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The effects of nutrient availability on tolerance to herbivory in a brown seaweed

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Summary

1. Plants can resist herbivory through mechanisms of resistance (traits that reduce the amount of tissue lost to herbivores) and tolerance (traits that reduce the negative effects of herbivory on plant fitness). The expression of such traits requires the allocation of plant resources, and consequently, trade-offs between tolerance and resistance can occur in plants. For marine algae, little is currently known about the expression of tolerance, the relationship between tolerance and resistance, and the impact of resource availability on these traits.

2. We manipulated herbivory (simulated or real) and nutrient availability in a series of laboratory and field experiments to test for tolerance to different types of damage under varying resource conditions in the brown alga *Sargassum* cf. *podacanthoides*. In addition to growth and photosynthetic responses of the alga, we measured the concentration of phlorotannins (secondary metabolites associated with resistance in many brown algae) to test for possible phenotypic trade-offs between traits of tolerance and resistance. Furthermore, we assessed whether the limiting resource model (LRM) could predict algal tolerance under different resource conditions.

3. *Sargassum* cf. *podacanthoides* expressed traits of tolerance (increased growth rates and reallocation of resources) that were specific to both the type and severity of herbivory encountered. Consistent with the predictions of the LRM, nitrogen enrichment did not alter the compensatory growth responses of the alga. Correlations between growth and phlorotannin content were variable, dependent on the measure of growth analysed, and only weakly influenced by nutrient availability. Nutrient enrichment, however, consistently changed within-alga patterns of tissue composition and photosynthetic activity.

4. *Synthesis*. Herbivory in the marine environment is often severe, and, similar to higher plants, tolerance to herbivory may be an important mechanism that maintains the productivity of marine algae. We demonstrate the potential applicability of the terrestrially derived LRM to predict algal tolerance under eutrophic conditions. Nutrient-induced changes to within-alga patterns of tissue composition should also be considered when evaluating the effects of nutrient enrichment on marine communities. Furthermore, as in higher plants, the relationships between traits of tolerance and resistance are variable in algae, and trade-offs cannot be assumed.

Key-words: amphipods, herbivory, limiting resource model, macroalgae, phlorotannins, plant–herbivore interactions, resistance, *Sargassum*, trade-offs

Introduction

Tolerance is the expression of plant traits that reduce the negative impact of tissue loss on plant fitness following herbivory (Strauss & Agrawal 1999; Tiffin 2000). Plants have evolved a diverse suite of tolerance traits including compensatory growth, increased photosynthesis, activation of dormant meristems and changes in resource allocation patterns and reproductive strategies (Strauss & Agrawal 1999; Tiffin 2000). While the ability to tolerate herbivory is affected by resource availability, simple models such as the growth rate model (Alward & Joern 1993) and compensatory continuum hypothesis

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(Hawkes & Sullivan 2001) have failed to predict the complexity of plant responses under different resource conditions (Hawkes & Sullivan 2001; Wise & Abrahamson 2007).

More recently, Wise & Abrahamson (2005, 2007) have developed the limiting resource model (LRM) that makes predictions of greater, lower or equal tolerance in either low- or high-resource environments. The LRM is based on three core questions that generate predictions of tolerance for seven possible scenarios. The questions include whether the resource differing between the environments and experimental treatments (the focal resource) is limiting plant fitness, whether herbivory primarily affects the acquisition of the focal resource or an alternative resource and, finally, whether herbivory causes the alternative resource to limit plant fitness (see Wise & Abrahamson 2007 for dichotomous key of predictions).

Along with tolerating herbivory, plants have evolved traits of resistance. Resistance traits reduce the amount of tissue lost to herbivores by either deterring herbivores or reducing the quality of the plant tissue as a food (e.g. secondary metabolites or morphological traits such as thorns) (Strauss & Agrawal 1999). Resistance can be expressed continuously (constitutive) or induced in response to herbivore damage (Karban, Agrawal & Mangel 1997).

Trade-offs between resistance and tolerance are predicted based on the assumptions that (i) plant resources are limited, (ii) the expression of resistance and tolerance require the allocation of resources within plants and (iii) resistance and tolerance serve similar functions for plant fitness (Meijden, Wijn & Verkaar 1988; Fineblum & Rausher 1995; Tiffin & Rausher 1999). When plant resources are limited, it is predicted that investment in resistance reduces the resources available to compensate for herbivore damage and vice versa. However, a meta-analysis of ecological and agricultural studies revealed variable evidence for trade-offs between resistance and tolerance in plants (Leimu & Koricheva 2006). The variation in the results was attributed to the measurement of phenotypic versus genotypic costs, the plant traits measured and variation in nutrient availability (Leimu & Koricheva 2006). To understand the variation in tolerance–resistance trade-offs, Leimu & Koricheva (2006) recommended that specific traits of resistance and tolerance should be examined under different nutrient conditions.

In the marine environment, herbivory is more severe than in terrestrial systems (Cry and Pace 1993), yet compared to the terrestrial literature (e.g. McNaughton 1979), the concept of tolerance to herbivory has only recently been applied to marine macroalgae. Although both chemical (Amsler 2008) and physical (Hay 1997) resistance are well studied in marine algae, it is unclear whether tolerance also helps maintain algal productivity under the pervasive influence of marine herbivory. The applicability of terrestrial models for marine algae should be tested as such models have the potential to advance our understanding of marine algal–herbivore interactions. Specifically, the LRM may provide insights into the effects of eutrophication on algal tolerance to herbivory.

Studies that have investigated the potential role of tolerance in macroalgae have shown variable results. Some coralline red algae have been found to be tolerant to molluscan (Littler, Littler & Taylor 1995) and urchin grazing (Wai & Williams 2005), whereas, in the few species of brown macroalgae examined, tolerance has varied with grazer identity (Rohde, Molis & Wahl 2004), damage type (Taylor, Sotka & Hay 2002), damage severity (Honkanen & Jormalainen 2002), depth (Jormalainen & Ramsay 2009) and the growth traits measured (Hemmi *et al.* 2005; Cerda *et al.* 2009).

The few studies that have investigated trade-offs between tolerance and resistance in brown macroalgae have also shown variable results, depending on the traits measured (Pfister 1992), the species investigated and the spatial and temporal scales of measurement (Steinberg 1995; Pavia, Toth & Aberg 1999). The variation seen in the expression of both tolerance and trade-offs between tolerance and resistance in macroalgae implies that, similar to terrestrial plants, resource availability can affect the expression and relative costs of tolerance and resistance in macroalgae. Therefore, the effects of resource availability on both algal tolerance and tolerance–resistance trade-offs should be investigated.

