Title: Control of grazing by light availability via light-dependent, wound-induced metabolites:
the role of reactive oxygen species

Running page head: Light can regulate grazing via ROS

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Abstract

Light may influence grazing through its effect on grazers or by influencing the
physiology and biochemistry of primary producers. It has recently been established that reactive
oxygen species (ROS) produced by a wounded macroalga depend on light via photosynthetic
electron transport. Since ROS can act as both chemical messengers and toxins, it is hypothesized

that the light-dependence of wound-induced ROS can have ecological consequences for plant-herbivore interactions. Through spin trapping and electron paramagnetic resonance, this study shows that wounded *Ascoseira mirabilis* produced ROS that were correlated with *A. mirabilis* wounding in light but not dark conditions. In feeding assays where sympatric amphipods consumed palatable macroalgae in the presence of wounded *A. mirabilis*, amphipod consumption was correlated with *A. mirabilis* wounding in light but not dark conditions. The nonlinear effect of ROS on grazing suggests that in ecology, as in physiology, low levels of ROS may act as messengers while high levels are toxic.

Key words: Algae; electron paramagnetic resonance (EPR); free radical; grazing; irradiance; light; oxidative burst; plant-herbivore interaction; reactive oxygen species (ROS); wound

Abbreviations: EPR, electron paramagnetic resonance; H₂O₂, hydrogen peroxide; O₂, molecular oxygen; O₂⁻, superoxide anion; OH[·], hydroxyl radical; ROS, reactive oxygen species

1. Introduction

The effect of light on plant-herbivore interactions is complex. Grazing strategies can be shaped by numerous factors including resource competition, predator avoidance, and the physiology of the food, each of which may be affected idiosyncratically by light. Yet the effect of light on grazing strategies is often viewed as a consequence solely of the ecology or physiology of herbivores as opposed to the physiology of the primary producers they consume (see Roberts and Paul, 2006). Although these are by no means mutually exclusive, it is important to remember that exposure to light produces unavoidable effects on the physiological state of primary producers, and that these effects may impact grazing. One such effect is the generation of reactive oxygen species (ROS) during photosynthetic electron transport (Asada, 2006). It is

not known whether the generation of ROS during photosynthetic electron transport – a simple consequence of light-exposure – may have repercussions on plant-herbivore interactions. If so, a purely physiological effect of light on primary producers may contribute to the evolution of feeding strategies in small primary consumers.

Reactive oxygen species are the partial reduction products of molecular oxygen (O_2) – superoxide ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2) and the hydroxyl radical ($OH^{\cdot}$) – and reactive derivatives such as peroxynitrite ($ONOO^{\cdot}$) and hypochlorous acid ($HOCl$) among many others (Halliwell and Gutteridge, 2007). While O_2 is potentially toxic but mostly unreactive and its full reduction product (H_2O) is nontoxic and unreactive, ROS are potentially toxic and many are quite reactive. In spite of and in fact owing to these attributes, ROS play important roles in the physiology and ecology of aerobic organisms (Halliwell, 2006). For example, the production of ROS in an “oxidative burst” is shared among algae, plants, and animals (Suzuki and Mittler, 2012; Potin 2008; Lamb and Dixon, 1997). The oxidative burst of animals, plants, and algae can be elicited upon wounding or herbivory (McDowell et al., 2014b; Suzuki and Mittler, 2012) and its role in mediating infection by microbial parasites and pathogens is well established (Yoo et al., 2012; Peng and Kuc, 1992).

Although far less well-studied than the mediation of infection, the wound-induced oxidative burst may also contribute to the mediation of grazing on sessile organisms. The incorporation of H_2O_2 into food or its elevated presence in seawater can decrease the grazing rate of small herbivores in both terrestrial and marine systems (McDowell et al., 2014a; Ramputh et al., 2002). Felton et al. (1994) found that insects that consumed previously wounded soybean plants had greater markers of oxidative damage in their gut and decreased growth compared to insects that consumed unwounded plants. Expressing a wheat oxalate oxidase gene in corn plants

both increased tissue H_2O_2 and decreased consumption by and growth of corn borers (Ramputh et al., 2002). Similarly, silencing a germin-like protein in *Nicotiana attenuata* both decreased tissue H_2O_2 and increased growth of larval insects on the plant (Lou and Baldwin, 2006). Deleterious effects of ROS on grazers may be due to direct toxicity and/or may arise downstream of ROS signaling from alteration in gene expression or metabolic pathways (e.g. Mao et al., 2007).

