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RESEARCH

The influence of light stress on bromoform synthesis and concentration in the red seaweed *Asparagopsis taxiformis*

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Abstract

The inclusion *Asparagopsis* spp. into the diet of ruminant animals has produced compelling data regarding the mitigation of agricultural methane emissions. This reduction is achieved via the action of brominated halogenated compounds, predominantly bromoform, which act to inhibit methanogenic enzymes in ruminant digestion. As such, there is great interest in the mass cultivation of *Asparagopsis* for use as a dietary supplement for livestock. However, data are still lacking on the basic biology of *Asparagopsis* relating to factors that influence the synthesis of bromoform, the key bioactive compound of interest. One of the two precursors for bromoform biosynthesis is hydrogen peroxide, while the other is bromide, a naturally occurring ion in seawater. Hydrogen peroxide is generated internally within the alga and can be stimulated by abiotic stress. Currently, the influence of temperature and external hydrogen peroxide addition on bromoform dynamics have been explored. The aim of this study is to explore how the stimulation of hydrogen peroxide by the application of light stress influences the dynamics of bromoform precursor uptake and production, as well as how this may drive changes in bromoform concentration and the persistence of gland cells, the cellular structures where bromoform is stored. While provision of light stress significantly stimulated an increase in hydrogen peroxide production, bromide dynamics were also significantly influenced, resulting in net bromide release, rather than uptake. Further, bromoform concentrations in algal tissue immediately declined after exposure to high light, from 4.5% to 2% (dry weight), while gland cell abundance declined from 95% to around 60%. Here we present data for dramatic alterations in bromoform dynamics after exposure to moderate increases in light intensity. These findings are strongly applicable to commercial *Asparagopsis* cultivation and will contribute to optimising algal quality during cultivation and harvest.

Keywords Aquaculture · Bromide · Gland cell · Methane mitigation · Ruminant · Seaweed

Introduction

The inclusion of the red alga *Asparagopsis taxiformis* (Delile) Trevisan in the diet of ruminant animals has been shown to inhibit enteric methane emissions dramatically in both in vivo (Roque et al. 2019) and in vitro (Machado et al. 2014) studies. While a suite of brominated halocarbons are responsible for this inhibition, bromoform makes up the greatest proportion of this group in *A. taxiformis,* while also serving marine chemical ecological function as an antimicrobial defence (Paul et al. 2006). As such, bromoform is the key bioactive compound of interest in driving methane emission reductions in cattle. The need to produce *Asparagopsis* tissue with consistently high bromoform has been identified as being crucial for the development of this industry (Glasson et al. 2022). As such, in order to make a meaningful reduction of methane emissions arising from ruminant animals, it is necessary to optimise *A. taxiformis* cultivation conditions to maximise biomass production as well as to understand the factors influencing bromoform synthesis, abundance and stability in farmed algal tissue. As with many species of red algae, *Asparagopsis* has a triphasic life cycle with heteromorphic alternation of generations. While both the larger and more complex gametophyte stage as well as the filamentous tetrasporophyte stage have been shown to

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reduce methane emissions in ruminants, land-based cultivation efforts have largely focused on the tetrasporophyte due to ease of growth via vegetative propagation. As this industry develops there is an urgent need to optimize the production of bromoform in cultivation settings. Increasing the bromoform content of the alga will reduce the total biomass required. As such, driving cultivation systems towards high bromoform levels may be equally important as maximising sheer biomass production alone.

While bromoform is a secondary metabolite produced by many macroalgae, the exceptionally high concentrations of this compound in *Asparagopsis* spp. occur due to the presence of unique cell structures known as gland cells, found in a small group of red algae (Womersley 1996). These structures allow for the internal accumulation, concentration and safe storage of volatile and potentially harmful compounds such as bromoform, beyond that of other macroalgae. In *Asparagopsis* these gland cells are connected to the outer wall of the pericentral cell by the presence of a stalk cell (Paul et al. 2006), which is thought to allow the release of stored metabolites from the alga. Paul et al. (2006) also note that gland cell size is highly variable, and additional reports suggest that there is a relationship between gland cell abundance and total bromoform content (Marshall et al. 2003). However, explorations of the impact of stress on gland cell dynamics and concurrent influence on bromoform production are currently lacking.