Brown macroalgae often form the base of coastal food webs and provide habitat for associated fauna (e.g. Christie, Norderhaug & Fredriksen 2009). Given the key ecological roles of brown macroalgae and the global issue of eutrophication of coastal ecosystems, it is important to identify whether tolerance is a mechanism that maintains algal productivity, how tolerance is influenced by resistance traits and how nutrient enrichment affects these traits. To test the interactive effects of herbivory and nitrogen enrichment on the expression of tolerance and resistance in a brown macroalga, we conducted a series of laboratory and field experiments using the subtropical alga, *Sargassum* cf. *podacanthoides* Kraft (2009) (henceforth referred to as *Sargassum*).

Experiments were designed to address the following specific questions: (i) Does *Sargassum* express changes in growth rates, photosynthetic activity or growth patterns (traits of tolerance) in response to herbivory? (ii) Does the expression of these tolerance traits differ among the types of tissue damaged or between simulated and natural herbivory? (iii) Is variation in tolerance traits associated with variation in phlorotannin content? (iv) Do tolerance traits, phlorotannin content or the relationship between these traits change with nitrogen availability? (v) Can the LRM successfully predict the response of tolerance under different resource conditions in a marine alga?

Materials and methods

STUDY ORGANISM, COLLECTION AND STUDY SITE

In this study, we used the large, subtropical brown alga *Sargassum* from Redcliffe, Moreton Bay, South East Queensland, Australia (27º13.2′ S; 153º06.7′ E). Voucher specimens of reproductive material have been deposited in the University of Melbourne (MELU) and Queensland (BRI) herbariums. *Sargassum* displays pseudo-annual growth and is the dominant alga of the shallow sub-tidal zone at Redcliffe over the austral summer. *Sargassum* exhibits modular growth and pieces can continue to grow and reproduce after being detached from the parent individual. All experiments in this study were conducted over the summer period of November 2007–March 2008. For each experiment, *Sargassum* was collected from a depth of 1–1.5 m.

At Redcliffe, the herbivorous amphipod *Cymadusa setosa* (Haswell 1879) is commonly associated with *Sargassum*. Although small (*c.* 1.5 cm), *C. setosa* can consume up to 1 cm^2 of a *Sargassum* blade in a 24-h period (K.B. Hay, A.G.B., Poore and C.E. Lovelock, unpublished data), and species in the genus *Cymadusa* are well-known herbivores of macroalgae world-wide (Poore, Hill & Sotka 2008). Evidence of grazing can be observed on all *Sargassum* plants in the field. Herbivory of apical tissues is particularly common, and *C. setosa* exhibits strong habitat and feeding preferences for apical tissues of *Sargassum* (K.B. Hay, A.G.B., Poore and C.E. Lovelock, unpublished data). Although less conspicuous, the herbivorous amphipod *Peramphithoe parmerong* and the fishes *Siganus fuscescens* and *Microcanthus strigatus* are also found at the study site. Echinoid herbivores are noticeably absent.

We conducted four laboratory and one field experiments using *Sargassum* from Redcliffe to investigate the expression of tolerance in brown algae under different resource conditions. In each of these experiments, we manipulated nutrient availability and varied damage by either simulated or real herbivory by *C. setosa*.

EFFECTS OF SIMULATED HERBIVORY AND NUTRIENT AVAILABILITY IN THE LABORATORY

To determine whether the expression of tolerance in *Sargassum* is specific to the type of damage encountered, we tested the effects of herbivory and nutrient availability on *Sargassum* in two laboratory experiments that utilized different simulated herbivory treatments. Tissue damage and nutrients were manipulated in a factorial design with two damage treatments (control and damage) and two nutrient conditions (low and high nitrogen availability).

For each experiment, eight *Sargassum* individuals were collected from Redcliffe by harvesting from above the holdfast and transported in natural sea water to the University of Queensland. To account for possible differences in growth and phlorotannin content owing to genotype (Jormalainen & Ramsay 2009), four side branches from each of the collected eight individuals were used for the experiments. Using a scalpel blade, the side branches were removed from the primary stipe of each individual and randomly allocated to one of the four treatment combinations. There were eight replicate branches per treatment combination.

In the first laboratory experiment, the damage treatments involved the removal of the primary apex (apical) and a control (Fig. 1). The apical treatment simulated the herbivory to apices that was commonly observed in the field. The low-nitrogen-availability treatment consisted of filtered sea water from Moreton Bay, where *Sargassum* growth is nitrogen-limited (ammonium concentrations below the detection level of 0.5 μM). The high-nitrogen-availability treatment consisted of filtered sea water with a spiked ammonium concentration of 28.5 μM. This high-nitrogen treatment is in excess of the *c.* 2 μM ammonium concentrations recorded within Moreton Bay (Dennison & Abal 1999). The damage treatments were inflicted on day 1 of the experiment. Following damage, each branch was spun dry with a salad spinner (80 revolutions) and weighed to obtain initial wet weight (mean ± SE = 1.198 ± 0.071 g). The initial length, number of nodes and number of blades were also recorded for each branch before adding the branches to the experimental system.

The algae were grown in 1-L Erlenmeyer flasks filled with filtered sea water and arranged in eight cooling basins. The four branches from each *Sargassum* individual were randomly assigned to a flask. The flasks were allocated in a block design so that each cooling basin housed four flasks representing each of the four treatments. Flasks were maintained under a photon density of 150 ± 50 μmol m^{-2} s^{-1} using a combination of halogen and fluorescent lights for a 12:12-h photoperiod. The temperature within the flasks was maintained at 26 ± 2 °C by adjusting the temperature within the cooling basins. Phosphorus and carbon were added to all flasks in the form of NaH_2PO_4 and $NaHCO_3$ at concentrations of 10 and 3 μM, respectively. Ammonium was added to the high-nitrogen-treatment flasks as NH_4Cl at a concentration of 28.5 μM. Low-nitrogen-treatment flasks received no added ammonium. The flasks were aerated to ensure water movement and the maintenance of aerobic conditions. The pH levels of all flasks were maintained between 8.1 and 8.3 by adding small amounts of hydrochloric acid. To maintain treatment conditions, water was exchanged every second day of the 8-day experiments.