The source of the oxidative burst is often a dedicated enzyme like an NADPH-oxidase (NOX; Bedard, 2007). Although it is well known that NOX and NOX homologs are very important in the wound response of both animals and plants (e.g. Miller et al., 2009; Niethammer et al., 2009), it has recently been shown in the kelp *Saccharina latissima* that wound-induced ROS ultimately depend on photosynthetic electron transport (McDowell et al., 2015). The potential involvement of a NOX homolog downstream of photosynthetic electron transport was not explored in McDowell et al. 2015 or the present study as the most common method of inhibiting NOX enzymes – diphenylene iodonium, or DPI – also inhibits photosynthetic electron transport (Chang et al., 2004; Ohtsuka et al., 2004; Corneille et al. 1998). Qualitative data indicate that disrupted photosynthetic electron transport may also contribute to the oxidative burst in Antarctic macroalgae (McDowell et al., 2015) and *Arabidopsis thaliana* (Morker and Roberts, 2011). If wound-induced ROS are light-dependent, can light availability control plant-herbivore interactions in the presence of wound-induced ROS?

Here, organisms from a well-studied ecosystem on the Western Antarctic Peninsula (Amsler et al., 2014) were used to investigate the hypothesis that plant-herbivore interactions in the presence of wound-induced ROS may be controlled by light. The omnivorous amphipod *Gondogeneia antarctica* readily consumes the palatable red macroalga *Palmaria decipiens* while

it does not consume the unpalatable brown macroalga *Ascoseira mirabilis* (Amsler et al., 2009). Neither macroalga contains secondary metabolites shown to be unpalatable to *G. antarctica* (Amsler et al., 2005), but both release ROS upon wounding (McDowell et al., 2014b), and preliminary evidence suggests that these ROS are light-dependent (McDowell et al., 2015). Finally, the presence of wounded *A. mirabilis* decreases grazing of *G. antarctica* on *P. decipiens* compared to the presence of intact *A. mirabilis* (experiment conducted under light conditions; McDowell et al., 2014a).

In this study, spin trapping and electron paramagnetic resonance (EPR) were used to show that wound-induced ROS from *A. mirabilis* are light-dependent. Wounded *A. mirabilis* produced less ROS in dark vs. light conditions, and wounding correlated with ROS in the light but not in the dark. Feeding assays were then conducted in which both macroalgal wounding and light were manipulated in order to manipulate wound-induced ROS. Amphipod consumption of the food alga *P. decipiens* in the presence of *A. mirabilis* was correlated with *A. mirabilis* wounding in light but not in dark conditions. These results suggest that light-dependence of plant physiology can translate into algal-herbivore interactions that are controlled by light. Therefore, light may play a more complex role in shaping the grazing strategies of small primary herbivores than previously recognized.

2. Materials and methods

2.1 Study area and species collection

Experiments were conducted at U.S. Palmer Station (64° 46' S, 64° 03' W) on Anvers Island off the west coast of the Antarctic Peninsula in April and May, 2013. All organisms were collected by hand within 3.5 km of Palmer Station using SCUBA from a depth of 5-20 m. The

amphipod *G. antarctica* was collected subtidally from the brown macroalga *Desmarestia menziesii* by collecting entire *D. menziesii* individuals in a mesh bag (cf. Huang et al., 2007). Amphipods were removed from the algae, sorted by species, and maintained in 2 L plastic bottles with mesh sides inside flowing ambient seawater tables. They were used for feeding assays within 7 days of collection and held without food for 2-3 days prior to the experiments. Individuals of the red macroalga *P. decipiens* and blades from individuals of the brown macroalga *A. mirabilis* were maintained in flowing ambient seawater tables and sampled within 7 days of collection. Samples of macroalgal thallus were excised 1-3 days before use in experiments.

