>14</sup>. In plants, the ROS hydrogen peroxide ( $\text{H}_2\text{O}_2$ ) is formed at various locations, including cell membranes, chloroplasts, mitochondria, and peroxisomes, often as a decay product of the ROS superoxide<sup>15</sup>. The accumulation of  $\text{H}_2\text{O}_2$  is hindered by a variety of enzymatic and non-enzymatic antioxidant mechanisms. However, when exposed to abiotic or biotic stressors, the production of ROS outpaces scavenging, leading to a swift increase in ROS concentrations<sup>16–18</sup>. The association between stress and increased concentrations of ROS have led to previous applications of ROS as biomarkers of human health<sup>19,20</sup>, and as stress indicators in plant studies<sup>21–25</sup>.

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Previous work has established the production of  $\text{H}_2\text{O}_2$ <sup>26–28</sup>, and presence of ROS-scavenging enzymes<sup>29</sup> in various macroalgae. An increase in ROS production is a common response to environmental stressors such as grazing/wounding, desiccation, and temperature shifts. For instance, rapid releases of  $\text{H}_2\text{O}_2$  have been observed in the red alga *G. conferta* when exposed to fragments of cell wall (oligoagars) or peptides from bacteria<sup>30,31</sup>, and in the brown alga *Laminaria digita* when exposed to alginates (which may be grazer or pathogen produced)<sup>22</sup>. Such oxidative bursts (encompassing a rapid release of  $\text{H}_2\text{O}_2$ ) are thought to be controlled by light<sup>32,33</sup> and serve as defense mechanisms against pathogens, prey, and surface colonization<sup>34–36</sup>. Increases in temperature may lead to more sustained elevated levels of ROS. For instance, in the red alga, *Kappacphycus alvarezii*, ROS production was noted to increase with temperature up to an experimental temperature of 40 °C<sup>37</sup>. The magnitude of the ROS response of seaweeds to desiccation may be influenced by their adaptation to particular habitats. For example, one study comparing  $\text{H}_2\text{O}_2$  release by subtidal and intertidal *Ulva lactuca* after 3 h of desiccation found notably higher levels of  $\text{H}_2\text{O}_2$  released by the subtidal species<sup>21</sup>.

In contrast to other evanescent ROS (e.g., superoxide, hydroxyl radical),  $\text{H}_2\text{O}_2$  has a sufficiently long lifetime allowing it to diffuse out and away from cells. This affords it the potential as a target for monitoring in the aqueous environment. While numerous studies have now demonstrated that seaweeds produce elevated concentrations of  $\text{H}_2\text{O}_2$  under certain stressors, many of these previous experiments studied intracellular concentrations, spanned just a few hours, used non-environmentally relevant conditions, or focused on segments rather than entire individuals. Thus, we lack a comprehensive understanding of how seaweeds control aqueous  $\text{H}_2\text{O}_2$  dynamics in their surroundings and whether measurements of  $\text{H}_2\text{O}_2$  can inform us about seaweed health.

Bromoform ( $\text{CHBr}_3$ ), is a halocarbon with ozone-depleting potential<sup>38,39</sup>, that is produced by macroalgae as part of an antioxidant response. The increased generation of  $\text{CHBr}_3$  during exposure to environmental stressors (e.g. nutrient depletion, and desiccation) has been observed in several studies<sup>40,41</sup>. Once released, halocarbons may help protect macroalgae from grazing and bacterial infection<sup>24,42</sup>. Macroalgal production of  $\text{CHBr}_3$  is thought to occur via the oxidation of bromide by bromoperoxidase (BrPO) with  $\text{H}_2\text{O}_2$  which generates a hypohalide that subsequently reacts with either DOM in the surrounding seawater, or ketones in the seaweed to produce bromoform<sup>43</sup>. A literature comparison of temperate, tropical, and polar macroalgae reveals a large range in bromoform production spanning from 0 to 6000 pmol g FW<sup>-1</sup> h<sup>-1</sup> for  $\text{CHBr}_3$ , with chlorophytes as the largest producers (up to 6000 pmol g FW<sup>-1</sup> h<sup>-1</sup>), followed by rhodophytes (up to 5000 pmol g FW<sup>-1</sup> h<sup>-1</sup>), and then phaeophytes (up to 3000 pmol g FW<sup>-1</sup> h<sup>-1</sup>)<sup>44</sup>. Although production of  $\text{CHBr}_3$  is well established across groups of macroalgae, a reaction for which  $\text{H}_2\text{O}_2$  is requisite, a direct quantitative tie to natural biological  $\text{H}_2\text{O}_2$  is still missing. Yet, contemporaneous analyses of  $\text{H}_2\text{O}_2$  and  $\text{CHBr}_3$  production by various macroalgal species could lead to a better understanding of the drivers behind the large ranges in observed production in culture studies and help constrain spatiotemporal trends in the fluxes of  $\text{CHBr}_3$  from the oceans to the atmosphere.

Determining whether  $\text{H}_2\text{O}_2$  and  $\text{CHBr}_3$  can indicate physiological stress in a manner that is distinguishable from fluctuations observed under ambient, non-stressed conditions will be essential to evaluating the potential of these compounds for early detection of stress in seaweed aquaculture. In this study we characterized the aqueous  $\text{H}_2\text{O}_2$  and  $\text{CHBr}_3$  dynamics associated with two temperate seaweeds, the chlorophyte *Ulva fenestrata* and the rhodophyte *Palmaria palmata* with the aim of determining whether aqueous  $\text{H}_2\text{O}_2$  measurements could be used to differentiate stress from baseline conditions. *U. fenestrata* and *P. palmata* are promising candidates for seaweed cultivation, and thus ideal model organisms for this type of study<sup>45–47</sup>. Specifically, we examined (1) temporal dynamics in  $\text{H}_2\text{O}_2$ , (2) responses to temperature, desiccation, and grazing, and (3)  $\text{CHBr}_3$  production, with the goal of examining the use of  $\text{H}_2\text{O}_2$  as a diagnostic management tool and the merit of monitoring natural  $\text{H}_2\text{O}_2$  and  $\text{CHBr}_3$  production in concert.

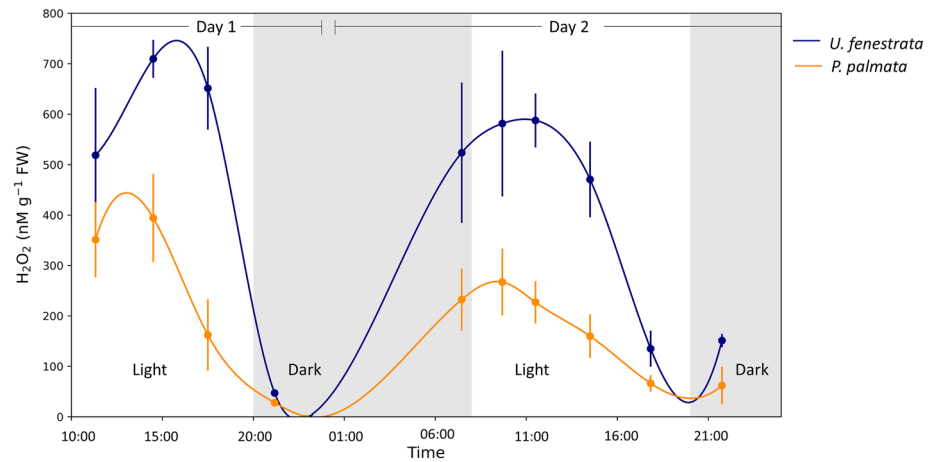
## Results

### $\text{H}_2\text{O}_2$ temporal dynamics

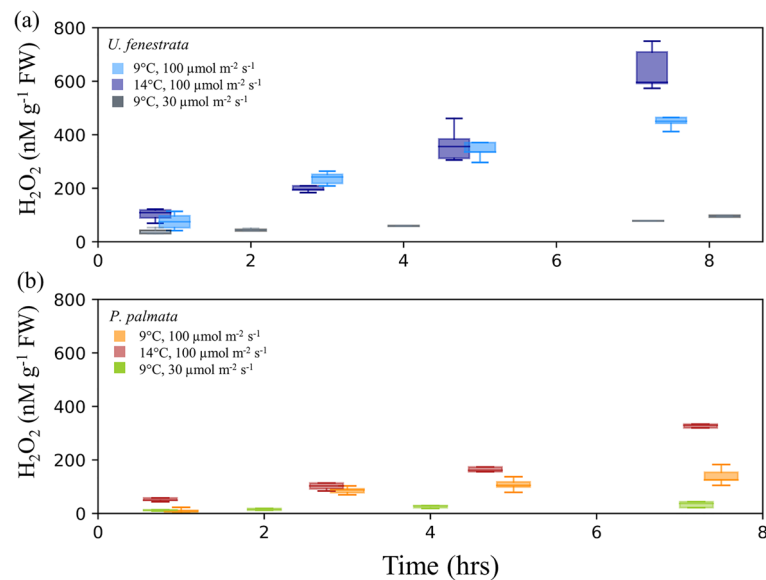
There were considerable fluctuations in the  $\text{H}_2\text{O}_2$  concentrations in incubations of *U. fenestrata* and *P. palmata* over the course of the day (see Fig. 1). The  $\text{H}_2\text{O}_2$  levels in the incubations of *U. fenestrata* were consistently higher than those for *P. palmata*. For both species,  $\text{H}_2\text{O}_2$  concentrations were at a maximum at 14:00 on the first day, and around 11:00 on the second day. The highest concentration in *U. fenestrata* incubations on the first day was 710 (± 38) nM g<sup>-1</sup> FW, while the maxima in *P. palmata* was 394 (± 87) nM g<sup>-1</sup> FW. A dramatic decrease in  $\text{H}_2\text{O}_2$  was observed prior to the onset of dark hours (set at 20:00) for both species with concentrations dropping to background  $\text{H}_2\text{O}_2$  levels. In contrast,  $\text{H}_2\text{O}_2$  concentrations in seaweed-free (control) tanks ranged only from 51 to 70 nM (see Figure S1).