After 8 days, the branches were re-weighed to obtain final wet weight. Length, number of nodes and number of blades were measured for each branch, and the relative growth rates (RGR) of each trait were calculated. To estimate the photosynthetic activity of the *Sargassum* branches, we used pulse-amplitude-modulated measurement of photosystem II chlorophyll *a* fluorescence with a PAM-2100 Chlorophyll Fluorometer (Heinz Walz GmbH, Effeltrich, Germany). Relative electron transport rates (ETR, μmol electrons m^{-2} s^{-1}) were measured following the general procedures described in algal studies (Franklin & Badger 2001). Measurements were taken under an actinic light of 270 μmol m^{-2} s^{-1} and indicate the tissues' maximum rate of photosynthesis. The branches were freeze-dried for 48 h and ground to a fine powder using a ball grinder. Near-infrared reflectance spectrometry (NIRS) was used to measure the tissue contents of the

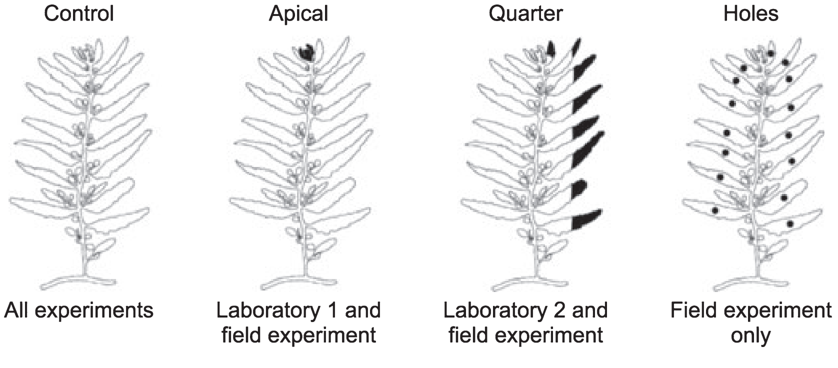

Fig. 1. Damage treatments used to simulate herbivory in laboratory and field experiments. Black sections represent *Sargassum* tissue removed.

ground algae. Samples were scanned using an NIR spectrophotometer (Model 6500; NIRSystems, Silver Springs, MD, USA) following the protocols in Hay *et al.* (2010). The content of phlorotannin, nitrogen and carbon in the samples were estimated with the NIRS software package VISION (Version 1.0; FOSS NIRSystems, Laurel, MD, USA), using spectra calibration models previously developed for each constituent (Hay *et al.* 2010).

To investigate the effects of differential tissue loss, a second experiment using greater amounts of blade tissue loss was conducted to simulate more intense fish herbivory. In the second experiment, we substituted the apical removal treatment with a 'quarter' treatment in which half the area of half of the blades was removed (Fig. 1). As in the first experiment, damage (control, quarter) and nutrient treatments (low, high) were manipulated in a fully crossed factorial design. The mean initial wet weight of the *Sargassum* branches was 1.093 ± 0.063 g, and the experiment was carried out using the same protocols and facilities as above.

EFFECTS OF SIMULATED HERBIVORY AND NUTRIENT AVAILABILITY IN THE FIELD

To investigate how *Sargassum* responds to herbivory under natural field conditions, we tested the effects of simulated damage and nutrient availability on *Sargassum in situ*. Because we were not constrained by the limited space of the culture facilities, a third damage treatment was added to the apical removal and quarter blade removal treatments in the experimental design to further test for the effects of differential herbivory. The third damage treatment ('holes') involved the removal of 5-mm-diameter holes from the centre of each blade using a hole punch (Fig. 1). Although the herbivore responsible for this damage type is unknown to us, this pattern of damage is observed at our field site. Two experimental levels of nutrient availability were used in the field experiment: high nutrients (*in situ* fertilization using slow-release NPK fertilizer) and ambient nutrients (ambient sea water). Nutrient treatments were manipulated along with the four damage treatments in a factorial design.

At Redcliffe, 20 *Sargassum* individuals were haphazardly tagged. From this pool of individuals, 10 were randomly allocated to the fertilization and ambient treatments. A brick, with a PVC pipe horizontally attached (150 mm in length × 20 mm diameter), was placed at the base of each tagged individual. PVC pipes allocated to *Sargassum* individuals in the fertilization treatment contained a nylon cloth holding 400 g of Osmocote® fertilizer with a 16 : 3.5 : 10 N/P/K ratio (Scotts Australia Pty Ltd, Sydney, Australia). PVC pipes allocated to the ambient treatment contained only the nylon cloth. Water adjacent to the fertilized plants had significantly higher concentrations of nitrogen oxides ($NO_x = 0.012$ mg L^{-1}) than water from the control plants ($NO_x = 0.009$ mg L^{-1}; $t = 3.74$, d.f. = 20, $P = 0.001$).

Four adjacent side branches from each individual (equivalent to those used in the laboratory experiments) were labelled with small cable ties at the base of the branch on the primary stipe. The four damage treatments (Fig. 1) were allocated randomly to the four side branches from each individual and assumed to be independent from each other owing to the modular nature of *Sargassum* (Ateweberhan, Bruggemann & Breeman 2008). To control for the effects of natural herbivory, only side branches without visible grazing scars or damage were used in the experiment. The length of each side branch was measured and recorded as the distance between the branch junction on the primary stipe and the apical growing point of the side branch. The number of nodes on each side branch was counted and recorded. The algae were harvested after 7 days. Each experimental side branch was scored for length, number of nodes and number of blades. Following the same protocols as in first experiment, the photosynthetic activity (ETR) and tissue content of each branch were measured. Branches that exhibited visible grazing damage from natural herbivores over the experimental period were excluded from the analyses.

EFFECTS OF NATURAL HERBIVORY AND NUTRIENT AVAILABILITY

Algal responses to simulated herbivory have been shown to differ from natural herbivory (Pavia & Toth 2000). We therefore conducted laboratory experiments using a natural herbivore of *Sargassum*, the amphipod *C. setosa*. The natural herbivory experiments used the same nutrient treatments (low and high NH_4^+), followed the same protocols and used the same facilities as the simulated herbivory experiments. The damage treatments in the natural herbivore experiments were the following: (i) control, *Sargassum* not exposed to amphipods and (ii) amphipod, *Sargassum* exposed to grazing by *C. setosa*. Nutrient and amphipod treatments were arranged in fully crossed factorial designs.

