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# Inducible versus constitutive antioxidant defenses in algae along an environmental stress gradient

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ABSTRACT: Optimal defense theory (ODT) predicts that antiherbivore defenses should be constitutive when plants are frequently attacked and inducible when the probability of attack is low. Like antiherbivore defenses, antioxidant defenses can be inducible or constitutive. We hypothesized the ODT predictions should apply to antioxidant defenses; thus, species inhabiting environments where oxidative stresses occur frequently should produce constitutive antioxidant defenses, whereas species in environments where stresses occur less frequently should produce inducible defenses. We tested this hypothesis by attempting to induce production of the antioxidant precursor dimethylsulfoniopropionate (DMSP) in 4 ulvoid algae species that experience different levels of environmental stress because they are zoned along a tidal gradient. The 2 lower intertidal species *Ulvaria obscura* and *Ulva fenestrata*, which experience oxidative stresses less frequently, induced DMSP production in response to applications of the chemical oxidant hydrogen peroxide within 7 d, whereas the higher intertidal species *Ulva linza* and *Ulva intestinalis*, which regularly experience oxidative stress, did not have increased DMSP concentrations. This study demonstrates a novel waterborne signaling mechanism for DMSP induction in marine macroalgae and provides evidence of selection for inducible antioxidant defenses in organisms experiencing less frequent environmental stresses.

KEY WORDS: Antioxidant · Environmental stress · Dimethylsulfoniopropionate · Hydrogen peroxide · Stress gradients · Macroalgae · Oxidative stress

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## 1. INTRODUCTION

Optimal defense theory (ODT) was proposed to explain variation in concentrations of chemical antiherbivore defenses among plants and their tissues (McKey 1974, Rhoades 1979). It predicts that plants should preferentially allocate resources to defending tissues that are most susceptible to attack because producing defenses incurs costs. Later work extended ODT to the evolution of induced and constitutive defenses, proposing that constitutive defenses should occur in plants or plant parts that are frequently attacked, whereas inducible defenses should occur in those that are less likely to be attacked (Zangerl & Rutledge 1996, Kaplan et al. 2008).

Antioxidant defenses that provide protection from environmental stress share many similarities with antiherbivore defenses. Like antiherbivore defenses (Simms & Rauscher 1987), producing antioxidant defenses has both benefits and costs. The primary benefit is reducing the detrimental effects of damage caused by environmental stress, which affect many aspects of organism function, including survival, growth, reproduction, resource acquisition, and immunological and defensive responses (Hurd et al. 2014). At a cellular level, environmental stress causes an overproduction of reactive oxygen species (ROS) (Sharma et al. 2012). ROS, such as superoxide ($O_2^{\bullet -}$), hydrogen peroxide ($H_2O_2$), and hydroxyl radicals ($OH^{\bullet}$), are produced during many metabolic processes, including photosynthesis and cellular respiration (Halliwell 1999), in chloroplasts, mitochondria, cell walls, endoplasmic reticula, apoplasts, membranes, and peroxisomes (Sharma et al. 2012). If ROS

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accumulation is not prevented, damage to DNA, proteins, and lipids occurs (Halliwell 1999).

In unstressed organisms, oxidative damage from ROS is prevented when the antioxidant capacity of enzymatic and non-enzymatic antioxidants exceeds the rate of ROS damage. When organisms are stressed, antioxidant defenses can be overwhelmed, resulting in damage to cellular components (Sies 1997). Therefore, organisms benefit by having enough antioxidants to balance the activity of the excess ROS produced in response to environmental stress. However, producing antioxidants also incurs costs (Benzie 2000); thus, producing excess antioxidants may divert resources from other functions, such as growth or reproduction, negatively affecting fitness.

Like antiherbivore defenses that are induced by herbivore attack, the production of antioxidant defenses can be induced by environmental stressors that create oxidative damage (Harris 1992), such as desiccation (França et al. 2007), hypoxia (Blokhina et al. 2003), increases in temperature (Hasanuzzaman et al. 2013), and hypersaline conditions (Sairam et al. 2002). However, some antioxidants are produced constitutively, and production is not upregulated in response to stress (Stepien & Klobus 2005, Türkan et al. 2005).