The results of one-way analysis of variance (ANOVA) tests indicate that  $\text{H}_2\text{O}_2$  concentrations in *U. fenestrata* and *P. palmata* are significantly different from one another as well as from control treatments not containing seaweeds across all timepoints apart from the measurement made at 21:10 on the first day of incubation (Table S2). At this timepoint, the  $\text{H}_2\text{O}_2$  in the *P. palmata* and the *U. fenestrata* incubations are not significantly different from that of the control aquaria (Control – *Palmaria*,  $P=0.0525$ ; Control – *Ulva*:  $P=0.776$ ).

Seaweeds grown in aquaculture are exposed to natural changes in temperature and light that occur over the course of a day or season. To better understand how shifts in these environmental conditions influence the production and decay of  $\text{H}_2\text{O}_2$  we incubated individuals of *U. fenestrata* and *P. palmata* at varying temperatures and light conditions (Fig. 2). In all experiments, except for the low light condition,  $\text{H}_2\text{O}_2$  concentrations in the seawater of *U. fenestrata* and *P. palmata* increased steadily with time. Experiments conducted under low light conditions (30  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) did not lead to notable increases or decreases in  $\text{H}_2\text{O}_2$  throughout the time-series for either species. We observed higher  $\text{H}_2\text{O}_2$  concentrations in the aquaria of all *P. palmata* individuals, and all but one *U. fenestrata* when incubated at 14 °C in comparison to incubations at 9 °C.



**Figure 1.** Diel  $\text{H}_2\text{O}_2$  concentrations.  $\text{H}_2\text{O}_2$  concentrations in seawater incubations of *U. fenestrata* (blue) and *P. palmata* (gold), shown in nM normalized per gram fresh weight of seaweed in the incubation. Data points display the average across four biological replicates, and the error bar represents  $\pm 1$  SD. Incubations of both species were done at  $9^\circ\text{C}$  under  $100\ \mu\text{mol m}^{-2}\ \text{s}^{-1}$  light and began at 8:00. Tabulated data available in Table S1. Control tanks without seaweed in Supplemental Fig. S1.



**Figure 2.** Effect of light and temperature on  $\text{H}_2\text{O}_2$ . Seawater  $\text{H}_2\text{O}_2$  concentrations (shown as boxplots) over time for incubations of (a) *U. fenestrata* and (b) *P. palmata*. The three conditions were: (1)  $9^\circ\text{C}$  and  $100\ \mu\text{mol m}^{-2}\ \text{s}^{-1}$  light, (2)  $14^\circ\text{C}$  and  $100\ \mu\text{mol m}^{-2}\ \text{s}^{-1}$  and (3)  $9^\circ\text{C}$  and  $30\ \mu\text{mol m}^{-2}\ \text{s}^{-1}$ . For each experimental condition there were five biological replicates. Tabulated data available in Tables S3, S4, and S5.

A control tank consisting of seawater without macroalgae incubated under the same temperature and light conditions as the macroalgae incubations ( $9^\circ\text{C}$  control =  $40.24 \pm 0.96$  nM;  $14^\circ\text{C}$  control =  $43.5 \pm 2.01$  nM; low light control =  $39.91 \pm 0.51$  nM) showed no significant differences between treatment ( $P = 0.0525$ ), nor across timepoints ( $P = 0.3255$ ). Across the experimental timeline, *U. fenestrata* and *P. palmata* incubations at  $14^\circ\text{C}$  and  $9^\circ\text{C}$  were significantly different from one another ( $P < 0.0001$ ). In contrast, between hours 2 and 8, the  $\text{H}_2\text{O}_2$  in incubations of *U. fenestrata* and *P. palmata* under low light ( $30\ \mu\text{mol m}^{-2}\ \text{s}^{-1}$ ,  $9^\circ\text{C}$ ) were not statistically distinguishable from each other, or from the seawater controls without seaweed. Results of the Student's T-Tests comparing experimental treatments are summarized in Table S6.

### Effects of temperature, grazing, and desiccation on $\text{H}_2\text{O}_2$ concentrations

To test the hypothesis that substantially elevated  $\text{H}_2\text{O}_2$  concentrations will be induced by conditions in which the seaweeds experience stress, we examined responses to acute heat shock, grazing, and desiccation. As seen in

**Fig. 3**, incubation at 20 °C led to far higher H<sub>2</sub>O<sub>2</sub> concentrations in seawater compared to the standard condition at 9 °C. After an hour of incubation, the seawater H<sub>2</sub>O<sub>2</sub> for *U. fenestrata* was 2853 (± 35) nM g<sup>-1</sup> FW, amounting to a roughly 26-fold increase over the 109 (± 19) nM g<sup>-1</sup> FW of the seawater in aquaria with individuals incubated under standard conditions. Similarly, incubations of *P. palmata* at 20 °C led to the accumulation of far higher H<sub>2</sub>O<sub>2</sub> in seawater over the period of an hour than when grown under standard conditions (3200 in comparison to 50 nM g<sup>-1</sup> FW). Across the experimental timeline, H<sub>2</sub>O<sub>2</sub> in the seawater in the elevated temperature condition incubations increased steadily reaching 5,644 (± 128) nM g<sup>-1</sup> FW and 5,200 (± 120) nM g<sup>-1</sup> FW for *U. fenestrata* and *P. palmata*, respectively, after 9 h of incubation.

To evaluate the effect of grazing pressure, we grew individuals in the presence of herbivorous snails (*L. littorea*). Seawater in incubations where *U. fenestrata* individuals were exposed to the snails showed a spike in H<sub>2</sub>O<sub>2</sub> levels within the first hour of incubation, reaching levels higher than under heat stress. In contrast to the observations of the temperature stress experiment, however, H<sub>2</sub>O<sub>2</sub> concentrations were drastically elevated after an hour of incubation and remained relatively constant across the 9 h incubation timeline, increasing from 5048 (± 577) nM g<sup>-1</sup> FW to 5899 (± 699) nM g<sup>-1</sup> FW. Of the three experimental conditions, the most variability between biological replicates was seen in the grazing experiment, and the least was observed under the heat stress condition.

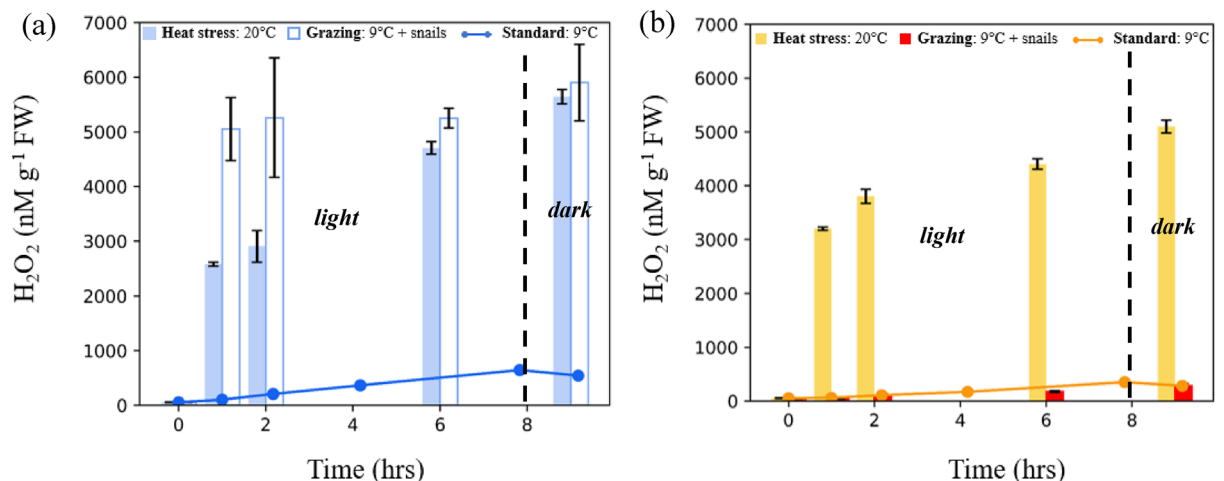
For all timepoints after one hour of incubation, the heat and grazing conditions resulted in H<sub>2</sub>O<sub>2</sub> concentrations in *U. fenestrata* that were significantly different ( $P < 0.001$ ) from the standard condition (Table S6). Similarly, the *P. palmata* heat treatment is significantly different ( $P < 0.001$ ) from the H<sub>2</sub>O<sub>2</sub> in the standard condition incubations. In contrast, the presence of *L. littorea* did not yield significantly different H<sub>2</sub>O<sub>2</sub> in *P. palmata* incubations from that in the standard condition. By the end of the nine hours of incubation, the H<sub>2</sub>O<sub>2</sub> levels in grazing and heat stress experiments were not statistically significant for *U. fenestrata* ( $P = 0.2333$ ). The pattern of H<sub>2</sub>O<sub>2</sub> accumulation in heat stress experiments of *U. fenestrata* and *P. palmata* was quite similar, but the total concentrations of H<sub>2</sub>O<sub>2</sub> were still significantly different between the species at most timepoints.