In terms of bromoform synthesis in *Asparagopsis*, the two precursors, bromide and hydrogen peroxide, are converted intracellularly to hypohalous acid and then bromoform (Beissner et al. 1981), catalysed by haloperoxidase enzymes (Butler and Carter-Franklin 2004; Thapa et al. 2020). Of these two precursors, bromide is abundantly available in oceanic water (Burton 1996) at concentrations of around 65 mg L^{-1} (Redondo and Lomax 2001). The requirement for bromide in bromoform synthesis has clearly been shown, where bromide deficiency results in a failure to produced gland cells (Paul et al. 2006). On the other hand, hydrogen peroxide concentrations in ambient seawater are low and variable, detectable on the nano-molar scale, and are assumed to be present predominantly in surface waters as a result of photooxidation (Cooper et al. 1988) by organisms such as phytoplankton. While bromide is taken up consistently by macroalgae in coastal waters (Christensen 2020), hydrogen peroxide is most likely produced internally via the formation of reactive oxygen species (ROS) (Cooper et al. 1988).

In plants and algae ROS formation is triggered by a range of stressors such as high light intensity, UV exposure and thermal extremes. While ROS have roles in cell signalling relating to basic functions such as growth and development, high levels of ROS are detrimental, resulting in damage to DNA and proteins, which in severe levels can result in cell death. Such damage occurs when the production of ROS, such as hydrogen peroxide, exceeds the ability for the cell to dissipate or scavenge ROS. As a result of such high light intensity, it has been observed that the production of halocarbons such as bromoform, by *Asparagopsis* spp. increases, as detected in the media around the algae under irradiances of 330 µmol photons m^{-2} s^{-1} compared to 30 µmol photons m^{-2} s^{-1} in laboratory conditions (Marshall et al. 1999). The involvement of hydrogen peroxide in bromoform synthesis has been demonstrated by Mata et al. (2011), where the external provision of hydrogen peroxide into the media caused an increase in the production of halogenated metabolites on the short term. The manipulation of temperature has also been seen to influence the growth and bromoform dynamics of *A. taxiformis,* where elevations in temperature were seen to cause reductions in botj halogenated compound concentration and growth rates (Mata et al. 2017). Currently, observations on bromoform production on the short term as measures of immediate responses to acute stressors are currently absent. Lastly, the influence of light stress on the production and uptake of the precursors relating to bromoform synthesis in *Asparagopsis* is unexplored to date.

The requirement of hydrogen peroxide in bromoform synthesis suggests a link between oxidative stress and the production of this metabolite. The aim of this study is to determine to what extent hydrogen peroxide concentrations vary as a result of light stress in *A. taxiformis*, as well as how the application of high light influences the uptake of bromide, and ultimately how light stress influences the production of bromoform. We hypothesise that increases in light intensity will stimulate the production of hydrogen peroxide, and as a result increasing bromide uptake and bromoform synthesis.

Methods

Experimental conditions

Asparagopsis taxiformis (Lineage 2) sporophytes were originally collected in the field on 1 January 2019, in San Diego, CA (32°51′59.2"N, 117°15′16.8"W), brought into the lab at Scripps Institution of Oceanography where they were sorted and cleaned using a dissecting microscope. Lineage was determined using the cox2-3 intergenic spacer, however only lineage 2 has been observed in the San Diego area to date. Individual branches were excised with surgical scissors under a dissecting microscope and were grown in well plates in sterilised seawater at 20 °C and purified through a number of steps of cutting clean new tips and discarding fouled material over the course of several months. After stable cultures were developed, they were kept in autoclaved seawater pumped from the Scripps Pier at densities of 8–10 g L^{-1} fresh weight and augmented with 100 µL L^{-1} per week of F/2 medium (Guillard 1975) under 12/12 day/night lights

kept at a maximal irradiance of 50 µmol photons m^{-2} s^{-1}. Water was changed biweekly for all algal cultures. At the beginning of the experimental work, sporophytes were transferred to 250 mL Erlenmeyer flasks containing 200 mL of nutrient enriched medium (F/2), filtered to 0.2 µm and UV treated, at a density of 1 g L fresh weight under 25 µmol photons m^{-2} s^{-1}, 20 °C and gentle aeration for 24 h, with a total of 28 flasks (n = 3 per time point) prior to experimental exposure. Immediately prior to providing elevated light conditions three flasks were sampled for hydrogen peroxide, bromoform and bromide content analysis to provide pre-exposure baseline levels. Light intensity was increased without ramping to 700 µmol photons m^{-2} s^{-1} (using Hydra 64HD LED lamps, AquaIllumination, USA) and flasks were sampled at time intervals of 1, 10, 30, 60 and 240 min post-exposure. Since we anticipated the responses to light stress to occur very quickly after irradiance was increased, these sampling times were selected to allow us to study the immediate effects of hydrogen peroxide stimulation on bromoform and gland cell dynamics. Further, we wished to explore whether these changes in bromoform concentration may occur fast enough to be problematic in a cultivation or harvest situation, where algae may undergo sudden but short-lived changes in light intensity, such as changes in density due to harvest or transfer between cultivation tanks.