2.2 Electron paramagnetic resonance

The spin trap 5,5-dimethyl-1-pyrroline-N-oxide (DMPO) was purchased from Enzo Life Sciences (ALX-430-090; Farmingdale, NY). Stock solution of DMPO (1 M) was made in ice cold DI water and stored at -80°C until use. Samples of *A. mirabilis* (2.5 cm²) were wounded using a razor blade to create a variable number of 2 cm-long parallel slices through the thallus, placed in 2 mL sterile filtered seawater (SFSW; 0.44 µm) containing 100 µM DMPO, and incubated at 1.5°C. After 20 minutes, seawater samples were immediately frozen in liquid nitrogen. All EPR sampling was conducted on May 4, 2013. Flash frozen samples were shipped on dry ice to the University of Alabama at Birmingham and stored at -80°C. Room temperature EPR analysis was conducted within four months of sampling. All samples were thawed at room temperature for 10 minutes and injected into a Bruker Elexys E500 Aqua-X sample cell (Billerica, MA). The timing for instrumental tuning was kept the same for all samples. Instrument settings were 20 mW power, 1 G modulation amplitude, 160 ms time constant, 320

ms conversion time, one scan. Spectra were analyzed by quantifying the intensity of the third peak (peak minus trough) in the quartet characteristic of DMPO/ OH .

2.3 Feeding assays

Fifteen *G. antarctica* were placed in 100 mL filtered seawater inside each of 20 Nalgene bottles inside a temperature-controlled room held at 1°C. Replicates of *A. mirabilis* (3 cm², n = 20), each from a different individual, were wounded by slicing through the thallus with a clean razor blade and one was placed in each bottle. Each *A. mirabilis* replicate received a variable number of 1 cm-long wounds, between 0 and 24. No number of wounds was repeated, and the order of wounding was randomized. Each *A. mirabilis* replicate had a paired sample from the same individual that was wounded an equal number of times and used as an autogenic control. Autogenic control samples were placed in bottles lacking amphipods and used to control for natural changes in food mass over the course of the feeding assay.

Using alginate-based artificial food in feeding assays with *G. antarctica* greatly increases weighing error compared with using fresh macroalgae (R. McDowell, personal observation). Therefore, one pre-weighed, 1 cm diameter disc of the palatable macroalga *P. decipiens* was placed as food in each bottle. *P. decipiens* food discs were not in contact with *A. mirabilis* samples in containers. *P. decipiens* discs for each experiment came from a single large individual in order to minimize any possible intra-individual differences in their relative palatability. Experiments were conducted using one “food alga” (*P. decipiens*) and a different, wounded alga (*A. mirabilis*) since *G. antarctica* feed preferentially on macroalgal edges (authors’ personal observations). Testing the effect of wounds on unpalatable *A. mirabilis* rather than on the food alga *P. decipiens* avoided potential variation in consumption due to physical differences in the

algal sample caused by wounding. Since the initial, light-dependent wound-induced macroalgal oxidative burst depends on disrupted photosynthetic electron transport (McDowell et al., 2015), it could theoretically be modified by exposure to herbivore-associated molecules that modify photosynthesis during grazing. Although this hypothesis was not explored in the present study, the wounded *A. mirabilis* were exposed to any such diffusible herbivore-associated molecules during the feeding assays.

Amphipods were allowed to graze for 3 hours, after which they were removed from bottles and *P. decipiens* discs were weighed. All weights were to 0.01 mg using a Mettler Toledo XP26 balance. The change in food mass during the feeding assay was corrected for autogenic changes using the formula $[(H_0 \times C_f/C_0) - H_f]$ where H_0 and H_f are disc masses before and after the feeding assay, respectively, and C_0 and C_f are autogenic control disc masses (Cronin and Hay, 1996). *G. antarctica* do not consume *A. mirabilis* as fresh tissue (Amsler et al., 2009), so it was not necessary to physically separate amphipods from *A. mirabilis* during feeding assays.

The “light” feeding assay was conducted during the day on April 26, 2013. All *A. mirabilis* samples were excised on April 25, 2013. The “dark” feeding assay was conducted during the day on April 29, 2013. Due to sampling constraints, 15 *A. mirabilis* replicates were samples excised on April 26, 2013, from the same individuals used for the light feeding assay. Five replicates were samples excised on April 28, 2013 from “new” plants that had been collected at the same time and site as the others. Feeding assays were designed to compare the slope of consumption as a function of *A. mirabilis* wounding between light and dark treatments but were not necessarily designed to compare mean consumption by *G. antarctica* between light and dark treatments since *G. antarctica* used in each assay were from different collections, and mean amphipod size may have differed between but not within assays.