The influence of desiccation on H<sub>2</sub>O<sub>2</sub> concentrations was studied by removing seaweed from water for 2- or 4-h periods and then rehydrating them. As seen in Fig. 4, the seawater H<sub>2</sub>O<sub>2</sub> levels measured 2 min after the introduction of seaweed was notably higher for incubations in which the individuals had been desiccated and scaled with the length of desiccation. In contrast to heat stress and grazing (Fig. 3), however, desiccation led to a far smaller shift from baseline H<sub>2</sub>O<sub>2</sub> levels.

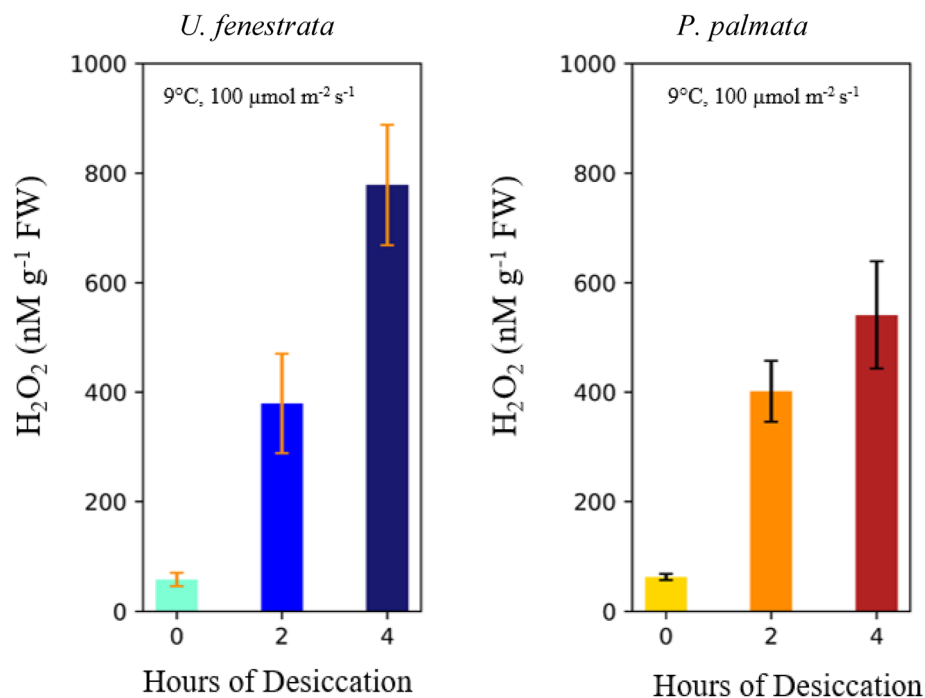
### Bromoform production

Seawater concentrations of CHBr<sub>3</sub> in closed-top incubations of *U. fenestrata* increased from 244 to 1067 ng g<sup>-1</sup> FW over the course of an 11-h period (Fig. 5). The corresponding H<sub>2</sub>O<sub>2</sub> concentrations in the incubation waters increased from 44 to 1645 nM g<sup>-1</sup> FW while the seaweeds were exposed to light (~ 1 h to 8.7 h of incubation), but dropped during the timepoint after the lights were turned off. In contrast to *U. fenestrata*, the seawater in which *P. palmata* was grown accumulated far less CHBr<sub>3</sub> over time, ranging between 20 and 200 ng g<sup>-1</sup> FW, and displays a general trend of increase. A similar pattern to *U. fenestrata* incubations, however, was observed for the seawater H<sub>2</sub>O<sub>2</sub> concentrations, with an increase from approximately 3.5 to 1615 nM g<sup>-1</sup> FW in the presence of light, and a drop to 588 nM g<sup>-1</sup> FW after an hour in the dark.

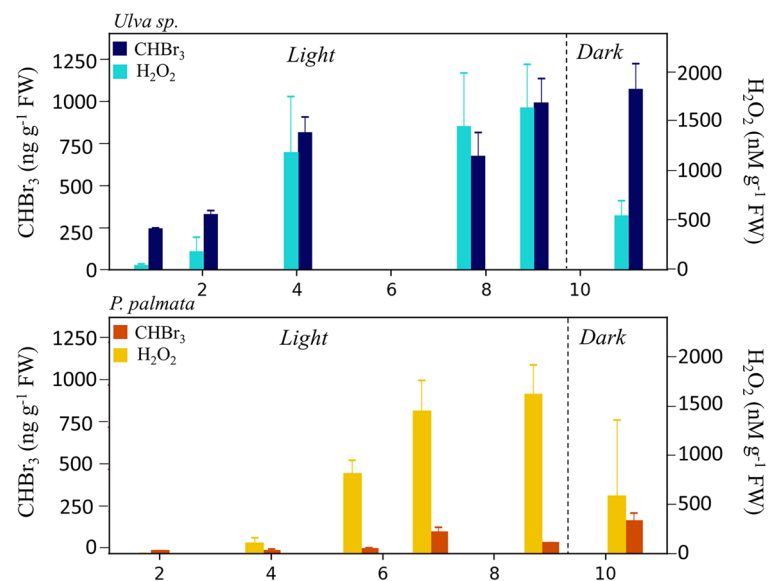
Incubation of *U. fenestrata* under both heat and grazing stress also greatly increased the concentrations of CHBr<sub>3</sub>. Seawater concentrations of CHBr<sub>3</sub> in jars of *U. fenestrata* grown at 20 °C had the most CHBr<sub>3</sub> (Fig. 6) of any condition at each timepoint and displayed an increase with length of time of incubation. Across biological replicates this resulted in CHBr<sub>3</sub> concentrations of 5030 (± 826) ng g<sup>-1</sup> FW after slightly over 6 h. *U. fenestrata*



**Figure 3.** Effect of grazing and temperature on H<sub>2</sub>O<sub>2</sub>. The evolution of H<sub>2</sub>O<sub>2</sub> in closed-top incubations of (a) *U. fenestrata* and (b) *P. palmata* over a period of approximately 9 h under three different conditions: standard (9 °C), acute heat stress at 20 °C, and when exposed to snails at 9 °C (not shown for *P. palmata*). The last hour of the incubation was in the dark. Incubations were done under 100 μmol m<sup>-2</sup> s<sup>-1</sup> light. The error bars show the ± 1 SD between biological replicates. Tabulated data available in Table S8.

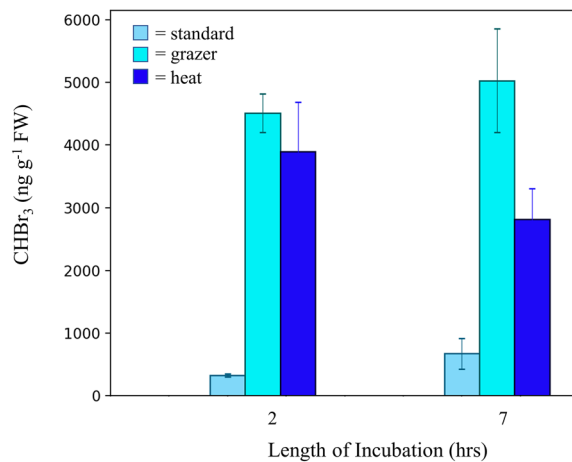


**Figure 4.** Effect of desiccation on H<sub>2</sub>O<sub>2</sub>. A comparison of seawater H<sub>2</sub>O<sub>2</sub> concentrations after introduction of seaweeds that had not been desiccated, desiccated for 2 h, and desiccated for 4 h. Data for *U. fenestrata* shown in the graph on the left, and *P. palmata* on the right. All incubations were done under 100  $\mu\text{mol m}^{-2} \text{s}^{-1}$  light. The error bars show the  $\pm 1$  SD of two biological replicates. Tabulated data available in Table S10.