Hydrogen peroxide analysis

Hydrogen peroxide concentration in *A. taxiformis* tissue was assessed at each timepoint using an Amplex Red hydrogen peroxide/peroxidase assay kit (Invitrogen, USA) where the suggested protocol for hydrogen peroxide quantification was followed precisely. *Asparagopsis taxiformis* tissue was harvested, blotted dry and 150 mg of fresh tissue was extracted via bead beating in 1.5 mL 5% trichloroacetic acid and 45 mg activated charcoal. The extract was centrifuged at 14,000 rpm for 10 min, supernatant was then transferred to a new tube and centrifuged for a further 5 min before filtration at 45 µm. A standard curve was produced to determine hydrogen peroxide concentrations of 0 to 10 µM. 50 µL of samples and standards were transferred to a 96 well plate and 50 µL of Amplex Red reagent and horseradish peroxidase working solution was added. Samples were incubated in darkness at room temperature for 30 min prior to analysis of absorbance at 560 nm.

Bromide analysis

Two separate bromide uptake studies were conducted, one at 1, 10, 30, 60 and 240 min post-exposure to 700 µmol photons m^{-2} s^{-1}, the other to assess bromide uptake under a range of light conditions of 25, 100, 250 and 500 µmol photons m^{-2} s^{-1} after 240 min. The post-light exposure samples were collected from the ambient seawater from the flasks in which the hydrogen peroxide trials were conducted, while the 25, 100, 250 and 500 µmol photons m^{-2} s^{-1} light intensity experiments were conducted after, to further shed light on bromide dynamics under sudden light elevation.

Bromide concentrations in seawater were measured using the phenol red colorimetric method exactly as outlined by Christensen (2020) where 0.3 mL of sample seawater was added to a 1.5 mL microcentrifuge tube along with 0.75 mL acetate buffer solution and 0.5 mL phenol red. Samples were chlorinated with 1 mL chloramine-T solution for exactly 10 s after which the sample solution was dechlorinated with 1 mL sodium thiosulfate. The samples were then read at 590 nm against a reagent blank and bromide concentrations were determined from a calibration curve using bromide standards (0–100 mg Br^{-} L^{-1}).

Bromoform analysis

Fresh tissue was strained from the culture flasks, blotted dry and immediately frozen at -80 °C prior to analysis. Samples were shipped on dry ice overnight to Georgia Institute of Technology where the tissue was then lyophilised, extracted and bromoform content was determined via GC–MS as outlined by Thapa et al. (2020).

Gland cell quantification

Gland cells were quantified from the 6-10th row of pericentral cells from the apical tip of each branch, ensuring the same cells were counted regardless of age, while avoiding the more recently developed cells which are smaller with absent or lesser developed gland cells. For each replicate flask a small portion of tissue was randomly collected and divided into four subsamples. For each subsample gland cells were counted in four separate branches, where the maximum number of cells from each branch could be 15, or 60 for each subsample. In total, 240 pericentral cells were assessed for gland cell presence in each biological replicate flask (n = 3 per timepoint) across the four subsamples.

Statistical analysis

All data were analysed using a one-way analysis of variance (ANOVA) after data were tested for normality and to meet the assumptions of ANOVA regarding homogeneity of variance. Where significant effects were detected, Student–Newman–Keuls post-hoc testing was performed to investigate differences between means among treatments. Logit transformation was applied to percent data prior to analysis.

Results

Hydrogen peroxide production

After exposure to elevated light conditions of 700 µmol photons m^{-2} s^{-1}, there was an immediate increase in hydrogen peroxide concentration in *A. taxiformis* tissue (Fig. 1, $F(5,12) = 24.45$, $P < 0.001$), rising from a pre-exposure level of 3.9 µM to a mean of 5.5 µM after one minute. Hydrogen peroxide concentrations declined significantly after 30 min compared to one minute but were not reduced to pre-exposure baseline levels. Concentrations increased back to initial 1 min exposure levels after one hour and did not significantly vary after four hours of exposure.