2.4 Light treatments

Both EPR sampling and the feeding assay were performed twice at approximately the same time during the day; once in dark conditions and once in light conditions. Both dark experiments were set up at approximately $0.1 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$ and subsequently covered when possible. Light experiments were set up beneath fluorescent overhead laboratory lighting, at approximately $8 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$. The light feeding assay was carried out under approximately $8 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$ while the light EPR samples were incubated under approximately $18 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$. Surface photosynthetically active radiation at Palmer Station can range from 400 to over 1200 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ in the austral summer (Helbling et al., 1996).

2.5 Statistical analyses

Statistical analyses were performed using SPSS Statistics 21 software (IBM, Armonk, NY, USA). Our data violated the assumptions of both linear and nonlinear ANCOVA since the form of the models between both dependent variables (EPR intensity and amphipod consumption) and *A. mirabilis* wounding were qualitatively different across light treatments with heterogeneous regression slopes. The relationship of these two dependent variables with *A. mirabilis* wounding were analyzed separately for each light level using linear regression, nonlinear regression, and/or correlation (Pearson product-moment) analyses, where appropriate. One EPR intensity data point under light conditions was identified as an extreme outlier using linear regression (studentized residual = -3.915, $p = 0.023$), and it was excluded from analysis. An independent sample t-test was used to compare the overall mean of ROS production when *A. mirabilis* was wounded in the dark vs. light. The effect of wounding was removed by adjusting

ROS production to the mean wounding level using the model of best fit for each individual light treatment. A second independent samples t-test was conducted on these corrected values when *A. mirabilis* was wounded in the dark vs. light. For both t-tests, unwounded (wounding level 0) samples were excluded from analysis.

3. Results

3.1 ROS release was correlated with algal wounding in the light but not in the dark.

Spin trapping and EPR were used to measure ROS released into seawater by wounded *A. mirabilis*. The use of off-line EPR assays by freezing spin-trapped samples has not been applied to macroalgal models, although these data show that it is a feasible and informative method. EPR spectra obtained from wounded *A. mirabilis* corresponded to the DMPO/·OH (hydroxyl radical) adduct (Fig 1; Zhao et al., 2001). All spectra showed a 1:2:2:1 absorbance quartet with equal coupling of the free electron to both the beta hydrogen of the pyrroline ring and the nitroxide nitrogen ($a^N = a^H = 14.9$ G). Enzymatic controls were not used to verify that all spectra were formed exclusively from ·OH. Since the DMPO/·OOH (superoxide) adduct decays with a half-life of ~35 s at pH 8 (Buettner and Oberley, 1978) and can break down into ·OH which may then react with unreacted DMPO (Finkelstein et al., 1982), some portion of these spectra may have been derived from $O_2^{\cdot-}$.

Significantly less ROS were detected in seawater surrounding wounded *A. mirabilis* in the dark compared to wounded *A. mirabilis* in the light (0.681 ± 0.065 vs. 1.006 ± 0.135 , mean $\pm$ SE; independent samples t-test, equal variances not assumed, $t_{25,935} = 2.161$, $p = 0.040$). ROS in seawater surrounding *A. mirabilis* wounded in the dark were not correlated with the amount of wounding ($r = 0.102$, $p = 0.669$) nor could ROS be predicted from the number of wounds on *A.*

mirabilis in the dark [Fig 2a; model of best fit = quadratic, $R^2 = 0.192$, $F(2,17) = 2.026$, $p = 0.162$]. In other words, wounding had no effect on ROS generation from *A. mirabilis* under dark conditions. In contrast, ROS detected in seawater surrounding *A. mirabilis* wounded in the presence of light were positively correlated with the amount of wounding ($r = 0.764$, $p < 0.001$). Using linear regression, ROS (y) could be predicted from the number of wounds on *A. mirabilis* (x) in the presence of light according to the expression $y = 0.077x + 0.301$ [Fig 2b; $R^2 = 0.583$, $F(1,17) = 23.777$, $p < 0.001$]. The effect of wounding was removed from the ROS data in order to compare the effect of light alone. After controlling for *A. mirabilis* wounding, mean ROS did not vary between light and dark conditions (1.01478 ± 0.092 vs. 0.809 ± 0.063 , mean $\pm$ SE; independent samples t-test, $t_{35} = 1.860$, $p = 0.071$), indicating that wounding alone was responsible for the increased ROS detected under light conditions.