**Figure 5.** Time-series data of H<sub>2</sub>O<sub>2</sub> and CHBr<sub>3</sub> from closed-top incubations of *U. fenestrata* (top) and *P. palmata* (bottom) at 9 °C in the presence of 130  $\mu\text{mol m}^{-2} \text{s}^{-1}$  light. The error bars show the  $\pm 1$  SD across three biological replicates. Tabulated data available in Table S7.

incubated with snails to induce a grazing pressure were also characterized by significantly elevated seawater CHBr<sub>3</sub> conditions, but unlike in the heat stress condition later incubations (6.3 h timepoint) showed a decline in bromoform from incubation jars sampled earlier (2.7 h). In contrast, heat stress did not lead to significantly increased CHBr<sub>3</sub> concentrations in incubation waters of *P. palmata* (after a 7 h incubation the CHBr<sub>3</sub> concentrations (nM g<sup>-1</sup> FW) were 57.53  $\pm$  18.83 under standard conditions, 55.89  $\pm$  14.84 under heat stress, and 60.12  $\pm$  21.56 under grazing stress).



**Figure 6.** Seawater bromoform concentrations accumulated in incubation jars of *U. fenestrata* under standard conditions (set as 9 °C, under heat stress at 20 °C, and in the presence of snails at 9 °C. All incubations were exposed to 100  $\mu\text{mol m}^{-2} \text{s}^{-1}$  light for the duration of the experimental timeline. The error bars show  $\pm 1$  SD of analytical and biological replicates. Tabulated data available in Table S11.

## Discussion

Here we show that *U. fenestrata* and *P. palmata* produce  $\text{H}_2\text{O}_2$  under ambient environmental conditions in the absence of stress (Fig. 1). These findings are in line with previous studies of a wide range of organisms, including a broad diversity of macroalgae<sup>26–28</sup>. The enzymatic production of  $\text{H}_2\text{O}_2$  in the absence of stress is linked to a suite of physiological processes in organisms, including cell signaling, cell differentiation, and nutrient acquisition. As such, we expected the basal levels of  $\text{H}_2\text{O}_2$  within the seaweeds here to have natural fluctuations in response to physiological activity and circadian rhythms.

Indeed, we observed diel patterns in  $\text{H}_2\text{O}_2$  concentrations in incubations of both *U. fenestrata* and *P. palmata*, ranging from 28 to 710  $\text{nM g}^{-1} \text{FW}$  (see Fig. 1). Concentrations were generally highest in the morning to early-afternoon and decreased around 15:00–16:00. A similar trend in  $\text{H}_2\text{O}_2$  but measured in greenhouse material of *Ulva rigida* over a 24-h period, and under different experimental conditions, was observed by Collén et al.<sup>48</sup>. As control incubations containing seawater without added algae did not show similar magnitudes to the seaweed incubations or significant variations with time (ranging only from 35 to 80 nM, see Fig. S1 and S2), we ascribe the observed  $\text{H}_2\text{O}_2$  pattern over the two-day timeline to the seaweed and associated microorganisms, and not to abiotic or planktonic microbe production pathways.

The aqueous dynamics of  $\text{H}_2\text{O}_2$  surrounding the seaweeds is a function of cumulative production and decay processes. Considering the ability of  $\text{H}_2\text{O}_2$  to diffuse through cell membranes, the origin of  $\text{H}_2\text{O}_2$  could be either intracellular or extracellular production pathways. To balance  $\text{H}_2\text{O}_2$  generation and maintain healthy levels of ROS, seaweed also possesses antioxidative enzymes which scavenge  $\text{H}_2\text{O}_2$ <sup>34</sup>. A strategy of the antioxidant response that is particularly strong in seaweeds is the production of halogenated hydrocarbons<sup>49</sup>. Previous studies have shown that apart from the seaweed itself, microbial communities (including bacteria, fungi, and spores of marine invertebrates) associated with the seaweed surface<sup>50–52</sup> can influence ROS dynamics in the external milieu. Species-specific differences in the relative strength of these production and decay pathways may account for the different, and generally higher concentrations of  $\text{H}_2\text{O}_2$  in incubations of *U. fenestrata* than *P. palmata*.

The shift from increasing to decreasing  $\text{H}_2\text{O}_2$  concentrations during our 2-day incubations signals the increasing strength of decay processes relative to production (Fig. 1). This change doesn't coincide directly with the light–dark cycle (light from 8:00 to 20:00) in our incubations, but the higher  $\text{H}_2\text{O}_2$  concentrations under light suggest that light-dependent processes are a primary driver of  $\text{H}_2\text{O}_2$  formation. This could occur through photosynthetic generation of NADPH which is then used as a substrate by transmembrane NADPH oxidases (NOX/DUOX) enzymes, via the Mehler reaction, or some combination. The importance of light in driving baseline  $\text{H}_2\text{O}_2$  dynamics was further supported by the observation of minimal accumulation of  $\text{H}_2\text{O}_2$  under lower light conditions (30  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) (Fig. 2).

Macroalgae in marine systems will experience a range in temperature and light conditions across growth seasons. For instance, sea-based aquaculture of *P. palmata* and *U. fenestrata* in northern Europe exposes seaweeds to natural variations in light availability, and a range of temperatures ( $\sim 0.5$  °C to 19 °C) throughout the year. To test for the response of  $\text{H}_2\text{O}_2$  to small shifts in seawater temperature we incubated individuals at 14 °C (Fig. 2). For most biological replicates of both *U. fenestrata* and *P. palmata* this led to higher accumulations of  $\text{H}_2\text{O}_2$  in the seawater after 5 h of incubation. One possible explanation for this is an increase in gross primary productivity, which has been recognized for many algal species at higher temperatures<sup>53,54</sup>.

We hypothesized that exposure to stressors would lead to significantly elevated concentrations in the seawater  $\text{H}_2\text{O}_2$ , that were distinguishable from natural fluctuations. Our results demonstrate drastic differences in the dynamics of seawater  $\text{H}_2\text{O}_2$  when *U. fenestrata* and *P. palmata* were exposed to acute heat stress (20 °C), and grazing pressure by snails (Fig. 3a,b). Herbivory and wounding are known to lead to oxidative bursts in plants<sup>23,55</sup>

a response thought to help sessile organisms fend off attackers based on observations of elevated  $\text{H}_2\text{O}_2$  in seawater leading to decreased grazing rates<sup>36</sup>. The primary source of ROS in the oxidative burst response is believed to be transmembrane NOX enzymes that coupled the oxidation of intracellular NADPH to the extracellular reduction of oxygen to superoxide and  $\text{H}_2\text{O}_2$ <sup>56</sup>. As previously seen in experiments with *U. lactuca*<sup>57</sup>, we observed that the snail *L. littorea* consumed *U. fenestrata*, leading to a significant increase in  $\text{H}_2\text{O}_2$  levels. In earlier studies, McDowell et al., (2014, 2015) found that ROS production upon wounding depends on the photosynthetic electron transport chain<sup>32,33</sup>. We observed a continued increase in seawater  $\text{H}_2\text{O}_2$  in incubations of *U. fenestrata* in the dark (see Fig. 4a), suggesting that photosynthetic activity is not requisite for  $\text{H}_2\text{O}_2$  production. However, since these measurements were made just 1.2 h after dark, there may have been continued diffusion out of the plant, or lagging  $\text{H}_2\text{O}_2$  production, and thus we cannot definitively rule out photosynthetic activity as a possible source of the observed dark  $\text{H}_2\text{O}_2$  at this time.

There was no significant difference in seawater  $\text{H}_2\text{O}_2$  concentrations when *P. palmata* was exposed to snails (*L. littorea*) from those under standard conditions (without grazers) (Fig. 3), which corresponded to the lack of observed grazing on and wounding of this species. Incubation at higher temperatures (20 °C) led to a considerable increase in  $\text{H}_2\text{O}_2$  in the seawater, with elevated production also under dark conditions. Although  $\text{H}_2\text{O}_2$  concentrations in the heat stress incubations were initially lower than under grazing pressure for *U. fenestrata*, the difference in response between the stress conditions became less notable after 6 h, perhaps pointing to a threshold  $\text{H}_2\text{O}_2$  stress level. Overall, these findings highlight that light levels are a dominant factor for  $\text{H}_2\text{O}_2$  production fluctuations under ambient conditions but become less influential in setting  $\text{H}_2\text{O}_2$  levels in the presence of heat and grazing stress.