Given the parallels in the costs, benefits, and inducibility of antiherbivore and antioxidant defenses, predictions regarding the occurrence of inducible antioxidant defenses should parallel predictions of ODT for antiherbivore defenses: antioxidant defenses should be constitutive in organisms living in environments where stresses occur regularly and inducible in organisms living in environments where these stresses occur infrequently. Empirical tests of predictions for the occurrence of inducible and constitutive antiherbivore defenses are few, especially for plants and marine organisms (Karban et al. 2006, Padilla & Savedo 2013). Here, we present novel empirical tests of these predictions for antioxidant defenses.

Marine intertidal environments are characterized by pronounced environmental stress gradients resulting from differences in the time organisms spend out of water during low tide (Dahlhoff et al. 2002, Tomanek & Helmuth 2002). Relative to high intertidal species, species living lower on the shore generally are subjected to less abiotic stress because tides low enough to expose them occur less frequently and emersion times in air are shorter. Very low intertidal species may only be emersed for brief periods during extreme spring tides, whereas high intertidal species may be emersed for several hours twice a day. As a result, intertidal species typically occur in zones whose locations are influenced by both biotic and abiotic factors (e.g. Lubchenco 1980, Somero 2002).

Because environmental stresses occur less frequently in the low intertidal zone, we predict that low intertidal species should produce inducible antioxidant defenses and high intertidal species, which experience oxidative stresses regularly, should produce constitutive defenses. To test this hypothesis, the inducibility of dimethylsulfoniopropionate (DMSP) in 4 closely related intertidal ulvoid (Phylum Chlorophyta, Order Ulvales) species that have different vertical distributions on the shore was examined. At our study site, all 4 species are present during the spring and summer (Van Alstyne et al. 2016). *Ulva intestinalis* L. occurs only in the high intertidal zone, often near freshwater seeps; *Ulva linza* L. occurs in the mid intertidal zone and overlaps in distribution with *Ulva fenestrata* Postels & Ruprecht, which occurs from the mid intertidal zone to the shallow subtidal zone. *Ulvaria obscura* var. *blytii* (Areschoug) Bliding overlaps with *Ulva fenestrata* but is more abundant in the shallow subtidal zone. During the spring, *Ulvaria obscura* occurs in the mid intertidal zone; however, in most years, its abundance is reduced in the mid intertidal zone during the late spring and summer, when it is primarily found in the low intertidal and subtidal zones (Nelson et al. 2003).

DMSP is a 'molecule of keystone significance' (Ferrer & Zimmer 2013) that occurs in all 4 species (Van Alstyne et al. 2016) along with a dithiomethylase enzyme (also known as DMSP lyase) that rapidly cleaves DMSP into DMS and acrylate or acrylic acid, depending on the pH (Bywood & Challenger 1953). Several lines of evidence suggest that DMSP in ulvoid algae may function as the precursor of an inducible antioxidant system that can be cued by waterborne oxidants. DMS, acrylate, and acrylic acid are potent antioxidants (Sunda et al. 2002, Ross & Van Alstyne 2007). Algae cannot store DMS and acrylic acid because they are volatile; however, DMSP is water-soluble and can be maintained in the cytoplasm. Thus, by maintaining DMSP and an enzyme that lyses it to generate antioxidants, algae can rapidly produce additional antioxidant capacity as needed. Furthermore, DMSP concentrations in ulvoid algae are affected by environmental stressors including light intensity, salinity, temperature, and nutrient limitation (Van Alstyne 2008, 2018). Stressors such as desiccation, high seawater temperatures, hyposaline conditions, and herbivory also cause DMSP to be split, resulting in the release of DMS (Van Alstyne et al. 2009, 2016). Finally, $H_2O_2$, which is released by ulvoid macroalgae

into the surrounding environment (van Hees & Van Alstyne 2013), induces DMSP lysis and generates DMS in the coccolithophore *Emiliania huxleyi*, but it does not increase cellular DMSP concentrations (Sunda et al. 2002). Similar inductions of DMSP concentrations or DMSP lysis are not yet known to occur in macroalgae.

In this study, we show that the production of DMSP is induced by waterborne $H_2O_2$ in intertidal macroalgae, but only in low intertidal species. These results support one of the predictions of ODT regarding the evolution of induced defenses and demonstrate that these predictions are applicable to antioxidant defenses as well as antiherbivore defenses.