In the first natural herbivory experiment, two individuals of *C. setosa* were added to *Sargassum* (mean initial wet weight of algae 1.251 ± 0.065 g) allocated to the amphipod treatments on day 3 of the experiment. The amphipods remained within the flasks until the algae were harvested on day 8. All tissues were analysed for growth, photosynthetic activity and tissue chemical content in the same manner as described in full for the first simulated herbivory experiment. A second natural herbivory experiment was run to allow the analysis of apical and basal tissue composition separately, given that the previous laboratory experiments found differences in the photosynthetic activity of apical and basal tissues within side branches of *Sargassum* (see Table S4). This second experiment with natural herbivores used the same four treatments as the first experiment (mean initial wet weight of 1.437 ± 0.056 g), but the two amphipods were added on day 5 of the 8-day experiment rather than day 3. Amphipods were added on day 5 (i.e. allowed to feed for only 3 days) to ensure enough algal tissue remained at the end of the experiment to analyse apical and basal tissues separately. On day 8, growth and photosynthetic measurements of the branches were made following the procedures described for the first experiment. Following these measurements, each *Sargassum* branch was cut in half to produce an apical section and basal section. These apical and basal sections were freeze-dried, ground to a fine powder and analysed for tissue content separately using NIRS.

RELATIONSHIPS BETWEEN GROWTH AND PHLOROTANNINS

To test for relationships between growth and phlorotannin content in *Sargassum* under different nutrient conditions, we conducted analyses of covariance (ANCOVA) using the data from the first two simulated herbivory and first natural herbivory experiments. However, given the high correlation between growth variables and the desire to avoid inflated type I error owing to the high number of statistical tests needed, we used a principal components analysis prior to the use of ANCOVA. The principal components analysis revealed that 79% of the total variance was explained by two principal components (see Table S1 in Supporting Information). Subsequently, principal component 1 (PC1) was used as a general predictor of *Sargassum* growth rate, and principal component 2 (PC2) was used as a general predictor of *Sargassum* biomass in the ANCOVA models.

STATISTICAL ANALYSES

The statistical software STATISTICA 8 (StatSoft Inc., Tulsa, OK, USA) was used for all two-way ANOVAS, repeated-measures ANOVAS and ANCOVAS. The statistical software R 2.8.0 (R Foundation for Statistical Computing, Vienna, Austria) was used to conduct a principal components analysis. All data were tested for normality and homogeneity of variances, and data were transformed when necessary. Following significant effects in ANOVA, Tukey's *post hoc* tests were used to contrast specific means.

Results

EFFECTS OF SIMULATED HERBIVORY AND NUTRIENT AVAILABILITY

Simulated herbivory had no effect on the relative growth rate (wet weight) of *Sargassum* in either laboratory experiments (Table S2). Simulated herbivory also had no effect on the dry weight of *Sargassum* in the field experiment (two-way ANOVA: $F_{3,\,66} = 1.7$, $P = 0.172$). However, in both the laboratory and field experiments, the removal of the primary apex prevented node initiation and increased the number of blades per node (Fig. 2, Table S2). Simulated herbivory had no effect on the photosynthetic electron transfer rate (ETR) of *Sargassum*.

Simulated herbivory had a limited and variable impact on the tissue composition of *Sargassum*. Removal of the primary apex significantly increased the C/N ratio of tissue in the first experiment (Table S3) but had no effect on C/N ratio in the field experiment. Quarter blade removal had no effect on tissue composition in the second laboratory experiment but significantly decreased the phlorotannin content of *Sargassum* tissue in the field experiment (Fig. 3f, Table S3).

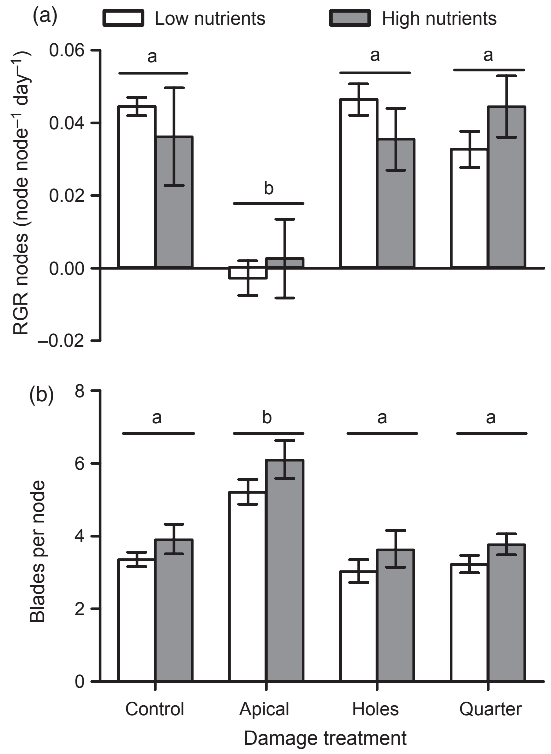

Fig. 2. Mean relative growth rate ± SE (RGR) of nodes (a) and number of blades per node (b) of *Sargassum* following simulated herbivory and nutrient enrichment in the field experiment. Damage treatments as in Fig. 1. Different letters above horizontal lines denote significant differences between damage treatments in *post hoc* tests.

Nitrogen availability increased at least one growth trait of *Sargassum* in all experiments (Fig. 2b, Table S2). Nitrogen availability had no main effect on the ETR of *Sargassum* tissue, but *Sargassum* tissue type (apical versus basal), and the interaction between nitrogen availability and tissue type, had significant effects on the ETR of tissues in the laboratory and field experiments (Table S4). In the laboratory experiments, the ETR of apical *Sargassum* tissue decreased under high nitrogen availability, and the ETR of basal tissue increased (Fig. S1).

Enhanced nitrogen availability increased tissue nitrogen and decreased the C/N ratio in all experiments (Fig. 3a–d, Table S3). Enhanced nutrient availability also decreased the phlorotannin content of *Sargassum* tissue in the field experiment but had no main effect in the laboratory experiments (Fig. 3f, Table S3).

Herbivory and nitrogen availability interacted to affect *Sargassum* tissue composition only in the second experiment (Table S3). Under low nitrogen availability, phlorotannin content increased following the quarter blade removal treatment, whereas under high nitrogen availability, phlorotannin content decreased following quarter blade removal (Fig. 3e).