Seaweeds may produce  $\text{CHBr}_3$  through the uptake and subsequent oxidation of bromide ( $\text{Br}^-$ ) from seawater with  $\text{H}_2\text{O}_2$ . This reaction is catalyzed by extracellular vanadium haloperoxidases (V-HPOs)<sup>43</sup>. Building on previous work demonstrating  $\text{CHBr}_3$  in incubation studies of various species of macroalgae<sup>58–60</sup>, we set out to examine possible covariations between  $\text{CHBr}_3$  and natural  $\text{H}_2\text{O}_2$ . Our experiments show that the seawater in which *U. fenestrata* were incubated displayed an increase in  $\text{CHBr}_3$  concentrations with incubation time in the light that tracked  $\text{H}_2\text{O}_2$ , while in the *P. palmata* aquaria the  $\text{CHBr}_3$  concentrations did not rise considerably. Since *P. palmata* do possess vanadium-containing bromoperoxidases (V-BrPO)<sup>61</sup>, it is possible that  $\text{H}_2\text{O}_2$  was preferentially used for the oxidation of other halides ( $\text{I}^-$ , or  $\text{Cl}^-$ ), also catalyzed by V-BrPO, or the production of brominated organic compounds other than  $\text{CHBr}_3$ . However, this would contradict previous studies which found that  $\text{CHBr}_3$  was the dominant halocarbon emitted by *P. palmata*<sup>62,63</sup>. For *U. fenestrata*, the relationship between seawater  $\text{CHBr}_3$  and  $\text{H}_2\text{O}_2$  concentrations under standard conditions can be captured by a linear trend ( $R^2 = 0.902$ ) (see Fig. S2), suggesting that species-specific predictions of total  $\text{CHBr}_3$  production could be made with knowledge of surrounding seawater  $\text{H}_2\text{O}_2$  concentrations in seaweed farms. Further experiments are required to elucidate the mechanisms which drive these species-specific variations in  $\text{CHBr}_3$  production under different conditions.

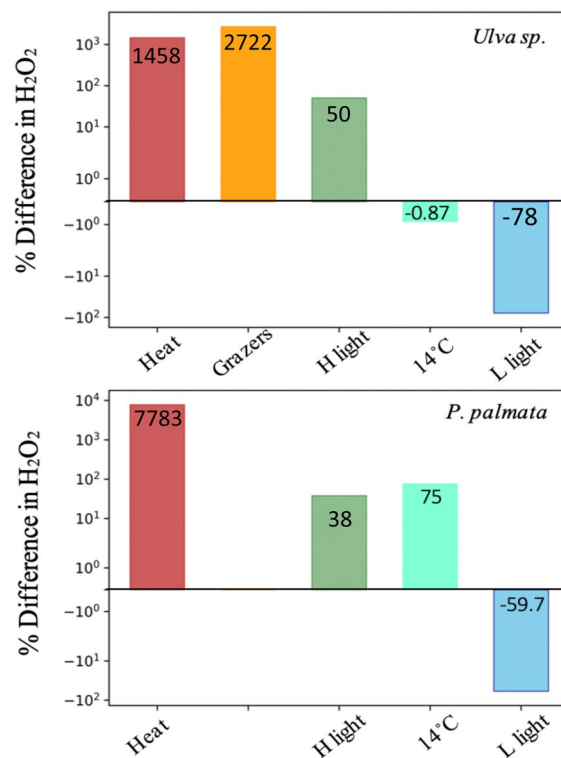
The increased production of  $\text{CHBr}_3$  has been linked to stress conditions such as nutrient depletion, and shifts in salinity, and temperature<sup>40,41</sup>. Confirming previous studies, we found that grazing and heat stress led to far greater production of  $\text{CHBr}_3$  in incubations of *U. fenestrata* (Fig. 6). The drop in  $\text{CHBr}_3$  in the grazer incubation between 2 and 6 h of incubation time could reflect biological variability, or absorptive or degradative processes occurring in the seawater. The significantly elevated  $\text{CHBr}_3$  concentrations in seawater that we witnessed for *U. fenestrata* grown under stress conditions suggests that  $\text{CHBr}_3$  could act as a secondary monitoring target in aquaculture farms of certain species. Beyond the production of  $\text{CHBr}_3$ , the elevated levels of ROS that we expect to accompany dense macroalgal growth may lead to other changes in the local coastal environment, for instance through alterations in the carbon flow, and redox state of metals.

A useful indicator of health for seaweed monitoring necessitates a marker molecule that is broadly applicable, reliably able to discern between standard and stressed conditions, and be readily measurable. Our data show that there are species-specific differences in the magnitude of  $\text{H}_2\text{O}_2$  produced under varying temperature (9 to 14 °C) and light regimes (30, 100, 130  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) that may occur during natural diel or seasonal cycles. However, as seen in Fig. 7, exposure to heat stress and grazing yield differences in seawater  $\text{H}_2\text{O}_2$  concentrations (1458 and 2722%) which are orders of magnitude larger than shifts seen in response to conditions falling within the expected natural environmental range. Elevated  $\text{H}_2\text{O}_2$  concentrations were visible within an hour of exposure to the tested stressor, which suggests that monitoring aqueous  $\text{H}_2\text{O}_2$  levels could serve an early indicator of stress conditions, and thus provide a rapid, and low-cost method to improve the biosecurity of sustainable seaweed farming in the future. We observed differences in  $\text{H}_2\text{O}_2$  and  $\text{CHBr}_3$  dynamics of *U. fenestrata* and *P. palmata* underlining the importance of characterizing expected threshold  $\text{H}_2\text{O}_2$  levels for each specific species that is being farmed. Future studies investigating time-series  $\text{H}_2\text{O}_2$  and  $\text{CHBr}_3$  levels under varying stress and disease conditions, and field-based studies to characterize the physical effects and natural seawater concentrations will help develop the robustness of  $\text{H}_2\text{O}_2$  as a monitoring method. Ultimately, the development of low-cost deployable  $\text{H}_2\text{O}_2$  and  $\text{CHBr}_3$  sensors could provide a promising strategy for monitoring the health of seaweeds in aquaculture farms.

## Methods

### Algal strains and culture conditions

This study was conducted with individuals of *P. palmata* and *U. fenestrata*, that were removed from aerated indoor cultivations at Tjärno Marine Laboratory. The original specimens for the cultivations were collected in the vicinity of the lab (58° 50.257' N 11° 2.570' E (WGS84 DDM) for *Palmaria*, and 58° 52.664' N 11° 6.804' E for *Ulva*). The *U. fenestrata* has been in cultivation for several generations (approximately for 1 year). The *P. palmata* proliferated vegetatively for approximately a 6-month period.



**Figure 7.** Percent difference in H<sub>2</sub>O<sub>2</sub>. Bars show the percent difference between stress and healthy treatments in aqueous H<sub>2</sub>O<sub>2</sub> (shown on a logarithmic y-axis) after two hours of incubation in each experimental condition (listed on x-axis) relative to standard conditions of 9 °C under 100  $\mu\text{mol m}^{-2} \text{s}^{-1}$  light. Heat: 20 °C, 100  $\mu\text{mol m}^{-2} \text{s}^{-1}$  light; Grazers: presence of snails (*Littorina littorea*), 100  $\mu\text{mol m}^{-2} \text{s}^{-1}$  light; H light (high light): 9 °C; 130  $\mu\text{mol m}^{-2} \text{s}^{-1}$  light, 14 °C: 14 °C, and 100  $\mu\text{mol m}^{-2} \text{s}^{-1}$  light; and L light (low light): 9 °C, and 30  $\mu\text{mol m}^{-2} \text{s}^{-1}$  light.

The seaweeds were grown in 90 L tanks provided with a filtered (5  $\mu\text{m}$  + UV) natural seawater without addition of nutrients in a flow through system (10–14 L h<sup>-1</sup>), where aeration provided water motion. The seaweeds were exposed to a 16:8 (L:D) cycle at an irradiance of 140  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$  (light source INDY 66 LED 60 W 4000 k 6000 lm). Both the temperature and salinity conditions in the greenhouse followed seasonal variations. Further conditions of the cultivations and methods for the molecular identification of the species are described in Toth et al.<sup>64</sup>.

### H<sub>2</sub>O<sub>2</sub> temporal dynamics

To characterize aqueous H<sub>2</sub>O<sub>2</sub> dynamics associated with the seaweeds *U. fenestrata* and *P. palmata*, we tracked the concentrations of H<sub>2</sub>O<sub>2</sub> in incubations at an ecologically relevant temperature of 9 ± 0.8 °C and light levels of 100  $\mu\text{mol m}^{-2} \text{s}^{-1}$  (12-h light and 12-h dark) over a 2-day diel cycle.

Seaweed individuals were incubated in open-top 1 L plastic aquaria containing 470 mL of filtered seawater. All aquaria were randomly distributed and placed on two shelves in a closed, temperature-controlled incubator. Lights were controlled from a timer operated from the top of the incubator to reach 100  $\mu\text{mol m}^{-2} \text{s}^{-1}$  white light. For each species, 5 aquaria with biological replicates were set up, and compared with 2 controls (containing just seawater). Aquaria were removed from the incubator for a short period of time 1–2 min for H<sub>2</sub>O<sub>2</sub> analyses and then immediately returned.