3.2 Amphipod consumption was correlated with algal wounding in the light but not in the dark.

Amphipod consumption of the palatable macroalga *P. decipiens* was measured in the presence of unpalatable *A. mirabilis* wounded under light and dark conditions. Consumption of the food alga *P. decipiens* in the dark was not correlated with the amount of *A. mirabilis* wounding ($r = 0.005$, $p = 0.982$) and could not be predicted from the number of wounds on *A. mirabilis* [Fig 3a; model of best fit = cubic, $R^2 = 0.083$, $F(3,16) = 0.482$, $p = 0.700$]. In contrast, amphipod consumption in the presence of light showed a significant curvilinear relationship with the number of wounds on *A. mirabilis*. In the light, consumption (y) could be predicted from the number of wounds on *A. mirabilis* (x) according to the quadratic expression $y = -0.029x^2 + 0.658x + 1.164$ [Fig 3b; $R^2 = 0.494$, $F(2,17) = 8.294$, $p = 0.003$].

4. Discussion

A. mirabilis produced wound-dependent ROS that were controlled by light (Figs 1, 2). These results are consistent with the recent report that the initial wound-induced macroalgal oxidative burst depends on disrupted photosynthetic electron transport (McDowell et al., 2015). Since the brown alga *Saccharina latissima* tested by McDowell et. al (2015) is separated phylogenetically and geographically from the brown alga *A. mirabilis* tested in this study, this mechanism may occur widely across the Phaeophyceae (brown macroalgae). Amphipod grazing in the presence of wounded *A. mirabilis* was light-dependent as well (Fig 3). Amphipod consumption depended on *A. mirabilis* wounding in light but not in dark conditions. This indicates that grazing was modified by a directly light-dependent (non-endogenously regulated), diffusible, wound-activated signal. Previous experiments showing that extracts of *A. mirabilis* do not deter *G. antarctica* grazing under light conditions suggest that this is not a constitutive secondary metabolite (Amsler et al., 2005). There are few grazing defenses besides ROS or molecules downstream of ROS that fulfill these criteria. Since elevated concentrations of ROS have been shown previously to affect feeding by the amphipod *G. antarctica* (McDowell et al., 2014a) and the present study shows that ROS are produced by *A. mirabilis* under the necessary light and wound conditions, all available evidence points to ROS as a mediator of this light and wound-dependent effect on grazing.

The correlation between ROS and amphipod grazing activity was curvilinear, and this is important for our understanding of ROS in ecology. As large amounts of ROS are toxic, we initially hypothesized that the relationship between amphipod grazing and *A. mirabilis* wounding would be linearly negative in the presence of light. The data obtained, however, show a more complex, nonlinear relationship between amphipod consumption and *A. mirabilis* wounding in

the light treatment where low amounts of ROS seemed to stimulate amphipod grazing until a threshold after which ROS began to negatively affect grazing. The involvement of ROS in physiological signaling at lower concentrations and toxicity at high concentrations is a paradigm in the field of free radical biology (e.g. Veal et al., 2007; Wink and Mitchell, 1998). It is intriguing that our results show a concentration-dependent effect consistent with this paradigm and suggest that it may be true ecologically as well. Low levels of ROS may act as an ecological signal which may stimulate amphipod activity or feeding while high levels become toxic and decrease grazing.

Although these experiments were not designed to interpret a difference in mean amphipod consumption between assays as different collections of amphipods were utilized for each assay, mean amphipod consumption in dark conditions was significantly greater than that in the presence of light, even after removing the effect of *A. mirabilis* wounding. Although this difference may simply reflect a difference in mean amphipod hunger or size between the two experiments, it is of note that if this effect was *not* due solely to uncontrolled variables, it supports our hypothesis. Preliminary evidence suggested that wound-induced ROS from the food alga *P. decipiens* are light-dependent (McDowell et al., 2014c), and these light-dependent, herbivory-induced ROS from *P. decipiens* could lead to decreased amphipod consumption in light vs. dark conditions, independent of *A. mirabilis* wounding.