## 2. MATERIALS AND METHODS

### 2.1. Induction experiments

*Ulva intestinalis*, *Ulva linza*, *Ulva fenestrata*, and *Ulvaria obscura* were collected by hand during low tides in the spring and summer of 2015 (see Table S1 in the Supplement at www.int-res.com/articles/suppl/m640p107_supp.pdf for collection dates) from Ship Harbor in Anacortes, Washington, USA (48° 30.32′ N, 122° 40.48′ W). The algae were transported to the Shannon Point Marine Center (SPMC) in Anacortes, where they were identified microscopically and used in induction experiments within a day of collection.

Eight separate experiments, 2 per species for each of the 4 species, were conducted to determine the effects of extracellular $H_2O_2$ on the inducibility of DMSP. Four of the experiments (single exposure experiments) consisted of single 3 h exposures to one of 4 concentrations of $H_2O_2$ (0, 5, 20, and 80 µM). These experiments simulated algae being exposed to $H_2O_2$ in a tide pool during a single low tide. The other 4 experiments (daily exposure experiments) consisted of exposing algae continuously to one of the same 4 $H_2O_2$ concentrations for 7 d to simulate conditions in an algal bed where $H_2O_2$ is regularly released. The concentrations used in these experiments were well below the maximum concentration (200 µM) measured in tide pools containing ulvoids at the site where the algae were collected (K. L. Van Alstyne & L. Sutton unpubl. data).

In each experiment, 96 pieces of algae were distributed amongst forty-eight 15 cm bowls (2 pieces per bowl). Each bowl contained 330 ml of f/2 culture medium (Anderson et al. 2005) to which $H_2O_2$ (Sigma Aldrich 216763) was added to generate concentrations of 0, 5, 20, and 80 µM $H_2O_2$ (n = 12 bowls per treatment). For the experiments involving *Ulva fenestrata*, *U. linza*, and *Ulvaria obscura*, the pieces consisted of 2.5 × 2.5 cm squares excised from the center of thalli that lacked obvious tears, holes, or macroscopic epiphytes. For the experiments involving *Ulva intestinalis*, pieces consisted of thalli cut to 13 cm lengths. While grazing by herbivores can stimulate increases in DMSP concentrations in *Ulva lactuca*, cutting the alga with a razor blade does not increase DMSP concentrations (K. L. Van Alstyne & N. Borgen unpubl. data).

In the single exposure experiments, algae were placed into bowls containing the 4 types of culture media, which were then placed in a lighted incubator (12°C, 12 h light:12 h dark, 110 µmol photons $m^{-2}$ $s^{-1}$). After 3 h, the media were discarded and replaced with fresh f/2 culture medium that lacked added $H_2O_2$. The bowls were then placed back in the incubator and the medium in each of the bowls was replaced every 2 d thereafter. In the daily exposure experiments, algae were placed into the bowls containing the 4 types of culture media and the bowls were placed in the incubator. The f/2 medium was changed daily and $H_2O_2$ was added to the bowls at the same time to bring the concentration back to the original amount.

Because the time course for induction was unknown, algae were sampled 3 and 7 d after the start of each experiment. On Day 3 after the start of each experiment, one of the 2 pieces of algae in each bowl was removed, blotted dry with a paper towel, weighed to obtain a fresh mass, and then placed in a 60°C oven. After allowing the algae to dry at least overnight, the pieces were reweighed and a dry to wet mass ratio was calculated. Approximately 10 mg of the dried piece was then weighed and placed into 4 ml of 4 N sodium hydroxide in a 30 ml vial. The vials were immediately sealed and stored in darkness overnight for measurements of DMSP. On Day 7 after the algae were initially exposed to $H_2O_2$, the remaining pieces were removed from the bowls and prepped for DMSP analyses in the same manner.

### 2.2. DMSP measurements

DMSP was measured in algal tissues as DMS following alkaline hydrolysis by injecting 10 µl headspace samples into an SRI (SRI Instruments) gas chromatograph (injection and oven temperatures: 90°C, flame photometric detector temperature: 120°C) equipped with a Chromasil 330 column (Supelco #11496). Commercially obtained DMSP

(Center for Analysis, Spectroscopy and Synthesis, University of Groningen) was used as a standard (standard range: 25–1000 µg).

### 2.3. Statistical analyses

Repeated measures analyses of variance were performed on Box-Cox transformed DMSP concentrations. Separate analyses were conducted for each experiment. Post-hoc analyses of changes in concentrations among treatments within experiments were conducted with Tukey's tests. Analyses were conducted in Minitab (version 16).