In all incubations (including those described in later sections), the pH of the seawater in each incubation vessel was measured at the start and end of each experiment but remained within the range of 8.12–8.23. At the end of each experiment the seaweeds in each incubation were blotted dry and weighed to obtain fresh weight data (grams). The weight of *U. fenestrata* in aquaria was between 3.2 and 4.5 g, and 5.2–6.7 g for *P. palmata*.

To test for differences in H<sub>2</sub>O<sub>2</sub> dynamics resulting from small shifts in temperature and light, three experiments were setup on sequential days: a comparison of *U. fenestrata* vs *P. palmata* at 9 °C under 100  $\mu\text{mol m}^{-2} \text{s}^{-1}$ , at 14 °C grown under 100  $\mu\text{mol m}^{-2} \text{s}^{-1}$ , and under low light conditions (30  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) at 9 °C. Each experiment lasted approximately 8 h, during which the seaweeds were exposed to a constant light level (either 100 or 30  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ). The incubations began between 7:00 and 8:00 in the morning. For each species five biological replicates were set up, as described above for the 2-day experiments. All experiments were conducted in the same incubator.



### Effects of temperature, grazing, and desiccation on H<sub>2</sub>O<sub>2</sub> concentrations

In heat stress experiments, the incubation temperature was set to 20 °C. This constitutes a temperature increase of ~ 11 °C from the standard cultivation, which is not likely to be realized in the natural environment but rather is meant to represent a scenario of acute stress. As in previous experiments, open-top 1 L plastic aquaria containing 470 mL of filtered seawater were prepared. To obtain the desired water temperature the aquaria were placed into the incubator 5 h prior to the start of the experiment. The seawater temperature was measured with a glass thermometer right before placing the seaweeds in to verify that the temperature was 20 °C (± 0.5 °C). Two aquaria of *U. fenestrata*, two aquaria of *P. palmata*, as well as two control aquaria that did not contain seaweeds were placed on the same shelf in the incubator under 100 μmol m<sup>-2</sup> s<sup>-1</sup> light. The experiments ran over slightly nine hours, the first eight hours of which were under light, and the last 1.2 h were in the dark (lights inside incubator toggled off on a timer). As in other experiments aquaria were briefly removed from the incubator for H<sub>2</sub>O<sub>2</sub> analysis.

Grazing experiments were conducted in a similar manner as the heat stress experiments described above but at a temperature of 9 °C, and with the addition of three *L. littorea* snails in each experimental aquarium. The snails were collected from the shore right outside the lab and were kept for two days under running seawater before being used in experiments. Two aquaria were set up for each species respectively, as well as two controls containing seawater with snails but no seaweed.

In desiccation experiments, seaweed individuals were removed from long-term cultivation tanks and left out to dry under ambient room conditions (18 °C) for 2 or 4 h respectively before being submerged in plastic 1 L aquaria containing 470 mL of filtered seawater. Two minutes after the seaweeds were submerged, the H<sub>2</sub>O<sub>2</sub> in the seawater was measured. A control was performed by removing the seaweed and directly placing it in the seawater (0 h of desiccation). Desiccation experiments were done in duplicates for each species.

### Bromofrom production

For experiments conducted for contemporaneous CHBr<sub>3</sub> and H<sub>2</sub>O<sub>2</sub> measurements, individuals were incubated in screw-top glass mason jars filled to the brim with 470 mL of filtered seawater. A piece of aluminum foil was placed over the top of the jar with the dull side facing the inside of the bottle, and the lid was tightly screwed shut paying attention that no creases formed. The purpose of the aluminum foil is to eliminate contact between the incubation water and the lid which may lead to extraction of CHBr<sub>3</sub> from the water.

Two experiments were done for contemporaneous CHBr<sub>3</sub> and H<sub>2</sub>O<sub>2</sub> analysis: a time-series experiment of both *U. fenestrata* and *P. palmata*, and a stress response experiment of *U. fenestrata*. In the time-series experiments, incubations were done at 9 °C and 130 μmol m<sup>-2</sup> s<sup>-1</sup> light. The first nine hours of the incubation were done in the presence of light, and the last two in the dark. The incubations were set up around 11:00 and dark conditions ensued at 20:00. Three incubation jars were set up per species per timepoint and were sacrificed at the end of the respective timepoint. Three controls containing just seawater but prepared in mason jars with aluminum foil lining as well were set up for each of two timepoints (1 h and 7.83 h incubation lengths). The jars were removed from the incubator at the time of measurement. Volatiles were sampled first (as described below), and then measured for H<sub>2</sub>O<sub>2</sub>. The same procedure was followed for volatile analysis in stress experiments of *U. fenestrata*. Sacrificed jars were prepared in duplicates for heat stress at 20 °C under 100 μmol m<sup>-2</sup> s<sup>-1</sup> light, and for grazing in which jars contained three *Littorina littorea* snails. These were compared with a standard condition in which individuals of *U. fenestrata* were incubated at 9 °C under 100 μmol m<sup>-2</sup> s<sup>-1</sup> light.

### Hydrogen peroxide analysis

Measurements of H<sub>2</sub>O<sub>2</sub> were made with the use of commercially available hydrogen peroxide micro- and macro-sensors (WPI ISO HPO 2, HPO 100) connected to a free radical analyzer (TBR 4100). Sensor readings were monitored in real-time through LabScribe software operated from a laptop.

Four-point calibration curves were made at the start of every day relating a known concentration of H<sub>2</sub>O<sub>2</sub> (nM) to a current (pA). Standard solutions were prepared with a 30% H<sub>2</sub>O<sub>2</sub> solution (VWR Chemicals) in filtered seawater which was also used for the incubations. Care was taken to calibrate the sensor in solutions at the temperature of the experiment being run to avoid any temperature-related effects in measurement.

Incubation waters were analyzed for H<sub>2</sub>O<sub>2</sub> concentration by submerging the tip of the sensor into the 1L aquarium or mason jar for a duration of approximately 1 min. To calculate the H<sub>2</sub>O<sub>2</sub> concentration in the incubation water the current readings between 20 and 60 s were averaged, allowing the first 20 s for stabilization of the sensor. Between measurements in different incubation containers the sensor was briefly cleaned by swirling it in deionized water.

### Statistical analyses of H<sub>2</sub>O<sub>2</sub> measurements

To determine whether experimental treatments (e.g. *Ulva* Heat Stress, *Palmaria* Grazing, *Ulva* Standard, *Palmaria* 14C, etc.) resulted in differences in H<sub>2</sub>O<sub>2</sub> that were statistically significant (using a significance level of 0.05) from one another, one-way analysis of variance (ANOVA) were performed at each timepoint to examine the differences in treatment. If the timepoints of comparison did not exactly align for each treatment, values were interpolated to predict the H<sub>2</sub>O<sub>2</sub> concentration at the chosen timepoint of examination. In situations where the results of the ANOVA revealed that some treatments were not significantly different (a *P* > 0.05), individual Student's T-Tests were done to compare means between individual treatments at specific timepoints. Levene's Tests were done to confirm equality in variances which is an assumption of a Student's T-Test. All statistical analyses were done in the software program JMP. The results of the tests are given in the Supplementary Information.

## Volatile organic compound sampling and analysis

At each experimental timepoint, two samples of water for volatile analysis were removed from the sealed incubation jars (covered with aluminum foil between water and lid) and transferred to 40 mL volatile organic analysis (VOA) vials. Samples were acidified to a pH < 2 with approximately 530  $\mu$ L of a 0.75 M HCl solution and stored in the cold (4 °C) and dark.

The halocarbon content of the sampled incubation seawater was analyzed within two weeks of collection using a purge and trap coupled with gas chromatography (Agilent 7890B) with electron capture detection (GC-ECD). The calculated maximum holding time for acid-preserved volatiles in water stored in 40 mL borosilicate vials was previously estimated at approximately 112 days<sup>65</sup>, which we are well under here. Volatiles were purged for 12 min with UHP nitrogen at a flow rate of 40 mL min<sup>-1</sup>. Upon completion of trapping, the sample was desorbed onto the column at 150 °C temp. The GC temperature was set at 45 °C during the injection and held for 5 min. The temperature was then ramped at 8 °C min<sup>-1</sup> to 130 °C, at 12 °C min<sup>-1</sup> to 150 °C and held for 2 min, and then at 15 °C min<sup>-1</sup> until 180 °C and held for 2 min. Samples were investigated primarily for bromoform content, which was identified by retention time and comparison to standards. The concentration of bromoform was quantified using a standard curve prepared with a commercially available standard in methanol.

## Data availability

Data available in tabulated form in the Supplementary Information.