All organisms were maintained at a natural light-dark cycle and both light and dark experiments were conducted at the same time of day. Since light was directly manipulated, the effects seen in these experiments were not endogenous. In addition, the light levels used in spin trapping and feeding assays (approximately 18 and 8 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$, respectively) were quite low. Surface photosynthetically active radiation at Palmer Station can range from ~ 400 to

greater than 1200 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ in the austral summer (Helbling et al., 1996). The depth ranges for *P. decipiens* and *A. mirabilis* near Palmer Station are fairly shallow; approximately 0-8 m and 7-10 m, respectively. Therefore, the light treatments were relevant and probably conservative. This experiment did not, however, take into account the effects of diffusion and water flow which would undoubtedly be important in a natural system.

The implications of the data shown here conflict with a 2005 meta-analysis (Hillebrand) of experiments that manipulated both irradiance and grazer presence. This meta-analysis found that higher light increased the effect size of the grazer treatment, indicating that grazers consumed a greater percentage of plant biomass at higher light. In many of the studies, however, the effect of light on grazing may have been complicated by the effect of light on the community composition, quality, and quantity of primary producers. In addition, the majority of the studies analyzed were conducted in fast-moving in situ or laboratory streams with currents ranging between 10 and 30 $\text{cm} \cdot \text{s}^{-1}$. Rapid water movement likely decreases the sensitivity of primary consumers to light-dependent ROS released during grazing. Furthermore, while the majority of the studies included in the meta-analysis utilized snails as grazers, the sensitivity of herbivores to grazing-induced ROS is likely to differ widely based on their size and grazing modality.

The data shown here indicate that the light-dependence of plant physiology can have a light-dependent effect on grazers, leading to repercussions on plant-herbivore interactions. The results of this study are consistent with the hypothesis that light-dependent, wound-induced metabolites such as ROS produced unavoidably by the disruption of photosynthetic electron transport during wounding can impact grazers. Furthermore, this study has shown a concentration-dependent ecological effect of ROS that parallels that seen in physiological ROS research, where low doses of ROS act as signals while higher doses act as toxins. The idea of

wound-induced ROS as an ecological signal at low doses is especially interesting as wound-induced ROS may not have been selected for as an ecological signal that depends on an injury recognition mechanism, signal transduction, and activation of an ROS source, but rather may have arisen as a simple consequence of physiology, in which injury causes an almost accidental leakage of ROS. Although the production of these ROS is dictated by physiology, the data shown here emphasize the importance of exploring the ecological roles of ROS in algal- and plant-herbivore interactions. For example, these data suggest that the light-dependent production of ROS during grazing injury may have contributed to the evolution of grazing strategies by very small primary consumers.

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Figure captions

Figure 1. Light-dependent ROS can be detected in seawater surrounding wounded *Ascoseira mirabilis*. Data points show ROS detected in the medium surrounding wounded *Ascoseira mirabilis* plotted as a function of the number of wounds on *A. mirabilis* (dark n = 20, light n = 19). ROS were measured using quantitative electron paramagnetic resonance (EPR). Data points are the intensity of the third peak in the characteristic DMPO/OH EPR quartet for each sample.

(A) Samples taken from seawater surrounding *A. mirabilis* wounded in the dark. (B) Samples taken from seawater surrounding *A. mirabilis* wounded in the light.

Figure 2. ROS are a function of algal wounding in the light but not in the dark. Data points show ROS in the medium surrounding wounded *Ascoseira mirabilis* plotted as a function of the number of wounds on *A. mirabilis* ($n = 20$). ROS were measured using quantitative electron paramagnetic resonance (EPR). Data points are the intensity of the third peak in the characteristic DMPO/•OH EPR quartet for each sample. (A) Samples taken from seawater surrounding *A. mirabilis* wounded in the dark. (B) Samples taken from seawater surrounding *A. mirabilis* wounded in the light.

Figure 3. Algal wounding is related to amphipod consumption in the light but not in the dark. Data points show consumption (mg) of amphipods feeding on the rhodophyte *Palmaria decipiens* in the presence of wounded *Ascoseira mirabilis* as a function of the number of wounds on *A. mirabilis* ($n = 20$). Assay duration was 3 hours. (A) Data from a feeding assay conducted in the dark. (B) Data from a feeding assay conducted in the light.