EFFECTS OF AMPHIPOD HERBIVORY AND NUTRIENT AVAILABILITY

In both natural herbivory experiments, amphipods were observed to actively feed on the *Sargassum* branches and, in other experiments, feeding rates have been shown not to differ between high- and low-nitrogen treatments (K.B. Hay, A.G.B., Poore and C.E. Lovelock, unpublished data). In both experiments, amphipod herbivory decreased the RGR of *Sargassum* wet weight (Fig. 4a, Table S2). Amphipod herbivory also decreased the RGR of blades in the second natural herbivory experiment (Fig. 4b, Table S2). Amphipods had no effect on the photosynthetic activity of *Sargassum* (Table S4). Amphipod herbivory also had no effect on the tissue N, C/N and phlorotannin content of *Sargassum* tissue (Table S3).

As in the simulated herbivory experiments, enhanced nitrogen availability increased the RGR of *Sargassum* wet weight in both amphipod experiments (Fig. 4a, Table S2). However, nitrogen availability had a variable effect on the RGR of blades between experiments: no effect in the first experiment (Fig. 4b) and a positive effect in the second experiment (Table S2). Also similar to the simulated experiments, the ETR of apical and basal tissues responded differently to nitrogen availability (Table S4). The ETR of apical tissue decreased under high nitrogen availability, while the ETR of basal tissue increased (e.g. Fig. S1).

Nitrogen enrichment increased tissue N, decreased C/N ratios and had no effect on the phlorotannin content of *Sargassum* in the first amphipod experiment (Fig. 5, Table S3) or the

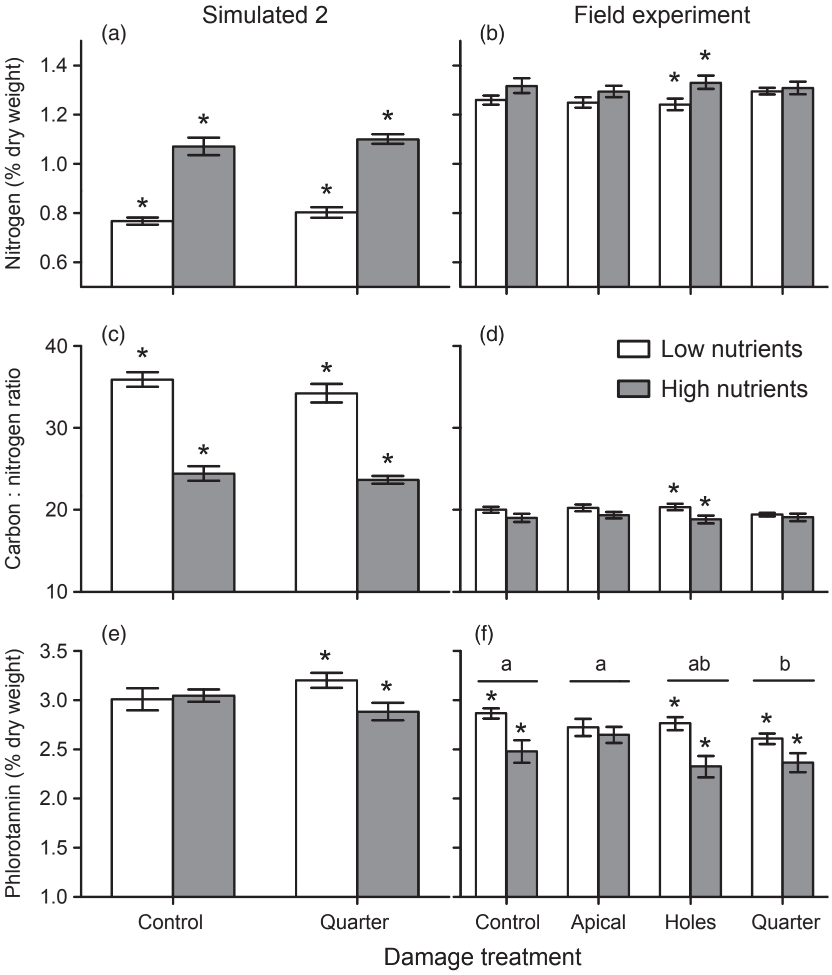

Fig. 3. Mean ± SE nitrogen content (a, b), carbon-to-nitrogen ratio (c, d) and phlorotannin content (e, f) of *Sargassum* following simulated herbivory treatments under experimentally manipulated low and high nutrient availability in the laboratory (left) and field (right). Damage treatments are as in Fig. 1. Different letters above horizontal lines signify significant differences between damage treatments, and asterisks above bars denote significantly different means between nutrient treatments following *post hoc* tests.

second (repeated-measures ANOVA: tissue N [$F_{1,\ 25} = 264.3$, $P < 0.001$], C/N [$F_{1,\ 25} = 274.7$, $P < 0.001$] and phlorotannin [$F_{1,\ 25} = 0.001$, $P = 0.970$]).

There were strong differences between the tissue composition of apical and basal sections of the branches and how the different tissue types responded to nutrient availability (Fig. 5). Overall, apical tissue had lower tissue N ($F_{1,\ 25} = 27.1$, $P < 0.001$; Fig. 5a) and higher C/N ratios ($F_{1,\ 25} = 60.2$, $P < 0.001$; Fig. 5b) than basal tissue. Under low-nitrogen conditions, apical tissue had lower tissue N, higher C/N ratios and lower phlorotannin content than basal tissue, but these differences were reduced under high nitrogen availability (Fig. 5). Under high nitrogen availability, the nitrogen content of apical tissue increased ($F_{1,\ 25} = 25.9$, $P < 0.001$; Fig. 5a), and the C/N ratio decreased ($F_{1,\ 25} = 39.4$, $P < 0.001$; Fig. 5b) more so than basal tissue. Furthermore, the phlorotannin content of apical tissue increased, whereas the phlorotannin content of basal tissue decreased ($F_{1,\ 25} = 9.4$, $P = 0.005$; Fig. 5c) under high nitrogen availability.