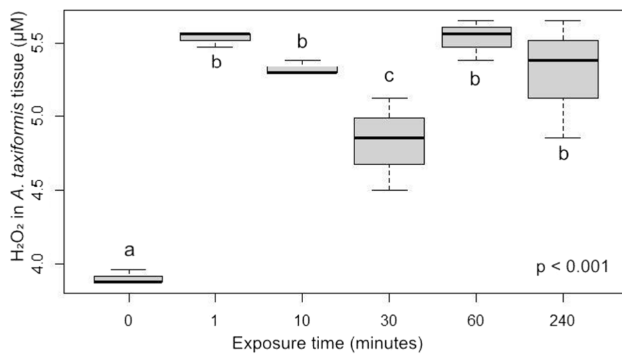

Fig. 1 Concentration of hydrogen peroxide (µM) in *A. taxiformis* tissue from one minute to four hours, and a pre-exposure baseline, after exposure to 700 µmol photons m^{-2} s^{-1}. Box plots display median values (thick black line), interquartile range between Q1 and Q3 (upper and lower limits of grey box), and maximum and minimum data points (whiskers). Lower case letters represent significant differences across time points

Bromide dynamics

Upon exposure to 700 µmol photons m^{-2} s^{-1}, there was a significant increase in bromide content in the seawater media (Fig. 2, $F(5,12) = 6.2$, $P = 0.005$). Bromide concentrations increased from 22 mg L^{-1} in the media prior to high light exposure, to a maximum of 29 mg L^{-1} after one hour of exposure. Significant increases of bromide in the media were observed after 10 min of exposure, but there were no further significant elevations beyond this time point.

Bromide uptake rates significantly varied with exposure to different light intensities (Fig. 3, $F(3,10) = 4.93$, $P = 0.024$), with a trend of decreasing uptake with increasing light intensity. After four hours of incubation under different light regimes, maximal levels of bromide uptake (6 mg g^{-1} FW h^{-1}) were detected at 25 µmol photons m^{-2} s^{-1}, the lowest light intensity, while minimal values (3.5 mg g^{-1} FW h^{-1}) were detected at 250 µmol photons m^{-2} s^{-1}, resulting in around a 40% reduction in bromide uptake with increasing light intensity. Further increases in light intensity did not cause a further significant reduction in bromide uptake rate over this timespan.

Bromoform content

The bromoform content of *A. taxiformis* tissue was strongly influenced by exposure to light stress (Fig. 4, $F(5,21) = 5.21$, $P = 0.009$). Initial baseline levels of 4.5% DW declined significantly by around half after one minute of exposure to increased high light intensity.

Gland cell presence

The proportion of gland cells located at the tips of the *A. taxiformis* tissue decreased significantly with exposure to 700 µmol photons m^{-2} s^{-1} (Fig. 5, $F(5,12) = 23.67$,

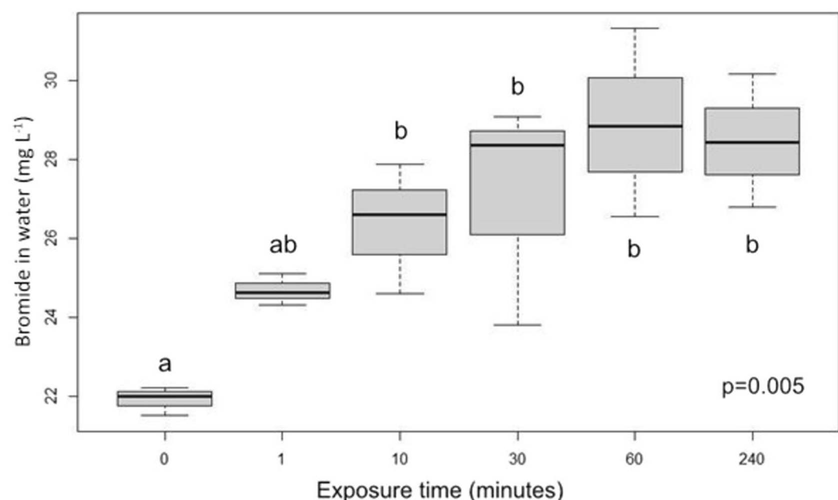

Fig. 2 Concentration of bromide (mg L^{-1}) in *A. taxiformis* growth media from one minute to four hours, and a pre-exposure baseline, after exposure to 700 µmol photons m^{-2} s^{-1}. Box plots display median values (thick black line), interquartile range between Q1 and Q3 (upper and lower limits of grey box), and maximum and minimum data points (whiskers). Lower case letters represent significant differences across time points