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## References

- United Nations, Department of Economic and Social Affairs, Population Division. World Population Prospects 2019: Data Booklet. ST/ESA/SER. A/424 (2019).
- Duarte, C. M., Bruhn, A. & Krause-Jensen, D. A seaweed aquaculture imperative to meet global sustainability targets. *Nat. Sustain.* **5**(3), 185–193. <https://doi.org/10.1038/s41893-021-00773-9> (2021).
- Holdt, S. L. & Kraan, S. Bioactive compounds in seaweed: Functional food applications and legislation. *J. Appl. Phycol.* **23**(3), 543–597. <https://doi.org/10.1007/s10811-010-9632-5> (2011).
- Makkar, H. P. S. *et al.* Seaweeds for livestock diets: A review. *Anim. Feed Sci. Technol.* **212**, 1–17. <https://doi.org/10.1016/j.anifeeds.2015.09.018> (2016).
- Guedes, A. C., Amaro, H. M., Sousa-Pinto, I. & Malcata, F. X. In *Biofuels from Algae* (eds Pandey, A. *et al.*) 397–433 (Elsevier, 2019).
- Cottier-Cook, E. J. *et al.* *Safeguarding the Future of the Global Seaweed Aquaculture Industry* (UNU-INWEH and SAMS, 2016).
- García-Poza, S. *et al.* The evolution road of seaweed aquaculture: Cultivation technologies and the Industry 4.0. *Int. J. Environ. Res. Public Health* **17**(18), 6528. <https://doi.org/10.3390/ijerph17186528> (2020).
- Campbell, I. *et al.* The Environmental risks associated with the development of seaweed farming in Europe—Prioritizing key knowledge gaps. *Front. Mar. Sci.* <https://doi.org/10.3389/fmars.2019.00107> (2019).
- Tsukidate, J. 1991. *Seaweed disease. Fish health management in Asia-Pacific*. Report on a regional study and workshop on fish disease and fish health management, pp. 397–408. Bangkok, Thailand, ADB/NACA.
- Kang, J. W. Some seaweed diseases occurring at seaweed farms along the South-Eastern coast of Korea. *Agris* **14**, 165–170 (1982).
- Endo, H. *et al.* Ocean warming combined with nutrient enrichment increases the risk of herbivory during cultivation of the marine macroalga *Undaria Pinnatifida*. *ICES J. Mar. Sci.* **78**, 402–409. <https://doi.org/10.1093/icesjms/fsaa069> (2021).
- Kim, G. H., Moon, K.-H., Kim, J.-Y., Shim, J. & Klochkova, T. A. A reevaluation of algal diseases in Korean Pyropia (Porphyra) sea farms and their economic impact. *Algae* **29**(4), 249–265. <https://doi.org/10.4490/algae.2014.29.4.249> (2014).
- Sugumaran, R. *et al.* A retrospective review of global commercial seaweed production—Current challenges, biosecurity and mitigation measures and prospects. *Int. J. Environ. Res. Public Health* **19**(12), 7087. <https://doi.org/10.3390/ijerph19127087> (2022).
- Hansel, C. M. & Diaz, J. M. Production of extracellular reactive oxygen species by marine biota. *Ann. Rev. Mar. Sci.* **13**(1), 177–200. <https://doi.org/10.1146/annurev-marine-041320-102550> (2021).
- Smirnov, N. & Arnaud, D. Hydrogen peroxide metabolism and functions in plants. *New Phytologist* **221**(3), 1197–1214. <https://doi.org/10.1111/nph.15488> (2019).
- Caverzan, A. *et al.* Plant responses to stresses: Role of ascorbate peroxidase in the antioxidant protection. *Genet. Mol. Biol.* **35**(4 suppl 1), 1011–1019. <https://doi.org/10.1590/S1415-47572012000600016> (2012).
- Sharma, P., Jha, A. B., Dubey, R. S. & Pessarakli, M. Reactive oxygen species, oxidative damage, and antioxidative defense mechanism in plants under stressful conditions. *J. Bot.* **2012**, 1–26. <https://doi.org/10.1155/2012/217037> (2012).
- Ugya, A. Y., Imam, T. S., Li, A., Ma, J. & Hua, X. Antioxidant response mechanism of freshwater microalgae species to reactive oxygen species production: A mini review. *Chem. Ecol.* **36**(2), 174–193. <https://doi.org/10.1080/02757540.2019.1688308> (2020).
- Gaikwad, R., Thangaraj, P. R. & Sen, A. K. Direct and rapid measurement of hydrogen peroxide in human blood using a microfluidic device. *Sci. Rep.* **11**(1), 2960. <https://doi.org/10.1038/s41598-021-82623-4> (2021).
- Maier, D. *et al.* Toward continuous monitoring of breath biochemistry: A paper-based wearable sensor for real-time hydrogen peroxide measurement in simulated breath. *ACS Sens.* **4**(11), 2945–2951 (2019).
- Ross, C. & van Alstyne, K. L. Intraspecific variation in stress-induced hydrogen peroxide scavenging by the Ulvoid macroalga *Ulva Lactuca*. *J. Phycol.* **43**(3), 466–474. <https://doi.org/10.1111/j.1529-8817.2007.00346.x> (2007).
- Küpper, F., Müller, D. G., Peters, A. F., Kloareg, B. & Potin, P. Oligoalginate recognition and oxidative burst play a key role in natural and induced resistance of the sporophytes of Laminariales. *J. Chem. Ecol.* **8**, 20572081. <https://doi.org/10.1023/A:1020706129624> (2002).
- McDowell, R. E., Amsler, C. D., Dickinson, D. A., McClintock, J. B. & Baker, B. J. Reactive oxygen species and the Antarctic macroalgal wound response. *J. Phycol.* **50**(1), 71–80. <https://doi.org/10.1111/jpy.12127> (2014).
- Weinberger, F. Pathogen-induced Defense and innate immunity in macroalgae. *Biol. Bull.* **213**(3), 290–302. <https://doi.org/10.2307/25066646> (2007).
- Asaeda, T., Rahman, M., Liping, X. & Schoelynck, J. Hydrogen peroxide variation patterns as abiotic stress responses of *Egeria densa*. *Front. Plant Sci.* <https://doi.org/10.3389/fpls.2022.855477> (2022).
- Collén, J., Ekdahl, A., Abrahamsson, K. & Pedersén, M. The involvement of hydrogen peroxide in the production of volatile halogenated compounds by *Meristiella gelidium*. *Phytochemistry* **36**(5), 1197–1202. [https://doi.org/10.1016/S0031-9422\(00\)89637-5](https://doi.org/10.1016/S0031-9422(00)89637-5) (1994).
- Collén, J. & Pedersén, M. Production, scavenging and toxicity of hydrogen peroxide in the green seaweed *Ulva rigida*. *Eur. J. Phycol.* **31**(3), 265–271. <https://doi.org/10.1080/09670269600651471> (1996).