## 3. RESULTS

Exposure to extracellular $H_2O_2$ altered concentrations of DMSP; however, the effects differed among species. DMSP concentrations in *Ulva intestinalis*, the seaweed that was zoned highest in the intertidal zone, did not significantly change in response to $H_2O_2$, regardless of whether the compound was administered in a single dose or on a daily basis (see Table S2 in the Supplement, Figs. 1a & 2a). Average DMSP concentrations did not change across sampling dates in the single exposure experiment, but they increased by 38% from Day 3 to Day 7 in the daily exposure experiment (Figs. 1a & 2a, Table S2).

In *Ulva linza*, an intertidal species zoned below *U. intestinalis*, DMSP concentrations decreased in response to single, 3 h exposures to $H_2O_2$ (Fig. 1b, see Table S3 in the Supplement). After 3 d, concentrations dropped by 18% in algae exposed to $H_2O_2$, relative to unexposed (0 µM) controls. On Day 7 after exposure, average DMSP concentrations in algae exposed to $H_2O_2$ were only 6% lower than concentrations in controls (Fig. 1b). Concentrations of DMSP rose an average of 60% from Day 3 to Day 7, regardless of the amount of $H_2O_2$ added (Fig. 1b, Table S3). Daily exposure to $H_2O_2$ did not have a significant effect on DMSP concentrations in *U. linza*, but concentrations increased by 46% from Day 3 to Day 7 (Fig. 2b, Table S3).

In *Ulva fenestrata*, single exposures to $H_2O_2$ did not result in significant increases in DMSP after 3 d. However, after 7 d, exposures to 20 and 80 µM $H_2O_2$ resulted in 24% higher concentrations of DMSP than in controls and algae exposed to 5 µM $H_2O_2$, which did not differ significantly from one another (Fig. 1c, see Table S4 in the Supplement). After 3 d of daily exposure to 20 µM $H_2O_2$, DMSP concentrations in *U.*