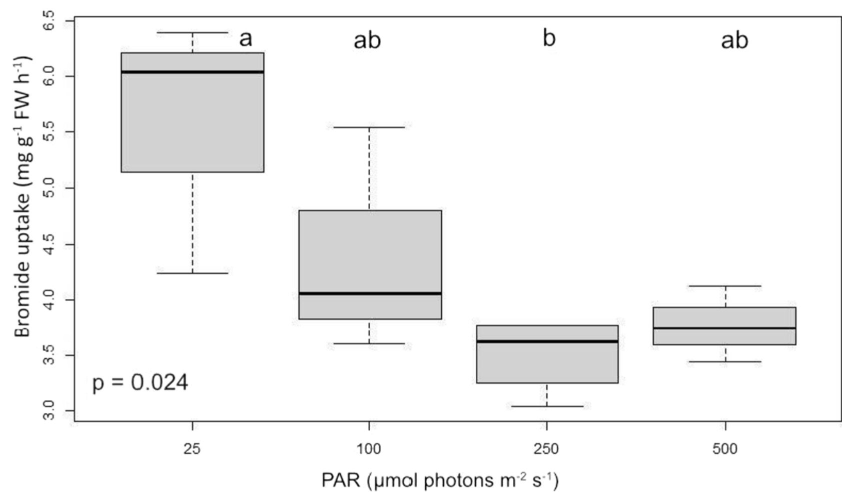

Fig. 3 Uptake rates of bromide (mg g^{-1} FW h^{-1}) by *A. taxiformis* across light intensities from 25—500 µmol photons m^{-2} s^{-1}. Box plots display median values (thick black line), interquartile range between Q1 and Q3 (upper and lower limits of grey box), and maximum and minimum data points (whiskers). Lower case letters represent significant differences across time points

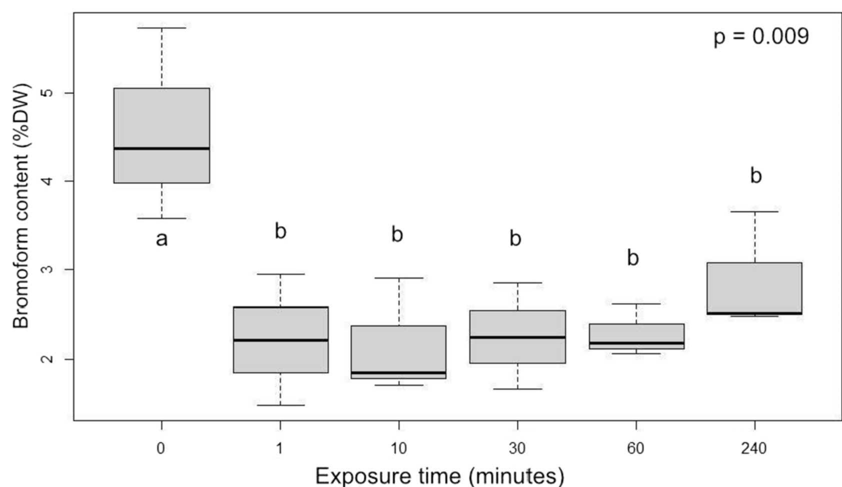

Fig. 4 Concentration of bromoform (% dry weight) in *A. taxiformis* tissue from one minute to four hours, and a pre-exposure baseline, after exposure to 700 µmol photons m^{-2} s^{-1}. Box plots display median values (thick black line), interquartile range between Q1 and Q3 (upper and lower limits of grey box), and maximum and minimum data points (whiskers). Lower case letters represent significant differences across time points

$P < 0.001$), from a mean baseline pre-treatment presence of 96% ± 3.4 SD to a mean of 54.8% ± 8.7 SD after 4 h of exposure. A decrease in the proportion of gland cells was apparent as soon as one minute after high light exposure, where gland cell presence declined by 7%. While the percentage of gland cells decreased linearly from one minute to one hour of exposure, there was no significant difference between the proportion of gland cells present in tissue after one or four hours of exposure to high light.

Discussion

While the provision of a light stress was observed to stimulate production of hydrogen peroxide, one known bromoform precursor in seaweeds, the uptake of the other precursor, bromide, was observed to be inhibited with increasing light stress and was ultimately released from the alga, rather than absorbed. The gland cells, in which bioactive metabolites such as bromoform are believed to be stored (Marshall et al. 2003), were seen to decline in number in the tips of *A. taxiformis* tissue when exposed to high light stress. Most importantly, light stress caused an immediate reduction in bromoform concentration in algal tissue that remained low for the duration of the experiment. The maximal peak in hydrogen peroxide coincided with the initial disappearance of bromoform from the tissue and apparent expulsion of bromide from the alga into the surrounding media. Such a response may indicate a rapid mechanism by which bromoform is released from the cell when exposed to a sudden, acute environmental stressor.