28. Mtolera, M. S. P. *et al.* Stress-induced production of volatile halogenated organic compounds in *Euclima denticulatum* (Rhodophyta) caused by elevated pH and high light intensities. *Eur. J. Phycol.* **31**(1), 89–95. <https://doi.org/10.1080/09670269006051241> (1996).
29. van Hees, D. H. & van Alstyne, K. L. Effects of emersion, temperature, dopamine, and hypoxia on the accumulation of extracellular oxidants surrounding the bloom-forming seaweeds *Ulva lactuca* and *Ulvaria obscura*. *J. Exp. Mar. Biol. Ecol.* **448**, 207–213. <https://doi.org/10.1016/j.jembe.2013.07.013> (2013).
30. Weinberger, F., Friedlander, M. & Hoppe, H.-G. Oligoagars elicit a physiological response in *Gracilaria conferta* (Rhodophyta). *J. Phycol.* **35**(4), 747–755. <https://doi.org/10.1046/j.1529-8817.1999.3540747.x> (1999).
31. Weinberger, F. & Friedlander, M. Response of *Gracilaria conferta* (Rhodophyta) to Oligoagars results in Defense against agar-degrading epiphytes. *J. Phycol.* **36**(6), 1079–1086. <https://doi.org/10.1046/j.1529-8817.2000.00003.x> (2000).
32. McDowell, R. E., Amsler, M. O., Li, Q., Lancaster, J. R. & Amsler, C. D. The immediate wound-induced oxidative burst of *Saccharina latissima* depends on light via photosynthetic electron transport. *J. Phycol.* **51**(3), 431–441. <https://doi.org/10.1111/jpy.12302> (2015).
33. McDowell, R. E., Amsler, C. D., Amsler, M. O., Li, Q. & Lancaster, J. R. Control of grazing by light availability via light-dependent, wound-induced metabolites: The role of reactive oxygen species. *J. Exp. Mar. Biol. Ecol.* **477**, 86–91. <https://doi.org/10.1016/j.jembe.2016.01.011> (2016).
34. Dring, M. J. Stress resistance and disease resistance in seaweeds: The role of reactive oxygen metabolism. *Adv. Bot. Res.* [https://doi.org/10.1016/S0065-2296\(05\)43004-9](https://doi.org/10.1016/S0065-2296(05)43004-9) (2005).
35. Steinberg, P. D. & de Nys, R. Chemical mediation of colonization of seaweed surfaces. *J. Phycol.* **38**(4), 621–629. <https://doi.org/10.1046/j.1529-8817.2002.02042.x> (2002).
36. McDowell, R. E., Amsler, C. D., McClintock, J. B. & Baker, B. J. Reactive oxygen species as a marine grazing defense: H<sub>2</sub>O<sub>2</sub> and wounded *Ascoseira mirabilis* both inhibit feeding by an amphipod grazer. *J. Exp. Mar. Biol. Ecol.* **458**, 34–38. <https://doi.org/10.1016/j.jembe.2014.04.012> (2014).
37. Kumar, Y. N. *et al.* Impact of elevated temperature on the physiological and biochemical responses of *Kappaphycus alvarezii* (Rhodophyta). *PLoS ONE* **15**(9), e0239097. <https://doi.org/10.1371/journal.pone.0239097> (2020).
38. Engel, A., Rigby, M., Burkholder, J. B., Fernandez, R. P., Froidevaux, L., Hall, B. D., Hossaini, R., Saito, T., Vollmer, M. K. & Yao, B. Update on Ozone-Depleting Substances (ODSs) and Other Gases of Interest to the Montreal Protocol, Chapter 1 in Scientific Assessment of Ozone Depletion: 2018, Global Ozone Research and Monitoring Project – Report No. 58 (World Meteorological Organization, 2018).
39. Zhang, J., Wuebbles, D. J., Kinnison, D. E. & Saiz-Lopez, A. Revising the ozone depletion potentials metric for short-lived chemicals such as CF<sub>3</sub>I and CH<sub>3</sub>I. *J. Geophys. Res. Atmos.* **125**, 9. <https://doi.org/10.1029/2020JD032414> (2020).
40. Bondu, S., Cocquempot, B., Deslandes, E. & Morin, P. Effects of salt and light stress on the release of volatile halogenated organic compounds by *Solieria chordalis*: A laboratory incubation study. *Botm* **51**(6), 485–492. <https://doi.org/10.1515/BOT.2008.056> (2008).
41. Mata, L., Gaspar, H. & Santos, R. Carbon/nutrient balance in relation to biomass production and halogenated compound content in the red alga *Asparagopsis taxiformis* (bonnemaisoniaceae). *J. Phycol.* **48**(1), 248–253. <https://doi.org/10.1111/j.1529-8817.2011.01083.x> (2012).
42. Paul, C. & Pohnert, G. Production and role of volatile halogenated compounds from marine algae. *Nat. Prod. Rep.* **28**(2), 186–195. <https://doi.org/10.1039/C0NP00043D> (2011).
43. Wever, R. & van der Horst, M. A. The role of vanadium haloperoxidases in the formation of volatile brominated compounds and their impact on the environment. *Dalton Trans.* **42**(33), 11778. <https://doi.org/10.1039/c3dt50525a> (2013).
44. Leedham, E. C. *et al.* Emission of atmospherically significant halocarbons by naturally occurring and farmed tropical macroalgae. *Biogeosciences* **10**(6), 3615–3633. <https://doi.org/10.5194/bg-10-3615-2013> (2013).
45. Grote, B. Recent developments in aquaculture of *Palmaria palmata* (Linnaeus) (Weber & Mohr 1805): cultivation and uses. *Rev. Aquacult.* **11**(1), 25–41. <https://doi.org/10.1111/raq.12224> (2019).
46. Zertuche-González, J. A. *et al.* Seasonal and interannual production of sea lettuce (*Ulva* sp.) in outdoor cultures based on commercial size ponds. *J. World Aquacul. Soc.* **52**(5), 1047–1058. <https://doi.org/10.1111/jwas.12773> (2021).
47. Steinhagen, S. *et al.* Sustainable large-scale aquaculture of the northern hemisphere sea lettuce, *Ulva fenestrata*, in an off-shore seafarm. *J. Mar. Sci. Eng.* **9**, 615. <https://doi.org/10.3390/jmse9060615> (2021).
48. Collén, J., Del Río, M. J., García-Reina, G. & Pedersen, M. Photosynthetic production of hydrogen peroxide by *Ulva rigida* C. Ag. (Chlorophyta). *Planta* **196**(2), 225–230 (1995).
49. Küpper, F. C. *et al.* Iodine uptake in Laminariales involves extracellular, haloperoxidase-mediated oxidation of iodide. *Planta* **207**(2), 163–171. <https://doi.org/10.1007/s004250050469> (1998).
50. Lachnit, T., Blümel, M., Imhoff, J. & Wahl, M. Specific epibacterial communities on macroalgae: Phylogeny matters more than habitat. *Aquat. Biol.* **5**(2), 181–186. <https://doi.org/10.3354/ab00149> (2009).
51. Goecke, F., Labes, A., Wiese, J. & Imhoff, J. Chemical interactions between marine macroalgae and bacteria. *Mar. Ecol. Progress Ser.* **409**, 267–299. <https://doi.org/10.3354/meps08607> (2010).
52. Burke, C., Steinberg, P., Rusch, D., Kjelleberg, S. & Thomas, T. Bacterial community assembly based on functional genes rather than species. *Proc. Natl. Acad. Sci.* **108**(34), 14288–14293. <https://doi.org/10.1073/pnas.1101591108> (2011).
53. Davison, I. R. Environmental effects on algal photosynthesis: Temperature. *J. Phycol.* **27**(1), 2–8. <https://doi.org/10.1111/j.0022-3646.1991.00002.x> (1991).
54. Lee, K.-S., Park, S. R. & Kim, Y. K. Effects of irradiance, temperature, and nutrients on growth dynamics of seagrasses: A review. *J. Exp. Mar. Biol. Ecol.* **350**(1–2), 144–175. <https://doi.org/10.1016/j.jembe.2007.06.016> (2007).
55. Suzuki, N. & Mittler, R. Reactive oxygen species-dependent wound responses in animals and plants. *Free Radic. Biol. Med.* **53**(12), 2269–2276. <https://doi.org/10.1016/j.freeradbiomed.2012.10.538> (2012).
56. Bedard, K. & Krause, K.-H. The NOX family of ROS-generating NADPH oxidases: Physiology and pathophysiology. *Physiol. Rev.* **87**(1), 245–313. <https://doi.org/10.1152/physrev.00044.2005> (2007).
57. Peckol, P. & Putnam, A. B. Differential toxic effects of *Ulva lactuca* (Chlorophyta) on the herbivorous gastropods, *Littorina littorea* and *L. obtusata* (Mollusca). *J. Phycol.* **53**(2), 361–367. <https://doi.org/10.1111/jpy.12507> (2017).
58. Carpenter, L. J. & Liss, P. S. On temperate sources of bromoform and other reactive organic bromine gases. *J. Geophys. Res. Atmos.* **105**(D16), 20539–20547. <https://doi.org/10.1029/2000JD900242> (2000).
59. Gschwend, P. M., MacFarlane, J. K. & Newman, K. A. Volatile halogenated organic compounds released to seawater from temperate marine macroalgae. *Science* **227**(4690), 1033–1035. <https://doi.org/10.1126/science.227.4690.1033> (1985).
60. Manley, S. L. & Dastoor, M. N. Methyl iodide (CH<sub>3</sub>I) production by kelp and associated microbes. *Mar. Biol.* **98**(4), 477–482. <https://doi.org/10.1007/BF00391538> (1988).
61. Bukin, V. A., Gladilin, A. K., Levitskii, V. Y., Guseva, O. A. & Levashov, A. V. Isolation, purification, and characterization of a vanadium-containing bromoperoxidase from red algae *Palmaria palmata*. *Appl. Biochem. Microbiol.* **33**, 241–248 (1997).
62. Bravo-Linares, C. M., Mudge, S. M. & Loyola-Sepulveda, R. H. Production of volatile organic compounds (VOCs) by temperate macroalgae. The use of solid phase microextraction (SPME) coupled to GC-MS as method of analysis. *J. Chil. Chem. Soc.* **55**, 227–332 (2010).

63. Keng, F.S.-L. *et al.* The emission of volatile halocarbons by seaweeds and their response towards environmental changes. *J. Appl. Phycol.* **32**(2), 1377–1394. <https://doi.org/10.1007/s10811-019-02026-x> (2020).
64. Toth, G. B. *et al.* Effects of irradiance, temperature, nutrients, and pCO<sub>2</sub> on the growth and biochemical composition of cultivated *Ulva fenestrata*. *J. Appl. Phycol.* **32**, 3243–3254. <https://doi.org/10.1007/s10811-020-02155-8> (2020).
65. Maskarinec, M. P. *et al.* Stability of volatile organic compounds in environmental water samples during transport and storage. *Environ. Sci. Technol.* **24**(11), 1665–1670. <https://doi.org/10.1021/es00081a006> (1990).

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### Author contributions

L.T. and C.M.H. conceived the experiment(s), L.T. and G.T. set-up the experiments, and L.T. conducted the experiments and analyzed the results. All authors contributing to the writing of the manuscript.

### Competing interests

The authors declare no competing interests.

### Additional information

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