RELATIONSHIPS BETWEEN GROWTH AND PHLOROTANNINS

Correlations between phlorotannin concentration and growth traits of *Sargassum* were variable, dependent on the growth traits and the experiment (Fig. 6, Table S5). The first principal component, PC 1, which is correlated with variables associated with relative growth rate, was significantly negatively correlated with phlorotannins in the second laboratory experiment, indicative of a trade-off between tolerance and phlorotannin production (Fig. 6, Table S5). In contrast, the second principal component, PC 2, which is correlated with dry mass and number of blades per node, tended to be positively correlated with phlorotannin content across experiments. The relationships between growth and phlorotannins remained similar under both low and high nitrogen availability (Fig. 6, Table S5), except in the first natural herbivory experiment. In this experiment, the positive relationship between PC 2 (biomass) and phlorotannin content was only present in *Sargassum* from the high-nitrogen treatment (Fig. 6).

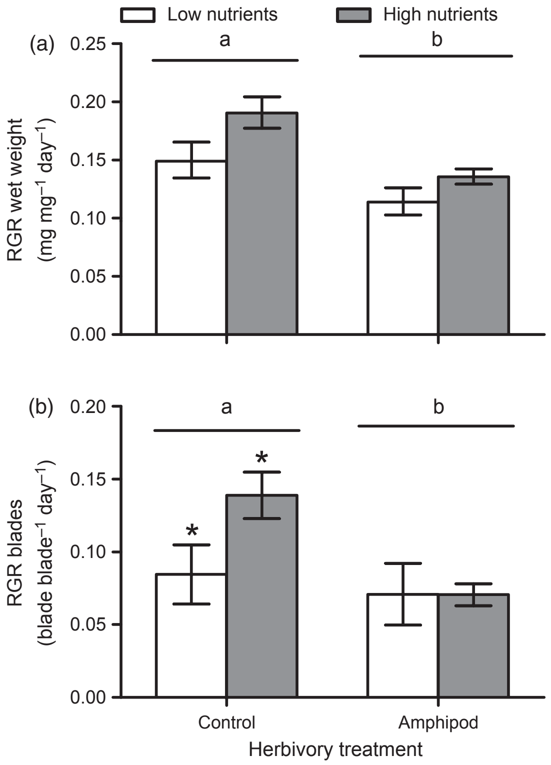

Fig. 4. The effects of amphipod herbivory on the mean ± SE *Sargassum* relative growth rates (RGR) of biomass (a) and blades (b) under different nutrient availability in the first natural herbivory experiment. Different letters above the horizontal lines denote significantly different means between herbivory treatments, and asterisks signify differences between low- and high-nutrient treatments.

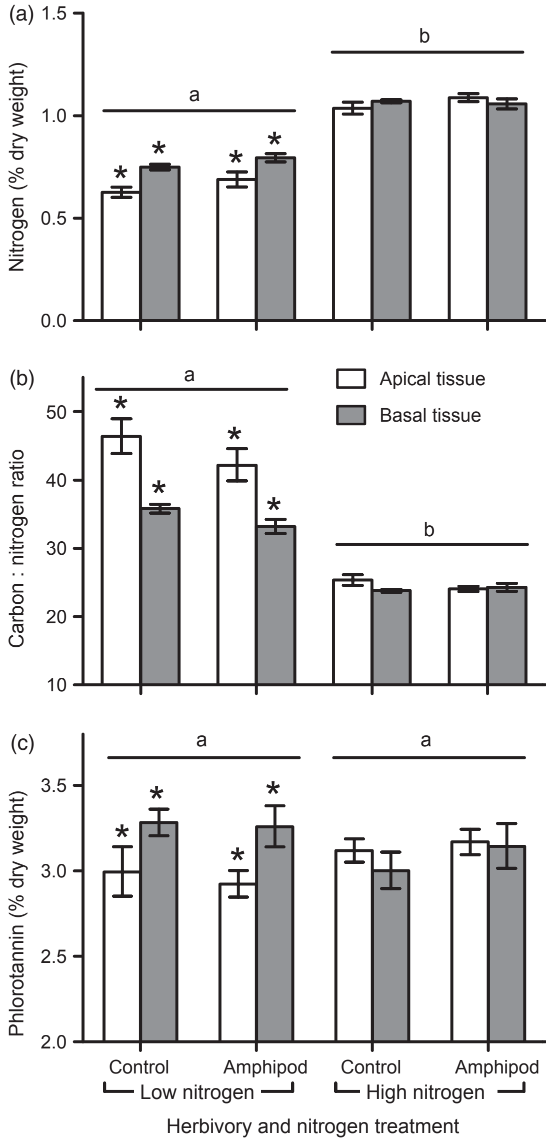

Fig. 5. The effects of amphipod herbivory and nitrogen availability on the nitrogen (a), carbon-to-nitrogen ratio (b) and phlorotannin content (c) of apical and basal *Sargassum* tissues (mean ± SE). Different letters above horizontal lines denote significant differences between nitrogen treatments, and asterisks indicate differences between apical and basal tissues.

Discussion

EXPRESSION OF TOLERANCE TRAITS

We show that, similar to plants, marine algae can express tolerance to herbivory. Therefore, algal tolerance, in addition to algal resistance, may maintain the productivity of marine communities where herbivory can be severe. Following the removal of primary apices, we observed that *Sargassum* consistently maximized the growth and development of secondary apices. Based on the high proportion of apices that are damaged in natural *Sargassum* populations (Ateweberhan, Bruggemann & Breeman 2005, 2006, 2008), and the preference of herbivorous amphipods for apical tissue (K.B. Hay, A.G.B., Poore and C.E. Lovelock, unpublished data), we believe that this compensatory response is important for the maintenance of *Sargassum* populations.

Compensatory growth is often supported by enhanced photosynthesis in higher plants (McNaughton 1979; Strauss & Agrawal 1999; Tiffin 2000). However, the observed compensatory growth responses in *Sargassum* were not associated with an increase in photosynthesis. This could indicate either an uncoupling between the timing of our measurements and the photosynthetic response of *Sargassum* (Retuerto, Fernandez-Lema & Obeso 2006) or that compensatory growth in *Sargassum* is underpinned by a mechanism other than photosynthetic enhancement (Vergés *et al.* 2008).