ROS production

The rapid increase of hydrogen peroxide after one minute of exposure to increased light intensity provides evidence for a rapid onset of photoinhibition in *A. taxiformis,* where an ecologically moderate illumination appears to have quickly overwhelmed the photosynthetic machinery. Since temperature remained constant during this study, it is likely that

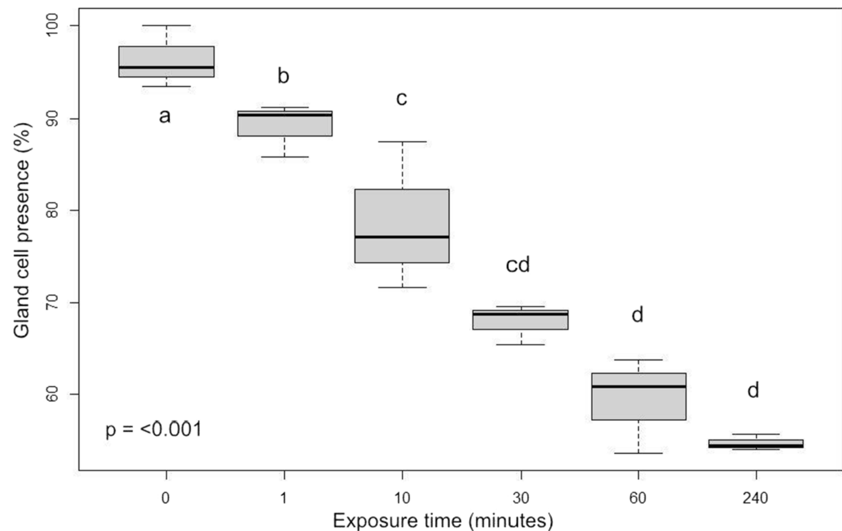

Fig. 5 Percentage of gland cells in *A. taxiformis* tips from one minute to four hours, and a pre-exposure baseline, after exposure to 700 µmol photons m^{-2} s^{-1}. Box plots display median values (thick black line), interquartile range between Q1 and Q3 (upper and lower limits of grey box, and maximum and minimum data points (whiskers). Lower case letters represent significant differences across time points

the increases of hydrogen peroxide observed originate from chloroplastic ROS generation rather than mitochondrial. This is perhaps surprising, given that shallow marine ecosystems where *Asparagopsis* spp. exist are likely to experience light levels significantly higher than the 700 µmol photons m^{-2} s^{-1} used as a stress treatment in this study. Recently, it has been shown that light intensities of 90 to 120 µmol photons m^{-2} s^{-1} are above the saturating irradiance for *A. taxiformis* at 1 – 2 g L^{-1} (Mata et al. 2023). Our study used a much higher light intensity of 700 µmol photons m^{-2} s^{-1} at a density of 1 g L^{-1}, so perhaps it is not surprising we observed such negative effects of high light. However, while the tissue used in these experiments originated from wild populations, the material was cultivated for several months under lower light conditions (~ 50 µmol photons m^{-2} s^{-1}), to which the alga may have become acclimated. Despite this, the marked decline in hydrogen peroxide over 30 min demonstrates a ROS scavenging response to the sudden peak in irradiance. There is an apparent decrease in hydrogen peroxide concentration after 30 min of exposure to high light, however maximal levels returned at both timepoints afterwards. It is not clear if this is a result of ROS scavenging or whether this datapoint is anomalous since no measures of ROS scavenging were made.

Bromide dynamics

The ionic precursor to bromoform, bromide, displayed interesting dynamics for *A. taxiformis* when exposed to increasing irradiance. It has been seen that bromoform production is light dependent, since levels have been observed to increase in algal tissue with increasing light exposure, for example Mata et al. (2023) observed higher bromoform levels at 90 µmol photons m^{-2} s^{-1} than at 30 or 60 µmol photons m^{-2} s^{-1}. In our study, the uptake of the bromide precursor was not enhanced by increasing light intensity. An increase in irradiance from 25 to 250 µmol photons m^{-2} s^{-1} caused a 45% reduction in bromide uptake rate, while the application of 700 µmol photons m^{-2} s^{-1} caused a net release of bromide rather than uptake. Küpper et al. (1998) observed that while iodide uptake was increased in the presence of hydrogen peroxide, oxidative stress resulted in the rapid release of iodide. In our study, the peak of hydrogen peroxide production over one to ten minutes coincides with the onset of bromide emission, likely driven by a similar mechanism as observed by Küpper et al. (1998). One potential explanation for the apparent increase in bromide in the media is via bromoform degradation. Marshall et al. (2003) suggest that bromoform can be degraded either via epiphytic microorganisms or biochemically by the alga itself, therefore it is possible that the detected increase in bromide in the medium may originate from the release and concurrent degradation of the emitted bromoform. However, the Marshall et al. (2003) study observed that this degradation occurred on a longer time scale, 48 h, while the data reported here suggest an increase in ambient bromide within 10 min (Fig. 2). Thus, it may be unreasonable to attribute the increased bromide in the medium to bromoform degradation alone in this comparatively short time. Further, epiphytic organisms were almost entirely absent in our experimental culture, which rules out epiphytic bromoform degradation. Bromide is an essential micronutrient for some red and brown algae, stored internally at higher concentrations than in ambient seawater (Küpper et al. 2014). As such, a more likely explanation is the release of bromide into the seawater media from internal stores within *A. taxiformis* tissue. It is possible that the bromide may also be stored in the gland cells, evidenced by the apparent synchronisation of the observed bromide increases in the media and the trends of gland cell loss in the algal tissue.