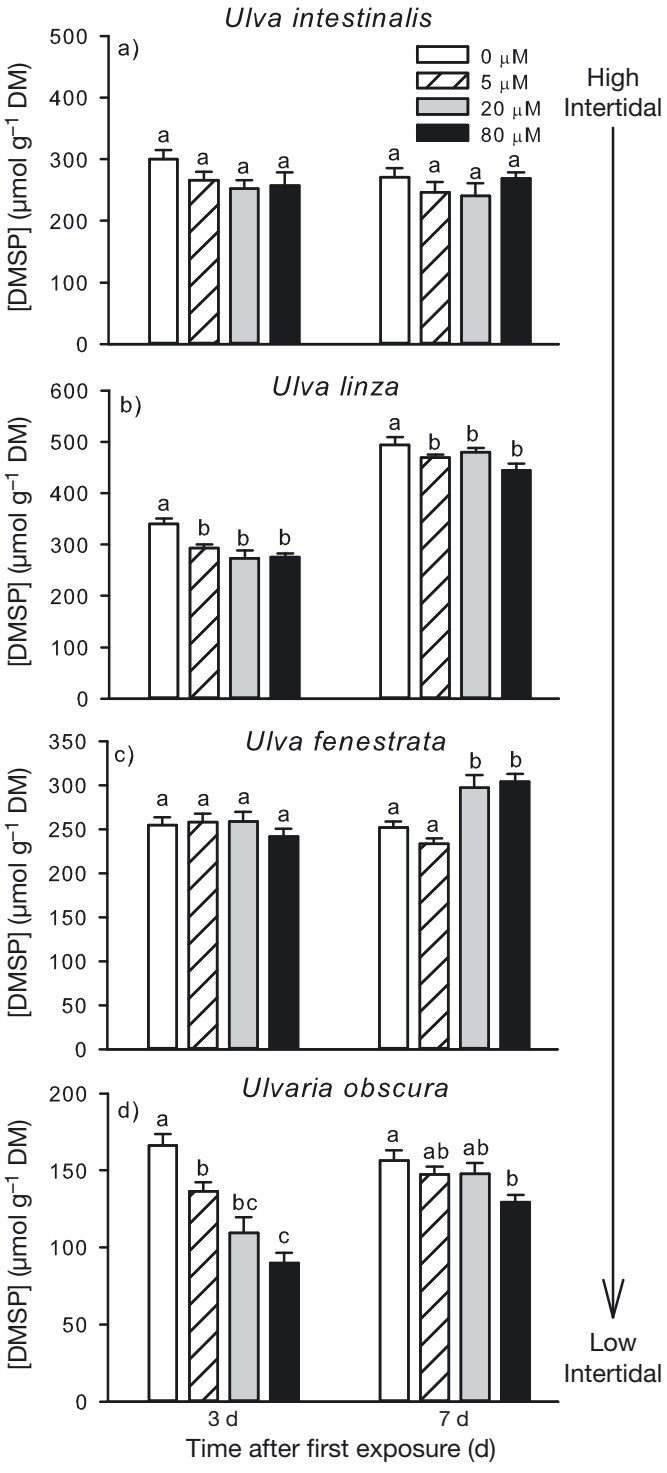


Fig. 1. DMSP concentrations (µmol DMSP $g^{-1}$ algal dry mass) of (a) *Ulva intestinalis*, (b) *Ulva linza*, (c) *Ulva fenestrata*, and (d) *Ulvaria obscura* exposed to single 3 h exposures of $H_2O_2$ at concentrations of 0, 5, 20, and 80 µM. DMSP measurements were taken 3 and 7 d after exposure to the $H_2O_2$. Data are means ±1 SE. Letters above the bars indicate results of Tukey's post hoc comparisons (normally distributed or transformed data) or Wilcoxon's tests (data that could not be transformed to meet the assumption of normality) ($p > 0.05$). Analyses were conducted separately for each species on each day

*fenestrata* decreased relative to unexposed controls (Fig. 2c, Table S4). On Day 3, a significant decrease of 22% occurred when thalli were exposed to 20 µM $H_2O_2$ (Fig. 2c). Exposure to 80 µM $H_2O_2$ resulted in an intermediate DMSP concentration that was not statistically distinguishable from concentrations in unexposed controls and thalli exposed to 20 µM $H_2O_2$ (Fig. 2c). On Day 7, exposure to 20 and 80 µM $H_2O_2$ caused DMSP concentrations to increase relative to unexposed controls; however, exposure to 5 µM $H_2O_2$ had no effect on DMSP concentrations (Fig. 2c). DMSP concentrations of unexposed controls were not significantly different between Days 3 and 7 in the single exposure experiment (Fig. 1c), but concentrations in controls decreased by 57% between Days 3 and 7 in the daily exposure experiment (Fig. 2c; 2-sample *t*-test: $t = 11.47$, $p < 0.001$).

In *Ulvaria obscura*, single exposures to $H_2O_2$ resulted in decreases in DMSP concentrations that were inversely correlated to the concentration of $H_2O_2$ added to the culture media (Fig. 1d, see Table S5 in the Supplement). The effect size was stronger on Day 3 (Pearson's correlation coefficient: −0.633, $p < 0.001$) than Day 7 (Pearson's correlation coefficient: −0.429, $p = 0.002$), and resulted in 46 and 17% decreases in DMSP in the 80 µM $H_2O_2$ treatments on Days 3 and 7, respectively, relative to controls. Daily additions of $H_2O_2$ resulted in the opposite pattern, with thalli exposed to 20 and 80 µM $H_2O_2$ having significantly higher concentrations of DMSP relative to controls (Fig. 2d, Table S5). The addition of 5 µM $H_2O_2$ to the culture medium had no effect on DMSP content in algal thalli (Tukey's test: $p > 0.05$); however, the addition of 20 and 80 µM $H_2O_2$ resulted in average increases of 41% on Day 3 and 16% on Day 7 (Fig. 2d). In both the single and daily exposure experiments, DMSP concentrations in unexposed control thalli did not change significantly between Days 3 and 7 (Tukey's test: $p > 0.05$).

## 4. DISCUSSION

Our study supports the hypothesis that an antioxidant defense precursor is produced constitutively in species inhabiting environments in which environmental stress occurs frequently and inducibly in species inhabiting environments in which environmental stress occurs infrequently. The 2 higher intertidal species, *Ulva intestinalis* and *Ulva linza*, had DMSP concentrations that were similar to unexposed controls when exposed to 20 and 80 µM $H_2O_2$ over 7 d. However, repeated exposure to 20 and 80 µM $H_2O_2$