Literature cited

- Amsler, C.D., McClintock, J.B., Baker, B.J., 2014. Chemical mediation of mutualistic interactions between macroalgae and mesograzers structure unique coastal communities along the western Antarctic Peninsula. *J. Phycol.* 50, 1–10.
- Amsler, C.D., Amsler, M.O., McClintock, J.B., Baker, B.J., 2009. Filamentous algal endophytes in macrophytic Antarctic algae: prevalence in hosts and palatability to mesoherbivores. *Phycologia* 48, 324–334.

- Amsler, C.D., Iken, K., McClintock, J.B., Amsler, M.O., Peters, K.J., Hubbard, J.M., Furrow, F.B., Baker, B.J., 2005. Comprehensive evaluation of the palatability and chemical defenses of subtidal macroalgae from the Antarctic Peninsula. *Mar. Ecol. Prog. Ser.* 294, 141–159.
- Asada, K., 2006. Production and scavenging of reactive oxygen species in chloroplasts and their functions. *Plant Physiol.* 141, 391–396.
- Bedard, K., Lardy, B., Krause, K.-H., 2007. NOX family NADPH oxidases: Not just in mammals. *Biochimie* 89, 1107–1112.
- Buettner, G.R., Oberley, L.W., 1978. Considerations in the spin trapping of superoxide and hydroxyl radical in aqueous systems using 5, 5-dimethyl-1-pyrroline-1-oxide. *Biochem. Biophys. Res. Commun.* 83, 69–74.
- Chang, C.C.-C., Ball, L., Fryer, M.J., Baker, N.R., Karpinski, S., Mullineaux, P.M. 2004. Induction of ascorbate peroxidase 2 expression in wounded *Arabidopsis* leaves does not involve known wound-signalling pathways but is associated with changes in photosynthesis. *Plant J.* 38, 499–511.
- Corneille, S., Cournac, L., Guedeney, G., Havaux, M., Peltier, G. 1998. Reduction of the plastoquinone pool by exogenous NADH and NADPH in higher plant chloroplasts: characterization of a NAD(P)H–plastoquinone oxidoreductase activity. *Biochim. Biophys. Acta* 1363, 59–69.
- Cronin, G., Hay, M.E., 1996. Induction of seaweed chemical defenses by amphipod grazing. *Ecology* 77, 2287–2301.
- Felton, G., Bi, J., Summers, C., Mueller, A., Duffey, S., 1994. Potential role of lipoxygenases in defense against insect herbivory. *J. Chem. Ecol.* 20, 651–666.

- Finkelstein, E., Rosen, G.M., Rauckman, E.J., 1982. Production of hydroxyl radical by decomposition of superoxide spin-trapped adducts. *Mol. Pharmacol.* 21, 262–265.
- Halliwell, B., 2006. Reactive species and antioxidants. Redox biology is a fundamental theme of aerobic life. *Plant Physiol.* 141, 312–322.
- Halliwell, B., Gutteridge, J., 2007. *Free Radicals in Biology and Medicine*. Fourth edition. Oxford University Press, New York, USA.
- Helbling, E.W., Chalker, B.E., Dunlap, W.C., Holm-Hansen, O., Villafañe, V.E., 1996. Photoacclimation of Antarctic marine diatoms to solar ultraviolet radiation. *J. Exp. Mar. Biol. Ecol.* 204, 85–101.
- Hillebrand, H., 2005. Light regime and consumer control of autotrophic biomass. *J. Ecol.* 93, 758–769.
- Lamb, C., Dixon, R.A., 1997. The oxidative burst in plant disease resistance. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 48, 251–275.
- Lou, Y.G., Baldwin, I.T., 2006. Silencing of a germin-like gene in *Nicotiana attenuata* improves performance of native herbivores. *Plant Physiol.* 140, 1126–1136.
- Mao, J., Burt, A.J., Ramputh, A.-I., Simmonds, J., Cass, L., Hubbard, K., Miller, S., Altosaar, I., Arnason, J. T., 2007. Diverted secondary metabolism and improved resistance to European corn borer (*Ostrinia nubilalis*) in maize (*Zea mays L.*) transformed with wheat oxalate oxidase. *J. Agric. Food Chem.* 55, 2582–2589.
- McDowell, R.E., Amsler, C.D., McClintock, J.B., Baker, B.J., 2014a. Reactive oxygen species as a marine grazing defense: H₂O₂ and wounded *Ascoscira mirabilis* both inhibit feeding by an amphipod grazer. *J. Exp. Mar. Biol. Ecol.* 458, 34–38.