Gland cell presence and persistence

The presence of gland cells, sometimes also referred to as vesicle cells or cellular inclusions, in *A. taxiformis* has been seen to correlate positively with bromoform production (Marshall et al. 2003). The trends reported here support this to an extent, where both gland cell persistence and bromoform concentration was observed to decline with increasing exposure to high light. However, the severity of these two separate declines displays a mismatch, where around 45% of the total bromoform was lost from the tissue in the first minute, only 7% of gland cells disappeared within this same timeframe. There was no further significant decline in bromoform content after one minute of exposure, while gland cell presence continued to decline for an hour. Despite the decline in visible gland cells (Supplementary Fig. 1), we observed no damage to the pericentral cells. There may be two explanations for the disappearance of gland cells, first the contents may be entirely released into the surrounding seawater via the stalk cells, as suggested by Paul et al. (2006), or the gland cells may have physically ruptured, releasing the contents into the surrounding pericentral cell. It has been suggested that bromoform production is light dependent, as increasing production has been observed in natural populations during noon and early afternoon when irradiance is higher (Ekdahl et al. 1998), which may provide evidence for the 'release' hypothesis. Rather than an increase in synthesis as a result of high light, the results of this study suggest that the 'production' of bromoform in the seawater media may be a result of a stress response where bromoform is expelled rapidly from the tissue.

Bromoform content

The concentration of bromoform in *Asparagopsis* tissue has been shown to vary widely in the literature, where Paul et al. (2006) report concentrations of up to 5% of dry mass while Zanolla et al. (2022) report values as low as 0.02% dry weight. As this compound is believed to be among the most effective methane reducing agents in ruminant animals, there is growing interest in understanding the drivers of this variability. Specifically for commercial cultivation there is not only a need to understand how cultivation conditions can maximise bromoform production but to also understand what stressors lead to degradation, decline, complete loss and even cell death and to further understand the time scale at which these responses may occur. The declines in tissue bromoform content observed in this study were rapid, with an onset of efflux occurring after just one minute of exposure to a moderately high light stress. Rather than stimulating bromoform production, it appears that light stress caused a release of bromoform from the algal tissue into the medium. Further, the emission of bromide from the alga into the surrounding medium suggests that bromoform synthesis was not occurring over the experimental period after stress induction, since bromide is actively taken up during brominated halogenated metabolite synthesis (Paul et al. 2006). A number of polar and Northern temperate macroalgae have also been observed to release bromoform, among other organohalogens, as a result of small increases in irradiance (up to 23 μmol photons m^{-2} s^{-1}), of which bromoform was among the major constituent of the halogenated compounds that were emitted (Laturnus et al. 2004). Other than bromoform, the release of iodine containing organohalogens was observed in kelps of *Laminaria* sp. as a result of stressful conditions, in this case elevated temperatures (Nitschke et al. 2013). Despite the occurrence of organohalogen release as seen in previous literature, the biochemical process or processes that drive the release of halogenated compounds is unknown. Our observations of gland cell decline and bromoform loss may suggest a benefit to rapid bromoform release. Speculatively, if the site of bromoform synthesis is within the gland cell, perhaps in relation to the peroxisome where hydrogen peroxide destruction has been seen to occur in seaweed (Keng et al. 2013), an accumulation of hydrogen peroxide may be expected here. Given that hydrogen peroxide is able to travel readily across membranes (Ledford and Niyogi 2005), it is possible that the rapid synthesis and release of bromoform may be a mechanism to limit hydrogen peroxide driven membrane damage. Such an increase in synthesis has already been observed by Mata et al. (2011) when bromoform concentrations increased quickly, as soon as 1 h after adding hydrogen peroxide to the growth medium of *A. taxiformis*. However, while Mata et al. (2011) found that external hydrogen peroxide provision stimulated bromoform concentrations in the cell, we found the opposite effect when hydrogen peroxide was stimulated internally. Alternatively, the pulse of hydrogen peroxide moving through the cell may act as a cell signal to activate stress responses. Hydrogen peroxide, being more stable than other ROS, has been observed to have a function in the signalling of many cellular processes (Mittler et al. 2011). In this case, perhaps a trigger for bromoform release.