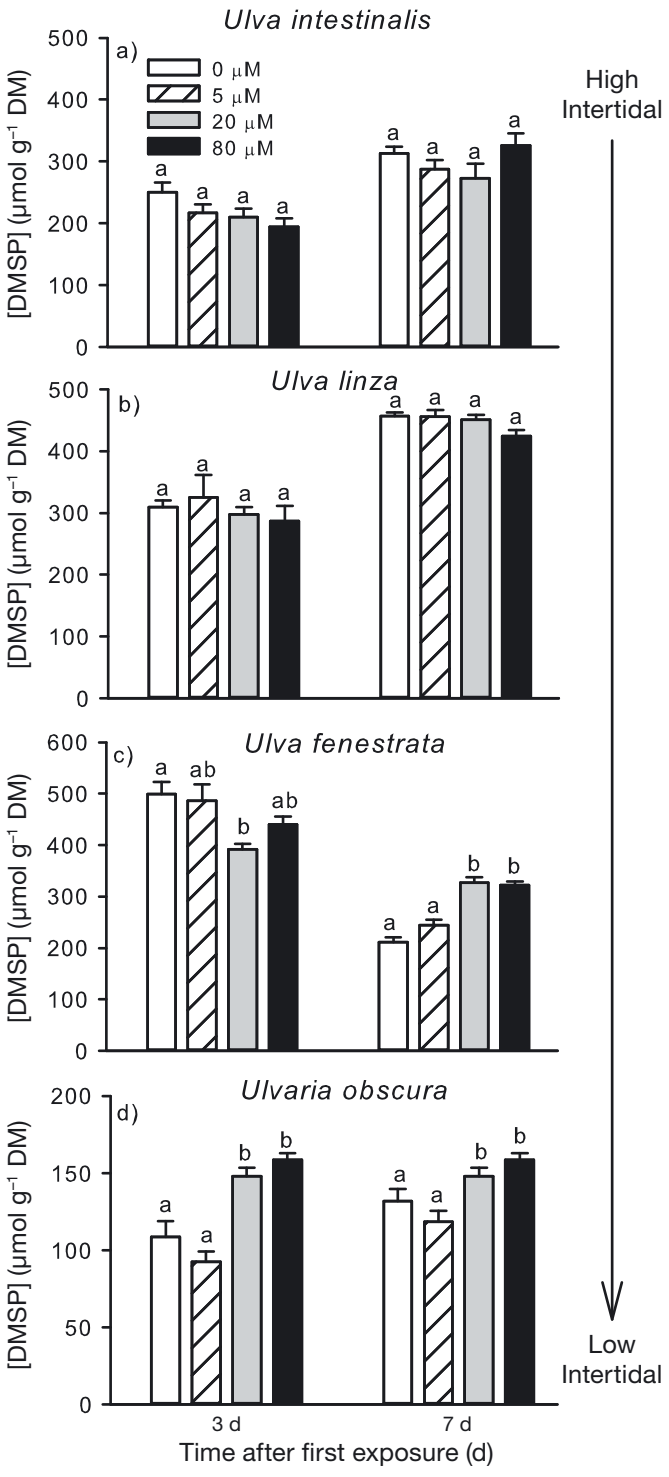


Fig. 2. DMSP concentrations (µmol DMSP $g^{-1}$ algal dry mass) of (a) *Ulva intestinalis*, (b) *Ulva linza*, (c) *Ulva fenestrata*, and (d) *Ulvaria obscura* continuously exposed to $H_2O_2$ at concentrations of 0, 5, 20, and 80 µM. DMSP measurements were made after 3 and 7 d. Letters above the bars indicate results of Tukey's post hoc comparisons (normally distributed or transformed data) or Wilcoxon's tests (data that could not be transformed to meet the assumption of normality) ($p > 0.05$). Analyses were conducted separately for each species on each day

resulted in significantly higher DMSP concentrations in lower-zoned *Ulva fenestrata* and *Ulvaria obscura*, relative to controls. These results suggest that this prediction of the ODT is applicable to antioxidant defenses as well as antiherbivore defenses.

In this study, we show for the first time that the ROS $H_2O_2$ functions as a waterborne signaling molecule to cue the upregulation of DMSP production in macroalgae. Although they are best known for their role in causing oxidative damage, ROS also have functional roles in plant metabolism and defense. For example, they combat pathogen infection when released in large quantities either directly into cells or into the extracellular environment during oxidative bursts (Lamb & Dixon 1997). In terrestrial plants, ROS are used intracellularly as signaling molecules, helping to regulate processes involved with development, growth, and programmed cell death (Shapiguzov et al. 2012). ROS produced in response to pathogens trigger antipathogen defense responses (Torres 2010) and ROS produced in response to stress trigger plant stress responses (Saxena et al. 2016).