The ability of *Sargassum* to compensate for simulated herbivory and not amphipod herbivory may be explained by differences in the treatments. In the simulated herbivory experiments, damage was inflicted at the beginning of the experimental period and *Sargassum* had the remainder of the experiment to compensate. In contrast, amphipod herbivory occurred until the end of the experimental period, resulting in no time after herbivory for compensatory growth. The amount of *Sargassum* tissue removed by amphipods (*c.* 21% and *c.* 17% wet weight loss) also exceeded the severity of the damage in the simulated herbivory treatments (apical *c.* 3%; holes

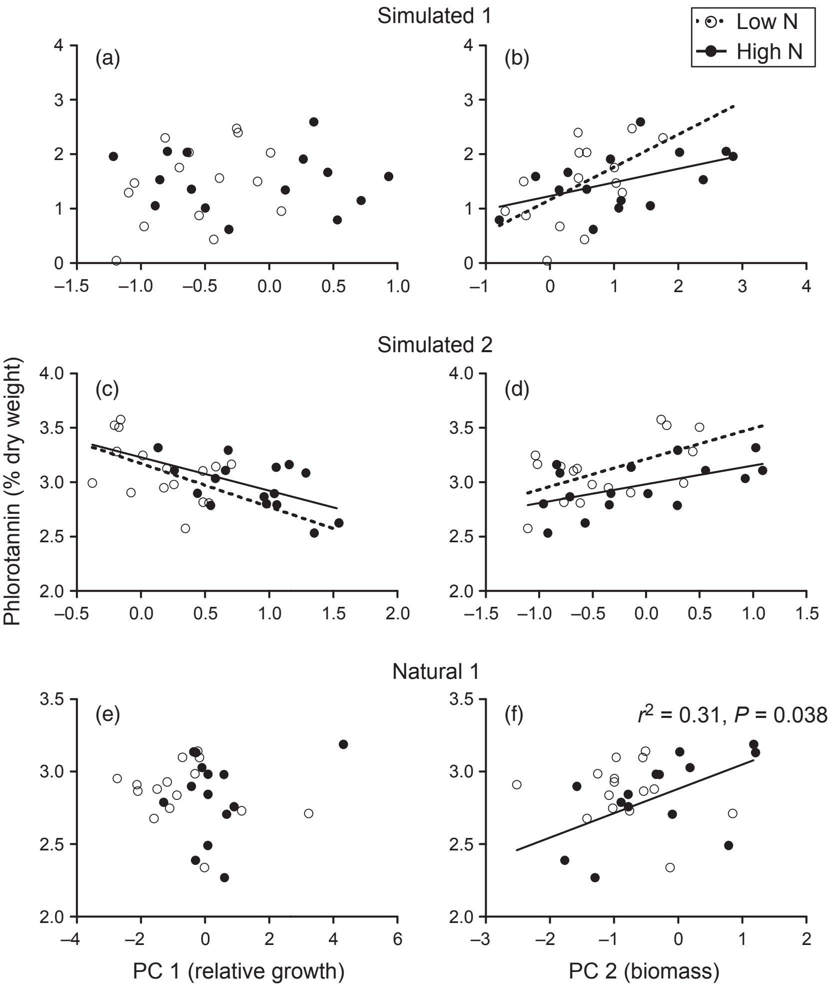

Fig. 6. Relationship between *Sargassum* phlorotannin concentration and two principal component factors summarizing *Sargassum* growth under high and low nitrogen availability from the simulated and natural herbivory laboratory experiments. PC 1 (left) is comprised of relative growth rate measures (wet weight, length, nodes and blades), and PC 2 (right) is comprised of dry mass and number of blades per node. Note the different scales. Regression lines show significant relationships determined by analysis of covariance (Table S5). A significant interaction was found for panel (f), where only the high-nitrogen treatment had a significant correlation with PC 2.

c. 9%; quarter *c*. 14%). The kelp *Macrocystis integrifolia* similarly expresses compensatory growth in response to only moderate herbivory by the amphipod *Peramphithoe femorata* (Cerda *et al*. 2009). However, in addition to exceeding the severity of the simulated herbivory treatments, amphipods also grazed over entire *Sargassum* branches, often damaging both apical and basal tissues simultaneously. Given that the growth responses of *Sargassum* to simulated herbivory were not a simple function of percentage tissue loss, with the pattern of damage having a significant influence on the growth response (e.g. the strong effects of apical loss versus blade loss), it is probable that both the greater severity and more diverse grazing patterns of the amphipods explain the observed difference between the responses of *Sargassum* to simulated and natural herbivory.

In addition to the severity and nature of herbivory, the site of damage within an alga can also affect the expression of tolerance in algae. Taylor, Sotka & Hay (2002) found that *Sargassum filipendula* had a greater ability to compensate for the removal of blades from the basal section of the alga than for the removal of blades from the apical section. We found strong differences between apical and basal sections of *Sargassum* branches in terms of tissue composition, photosynthetic activity and their responses to nitrogen availability (Figs S1 and 5). These strong within-alga differences demonstrate how the expression of tolerance in algae can be dependent on the site of herbivory.

Given the potential importance of the site, nature and severity of damage, differences in within-alga feeding patterns of herbivores are likely to explain why outcomes of algal herbivory are often dependent on herbivore identity (e.g. Toth, Karlsson & Pavia 2007). Within-alga differences in tolerance capabilities may also give rise to small herbivores having disproportionate effects on algae, similar to those found in terrestrial plants (Crawley 1985). Small herbivores, such as amphipods, can feed selectively on small spatial scales, targeting specific tissue types within a plant (Poore 1994). Although the amount of tissue removed is usually small, such targeted herbivory on specific tissues with lower compensatory abilities could have negative effects on algal fitness.

Under eutrophic conditions, the differential response of apical and basal *Sargassum* tissue composition to nitrogen availability observed in this study may also result in changes to the palatability of the different tissue types to herbivores. Under high nitrogen availability, differences in the phlorotannin content of apical and basal tissues were suppressed. If such nutrient-induced changes are linked to changes in herbivore preferences for specific tissues, then the ability of algae to tolerate herbivory under eutrophic conditions may be affected if the newly targeted tissues have different compensatory abilities.

USING THE LIMITING RESOURCE MODEL TO PREDICT ALGAL TOLERANCE UNDER DIFFERENT NUTRIENT CONDITIONS

To our knowledge, this is the first study to use the LRM (Wise & Abrahamson 2005) to predict the effects of nutrient enrichment on the expression of tolerance in marine macroalgae. The LRM successfully predicted that phenotypic tolerance in *Sargassum* would not vary with nitrogen availability. Consequently, we believe the LRM may be a useful tool to predict the effects of resource availability not only in terrestrial plants (Wise & Abrahamson 2007) but also for marine macroalgae.