Here we observed that the majority of bromoform release in *A. taxiformis* (around 45% of the total) occurred after 1 min of exposure to high light, however in this time only 7% of the gland cells were lost, compared to ~ 40% after one hour. By emitting around half of the total intracellular bromoform almost instantly, a significant amount of autotoxicity may have been avoided, as would have occurred after an hour when most gland cells disappeared had a large proportion of bromoform not been emitted. Gland cells in *Asparagopsis,* and in other species, are believed to allow for the safe storage of harmful bioactive compounds which are isolated from other cellular constituents (Young and West 1979). While structures exist to isolate and concentrate harmful

compounds, as well as to export these compounds out of the alga, such as gland cells and stalk cells, it is possible that this allows the alga to rapidly release such compounds to prevent autotoxicity in the event that stress may cause mechanical damage to cellular structures.

Conclusion

With the growing need to implement greenhouse gas emissions reductions programs, the use of seaweed to mitigate methane emissions in livestock has the potential to have a real impact and help the planet achieve the global methane pledge of reducing methane emissions by 30% by 2030. With this methane mitigating seaweed solution, there is not only a need to understand how to cultivate it at scale but to produce a high quality product. Many factors are being explored that may help maximise production of the bioactive compound bromoform, but fewer studies have focused on how short-term exposure to environmental stressors may lead to a decline in seaweed quality. Here we show that while short term exposure to high irradiance does stimulate an increase in hydrogen peroxide in *A. taxiformis*, this rise does not stimulate bromoform production. In fact, we documented a rapid decline in bromoform content in the algal tissue and inhibition of bromide uptake or release of bromide into the surrounding seawater. This runs contrary to our hypothesis, where we theorised an increase in bromoform production as a result of enhancing internal hydrogen peroxide concentrations. Rather than stimulating bromide uptake and bromoform production, it appears the sudden provision of light caused stressful conditions within the alga, beyond oxidative stress, that resulted in physical changes to the cells. This is seen most clearly in the decline in gland cell presence, which provides evidence for cellular damage as a result of light stress. This study thus highlights the importance of controlling specific factors, namely exposure to high light, as even brief exposure to extreme light intensities could reduce the effectiveness of the seaweed to mitigate methane by over 50% due to loss of the key bioactive compound. While we observed strong responses to light stress with regard to potential oxidative stress, declines in bromide ion uptake, and changes to cell structure via reduction in gland cells, we are unable to say whether these factors had any negative effects on the overall health of the algae, or how it may recover from the evidence light stress without directly measuring these factors. Lastly, our results suggest that in cultivation settings if the goal is to produce a seaweed product with high concentrations of bromoform, exposure to high light and UV during harvest should be avoided.

There are numerous situations in cultivation where light stress may occur, such as sudden changes in algal density via transition to larger vessels, transition from indoor hatchery conditions to outdoor tanks, and final harvest. The harvest conditions may prove the most crucial, since this is the point at which peak bromoform content should be captured within the biomass. More research is needed to better understand the roles of other environmental stressors on bromoform production, storage and release in *A. taxiformis*, particularly in regard to cultivation, where the maintenance of a high bromoform content is crucial. Further, the extent to which gland cells and bromoform concentrations can recover after losses due to light stress, and how rapid this recovery process must be addressed. If recovery proves slow or non-existent, then light stress at vulnerable stages in the cultivation and harvest of this species must be strongly avoided.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s10811-023-03129-2.

Authors' contributions M.H.: Conceptualization, Formal analysis, Investigation, Methodology, Validation, Visualization, Writing – original draft.

S.I.: Conceptualization, Investigation, Methodology.

V.A.: Conceptualization, Methodology, Project administration, Resources, Supervision, Writing – review & editing.

J.S.: Conceptualization, Methodology, Sample collection, Project administration, Resources, Supervision, Writing – review & editing.

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Data availability Data are available upon kind request to the authors.

Declarations

Competing interests The authors confirm there are no competing interests relating to the submission of this manuscript for publication.

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