Because they can be transported by water, ROS released into aquatic environments may function as both intraspecific and interspecific signaling molecules. Macroalgae release excess ROS into the extracellular environment (van Hees & Van Alstyne 2013), and their concentrations can exceed 200 µM in tidepools with ulvoids (K. L. Van Alstyne & L. Sutton unpubl. data). Macroalgae are known to detect waterborne signals and respond to them by altering morphology (Grueneberg et al. 2016), increasing concentrations of herbivore-deterrent chemicals (Toth & Pavia 2000), or becoming less palatable to herbivores (Rohde et al. 2004). Releasing a signal into the environment, where it is transported to other parts of the thallus, may be a way to induce a systemic response across the entire thallus, similar to the use of airborne volatile compounds for inducing systemic antiherbivore defenses in vascular plants lacking well-integrated vascular systems (Karban 2011). Like most macroalgae, ulvoids lack a vascular system; therefore, their ability to transport signals within individuals is very limited.

The induction of DMSP production by $H_2O_2$ provides further support for an antioxidant function for DMSP in taxonomically diverse marine organisms. DMSP is produced by many marine organisms, including macroalgae (Van Alstyne 2008), bacteria (Curson et al. 2017), phytoplankton (Simó 2001), endosymbiotic zooxanthellae (Deschaseaux et al. 2015), corals (Raina et al. 2013), and some salt-tolerant vascular plants (Otte et al. 2004). Its widespread distribution in marine organisms may reflect a general need for ROS scavengers as well as selection for the production of DMSP for other functions. DMSP is used as a compatible solute during osmotic acclimation, a cryoprotectant, a foraging cue, and possibly a storage mechanism for carbon, sulfur, and energy (Stefels 2000). Its breakdown products, DMS and acrylic acid, function as antibiotics, foraging cues for vertebrates, herbivore deterrents, and antioxidants (Van Alstyne 2008).

The DMSP breakdown products, DMS and acrylic acid, are 2 of many antioxidants produced by ulvoid algae. Others include catalase, superoxide dismutase, glutathione reductase, and peroxidase enzymes, as well as non-enzymatic antioxidants such as ulvans, pigments, ascorbic acid (vitamin C), and α-tocopherol (Vitamin E) (Qi et al. 2005, Ortiz et al. 2006, Sung et al. 2009, Contreras-Porcia et al. 2011). Although DMSP was produced constitutively by *Ulva intestinalis* and *U. linza*, it is possible that these other compounds are upregulated in response to $H_2O_2$.

As for almost all studies of induced chemical defenses, the DMSP measurements made in this study provide only a snapshot of changes in the antioxidant defensive capacity of the study organisms. Our measurements only quantified the amounts of DMSP in the algae, which are a function of both its production and lysis. By growing *E. huxleyi* in tightly closed containers, Sunda et al. (2002) demonstrated that exposure to $H_2O_2$ increases DMS production but does not alter cellular DMSP concentrations, suggesting DMS loss due to DMSP lysis in *E. huxleyi* is equivalent to DMSP production. DMS production in our experiments could not be measured because algae were not grown in axenic cultures; therefore, released DMS would likely be metabolized by bacteria during our week-long experiments. Also, growing algae in the absence of bacteria might alter how algae respond to $H_2O_2$. In ulvoid algae, developmental and possibly other processes are altered by the presence of bacteria. For example, *Ulva mutabilis* grown in axenic cultures exhibit unusual morphologies because bacteria associated with the algae produce morphogens that allow it to grow in its normal morphology (Spoerner et al. 2012).

Each ulvoid species had unique responses with respect to the timing, direction and magnitude of DMSP increases or decreases. While *U. intestinalis* had the same DMSP concentrations regardless of the concentration or duration of exposure to $H_2O_2$, DMSP concentrations in *U. linza* were lower relative to controls in all single exposure treatments. These reductions were most likely caused by DMSP being lysed and

generating antioxidants to scavenge $H_2O_2$. Although DMSP lysis was not measured, intertidal *U. lactuca* from Florida release DMS within 3 h of exposure to very high $H_2O_2$ concentrations, causing decreases in thallus DMSP concentrations (Ross & Van Alstyne 2007). The lack of differences in DMSP concentrations in the *U. linza* daily exposure experiment suggest that continuous exposure to $H_2O_2$ causes algae to increase DMSP production and bring concentrations back to levels comparable to those in unexposed controls.