We applied three premises to the LRM to predict that nitrogen enrichment would not affect tolerance in *Sargassum*. Premise one was that *Sargassum* growth would be limited by the focal resource of nitrogen in the low-nitrogen treatment. Premise two was that carbon acquisition via loss of photosynthetic area would be more affected by herbivory than nitrogen acquisition, based on the lack of photosynthetic plasticity in response to herbivory observed in *Sargassum* and *Fucus gardneri* (Dethier, Willians & Freeman 2005), and the high plasticity of nitrogen transport across membranes in algae (e.g. Wallentinus 1984; Schaffelke & Klumpp 1998). Premise three was that carbon would not limit *Sargassum* in the high-nutrient treatment (Beer & Koch 1996).

The results from Pavia & Brock (2000), in which nitrogen availability limited macroalgal growth but did not alter the growth response of *Ascophyllum nodosum* to snail herbivory, seems also to offer support for the LRM. Jormalainen & Ramsay (2009) suggest that depth (i.e. light) rather than nutrients limited growth of *Fucus vesiculosus* in their study. If light is considered as the focal resource that limits growth in the low-resource environment (3 m depth), and grazing by isopods reduces the acquisition of light by removing photosynthetic tissue, the LRM predicts that tolerance to herbivory will be higher in the high-resource environment (1 m depth). As predicted by the LRM, tolerance to isopod grazing was higher at 1 m depth where light was not limiting (Jormalainen & Ramsay 2009). Although Jormalainen & Ramsay (2009) proposed a number of additional factors that may be responsible for the difference in tolerance observed, it appears the LRM may also help explain their results.

Wise & Abrahamson (2008) also demonstrated that the LRM can be applied to predict tolerance to apical meristem damage in plants by considering active meristems as a resource. Given the significant compensatory response observed in *Sargassum* following apical damage, we followed the guidelines of Wise & Abrahamson (2008) to test whether the LRM could be used to predict tolerance to apical damage in *Sargassum* under different nitrogen conditions. First, we assumed that nitrogen (the focal resource) limits growth of *Sargassum* in the low-nitrogen treatment. Secondly, that damage to apices primarily affects the number of apices (i.e. the alternative resource) the alga has. Thirdly, we assumed that the alternative resource affected by herbivory (i.e. apical meristems) does not limit plant fitness in the high-nitrogen environment. We based this on the large number of active growing points present in *Sargassum*, and the continuous branching and fruiting nature of *Sargassum*. Based on these assumptions, the LRM successfully predicted equal tolerance to apical damage in *Sargassum* under both high- and low-nitrogen environments. This supports the notion that the LRM has the potential to be widely applied to plants and algae. Consequently, the ability of the LRM to predict the effects of eutrophication on marine communities via changes to algal tolerance should be assessed further as it presents as a potentially powerful model.

TRADE-OFFS BETWEEN TOLERANCE AND RESISTANCE

We found variable correlations between *Sargassum* growth and phlorotannin content, and the nature of the relationship was dependent on the growth traits measured. Overall, phlorotannins tended to be positively correlated with traits associated with overall biomass. In contrast, phlorotannins were either negatively correlated or not correlated with traits of RGR. Nutrient availability had little effect on the nature of the relationships, which suggests trade-offs between algal traits may not be strongly influenced by nutrient enrichment.

Given that trade-offs between tolerance and resistance are predicted to exist (Coley, Bryant & Chapin 1985; Herms & Mattson 1992), the positive correlation between overall biomass traits and phlorotannin content in *Sargassum* was unexpected. We, however, are not the first to report positive correlations between growth and a trait associated with resistance in marine algae (Jormalainen *et al.* 2003; Dworjanyn *et al.* 2006; Jormalainen, Wikstrom & Honkanen 2008). Furthermore, both non-significant (Hemmi *et al.* 2005; Honkanen & Jormalainen 2005) and variable correlations (Steinberg 1995; Pavia, Toth & Aberg 1999; Jormalainen & Ramsay 2009) between traits of growth and resistance in algae have also been found. Accordingly, similar to terrestrial plants (Leimu & Koricheva 2006), trade-offs between tolerance and resistance in algae may be limited and should not be assumed based on theoretical expectations alone.

In particular, this study demonstrates the need to consider the type of growth trait measured when assessing trade-offs as this may result in strikingly different conclusions. In one experiment, we simultaneously observed both a positive relationship between phlorotannin concentration and *Sargassum* biomass and a negative correlation between phlorotannin concentration and *Sargassum* relative growth rate (Fig. 6c,d). Similar to our findings, correlations between growth and phlorotannin content in the kelp *Alaria nana* (Pfister 1992) and bladder wrack *Fucus vesiculosus* (Jormalainen & Ramsay 2009) were dependent on the tissues measured and scales of measurement. These results demonstrate that while trade-offs between resistance and overall growth can be absent, phenotypic trade-offs at the smaller scale of RGR of plant components (e.g. leaves, blades and nodes) may be present.

The type of resistance trait measured may also influence the tolerance–resistance relationship observed in algae. For example, owing to the dual primary and secondary roles of phlorotannins in algae, costs of production for resistance may not be realized (Arnold & Targett 2003). Consequently, future studies that measure resistance traits directly using herbivores rather than inferring resistance from algal traits will help further address the question of tolerance–resistance trade-offs in algae.

In summary, we found *Sargassum* expressed specific tolerance to moderate herbivory and show the potential applicability of the LRM to predict tolerance in marine macroalgae under different resource conditions. We also show that relationships between traits of tolerance and resistance are variable in algae and that trade-offs cannot be assumed. Finally, although nutrient availability affects the growth and tissue composition of *Sargassum*, it had little effect on the expression of tolerance, or the nature of the relationship between traits of tolerance and resistance.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Principal components analysis of *Sargassum* growth traits.

Table S2. Two-way ANOVA testing the effects of herbivory and nitrogen availability on the relative growth rates of *Sargassum*.

Table S3. Two-way ANOVA testing the effects of herbivory and nitrogen availability on the tissue composition of *Sargassum*.

Table S4. Repeated measures ANOVA testing the effects of herbivory and nitrogen availability on the photosynthetic electron transport rate of *Sargassum*.

Table S5. ANCOVA results testing the relationship between growth and phlorotannin content of *Sargassum* under different nitrogen conditions.

Figure S1. Effects of simulated herbivory and nitrogen availability on the photosynthetic rate of *Sargassum* tissue.