- 399 McDowell, R.E., Amsler, C.D., McClintock, J.B., Baker, B.J., 2014b. Reactive oxygen species
400 and the Antarctic macroalgal wound response. *J. Phycol.* 50, 71–80.
- 401 McDowell, R.E., Amsler, M.O., Li, Q., Lancaster Jr., J.R., Amsler, C.D., 2015. The immediate
402 wound-induced oxidative burst of *Saccharina latissima* depends on light via
403 photosynthetic electron transport. *J. Phycol.* 51, 431–441.
- 404 Miller, G., Schlauch, K., Tam, R., Cortes, D., Torres, M.A., Shulaev, V., Dangel, J. L., Mittler, R.,
405 2009. The plant NADPH oxidase RBOHD mediates rapid systemic signaling in response
406 to diverse stimuli. *Sci. Signal.* 2:ra45.
- 407 Morker, K.H., Roberts, M.R., 2011. Light as both an input and an output of wound-induced
408 reactive oxygen formation in *Arabidopsis* leaves. *Plant Signal. Behav.* 6, 1087–1089.
- 409 Niethammer, P., Grabher, C., Look, A.T., Mitchison, T.J., 2009. A tissue-scale gradient of
410 hydrogen peroxide mediates rapid wound detection in zebrafish. *Nature* 459, 996–999.
- 411 Ohtsuka, M., Oyabu, J., Kashino, Y., Satoh, K., Koike, H. 2004. Inactivation of *ycf33* results in
412 an altered cyclic electron transport pathway around photosystem I in *Synechocystis* sp.
413 PCC6803. *Plant Cell Physiol.* 45, 1243–1251.
- 414 Peng, M., Kuc, J., 1992. Peroxidase-generated hydrogen peroxide as a source of antifungal
415 activity in vitro and on tobacco leaf disks. *Phytopathology* 82, 696–699.
- 416 Potin, P., 2008. Oxidative burst and related responses in biotic interactions of algae, in: Amsler,
417 C.D. (Ed.), *Algal Chemical Ecology*. Springer-Verlag, Berlin, pp. 245–271.
- 418 Ramputh, A.I., Arnason, J.T., Cass, L., Simmonds, J.A., 2002. Reduced herbivory of the
419 European corn borer (*Ostrinia nubilalis*) on corn transformed with germin, a wheat
420 oxalate oxidase gene. *Plant Sci.* 162, 431–440.

- 421 Roberts, M.R., Paul, N.D., 2006. Seduced by the dark side: integrating molecular and ecological
422 perspectives on the influence of light on plant defence against pests and pathogens. *New*
423 *Phytol.* 170, 677–699.
- 424 Suzuki, N., Mittler, R., 2012. Reactive oxygen species-dependent wound responses in animals
425 and plants. *Free Radic. Biol. Med.* 53, 2269–2276.
- 426 Veal, E.A., Day, A.M., Morgan, B.A., 2007. Hydrogen peroxide sensing and signaling. *Mol. Cell*
427 26, 1–14.
- 428 Wink, D.A., Mitchell, J.B., 1998. Chemical biology of nitric oxide: insights into regulatory,
429 cytotoxic, and cytoprotective mechanisms of nitric oxide. *Free Radic. Biol. Med.* 25,
430 434–456.
- 431 Yoo, S.K., Freisinger, C.M., LeBert, D.C., Huttenlocher, A., 2012. Early redox, Src family
432 kinase, and calcium signaling integrate wound responses and tissue regeneration in
433 zebrafish. *J. Cell Biol.* 199, 225–234.
- 434 Zhao, H., Joseph, J., Zhang, H., Karoui, H., Kalyanaraman, B., 2001. Synthesis and biochemical
435 applications of a solid cyclic nitron spin trap: a relatively superior trap for detecting
436 superoxide anions and glutathionyl radicals. *Free Radic. Biol. Med.* 31, 599–606.

Figure 1 final

Figure 2 final

Figure 3 final