In *Ulva fenestrata* and *Ulvaria obscura,* the presence and types of responses to the added $H_2O_2$ also differed. Induction occurred within 7 d in response to both single and daily exposures to the 2 higher $H_2O_2$ concentrations in *Ulva fenestrata*, but in *Ulvaria obscura*, induction only occurred in response to daily $H_2O_2$ exposures. In *Ulva fenestrata*, daily exposure to higher $H_2O_2$ concentrations resulted in initial drops in DMSP concentrations, most likely because DMSP was lysed. In contrast, no drop in DMSP concentration was seen 3 d after a single exposure to $H_2O_2$. This suggests that a relatively strong or prolonged oxidative stress is required to initiate DMSP lysis in *U. fenestrata*, but that smaller amounts of oxidative stress can cue DMSP induction. Alternatively, some lysis could have occurred in the single exposure experiment, if the lysed DMSP was replaced within 3 d.

Responses to single exposures of $H_2O_2$ were different in *Ulvaria obscura*, possibly because *U. obscura* contains dopamine in its tissues, whereas *Ulva fenestrata* does not. After 3 d, single exposures to $H_2O_2$ resulted in lower DMSP concentrations in *Ulvaria obscura*. These decreases increased in proportion to the amount of $H_2O_2$ the algae were exposed to, suggesting that the algae were countering higher amounts of oxidative stress with higher rates of DMSP lysis. The decreases in DMSP in *U. obscura* in response to single exposures of $H_2O_2$ were also proportionately higher than any of the decreases that occurred in *Ulva linza* or *U. fenestrata*. Stronger responses by *Ulvaria obscura* to high $H_2O_2$ concentrations might result from interactions between hydroxyl radicals formed from $H_2O_2$ and dopamine, which cause dopamine to oxidize and form additional ROS (Slivka & Cohen 1985). Dopamine is found in high concentrations in *U. obscura* and is released in response to tissue damage from desiccation (Van Alstyne et al. 2013). DMSP induction in *U. obscura* in response to continuous exposure to $H_2O_2$ also occurred more rapidly than in *Ulva fenestrata*, with increased concentrations occurring after 3 d for *Ulvaria obscura* and 7 d for *Ulva fenestrata*. Furthermore, in *Ulvaria obscura*, the relative differences in DMSP concentrations between the induced algae and controls were higher at 3 d than at 7 d, suggesting a relaxation of DMSP production between Days 3 and 7. Similarly, phlorotannin induction in brown algae as a response to mechanical damage occurs within 1 to 3 d and often, though not always, relaxes within 7 d (Hammerstrom et al. 1998).

We conducted the experiments on the lower zoned *U. obscura* in the spring because *U. obscura* is abundant in the spring and can be much rarer in late summer (Nelson et al. 2003). Likewise, we conducted experiments with the higher intertidal *Ulva linza* in the summer because it is uncommon in the spring (K. Van Alstyne, pers. obs.). It is possible that the timing of the experiments could have affected responses to DMSP induction. There is little known about seasonal patterns in DMSP concentrations in green macroalgae and the patterns that are known are not consistent. For example, *Codium fragile* in Nova Scotia, Canada has peak DMSP concentrations in the winter because its DMSP production increases in colder temperatures (Lyons et al. 2010); however, several Antarctic green macroalgae have peak DMSP concentrations in the summer due to higher light levels (Karsten et al. 1990). Whether responses to waterborne cues differ seasonally is unknown and would be an interesting area for future exploration.

Although ODT was proposed to predict the allocation of resources to antiherbivore defenses, we show here that its predictions are more broadly applicable to other defense types, including the production of antioxidant defenses. The study of resource allocation to plant-herbivore defenses has yielded a rich body of ecological and evolutionary theory. Theories such as the growth differentiation balance hypothesis (Herms & Mattson 1992), optimal defense theory (McKey 1974, Rhoades 1979), the plant apparency hypothesis (Feeny 1976), the carbon-nutrient balance hypothesis (Bryant et al. 1983), and the resource availability hypothesis (Grime 1977, Coley et al. 1985) have generated testable hypotheses that have spurred research in marine, freshwater, and terrestrial systems. While not all these theories will be applicable to other defense types, many may prove useful in understanding and predicting how plants will allocate resources to antioxidant defenses, a topic that is particularly relevant during these times of environmental change.

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