PLANT ANIMAL INTERACTION

Mesoherbivores reduce net growth and induce chemical resistance in natural seaweed populations

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Abstract Herbivory on marine macroalgae (seaweeds) in temperate areas is often dominated by relatively small gastropods and crustaceans (mesoherbivores). The effects of these herbivores on the performance of adult seaweeds have so far been almost exclusively investigated under artificial laboratory conditions. Furthermore, several recent laboratory studies with mesoherbivores indicate that inducible chemical resistance may be as common in seaweeds as in vascular plants. However, in order to further explore and test the possible ecological significance of induced chemical resistance in temperate seaweeds, data are needed that address this issue in natural populations. We investigated the effect of grazing by littorinid herbivorous snails (Littorina spp.) on the individual net growth of the brown seaweed Ascophyllum nodosum in natural field populations. Furthermore, the capacity for induced resistance in the seaweeds was assessed by removing herbivores and assaying for relaxation of defences. We found that ambient densities of gastropod herbivores significantly reduced net growth by 45% in natural field populations of A. nodosum. Seaweeds previously exposed to grazing in the field were less consumed by gastropod herbivores in feeding bioassays. Furthermore, the concentration of phlorotannins (polyphenolics), which have been shown to deter gastropod herbivores, was higher in the seaweeds that

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G. B. Toth (⊠) · M. Karlsson · H. Pavia Department of Marine Ecology, Tjärnö Marine Biological Laboratory, Göteborg University, Strömstad 452 96, Sweden e-mail: gunilla.toth@tmbl.gu.se were exposed to gastropod herbivores in the field. This field study corroborates earlier laboratory experiments and demonstrates that it is important to make sure that the lack of experimental field data on marine mesoherbivory does not lead to rash conclusions about the lack of significant effects of these herbivores on seaweed performance. The results strongly suggest that gastropods exert a significant selection pressure on the evolution of defensive traits in the seaweeds, and that brown seaweeds can respond to attacks by natural densities of these herbivores through increased chemical resistance to further grazing.

Keywords Ascophyllum · Gastropod · Littorina · Nutrients · Phlorotannins

Introduction

One important assumption behind theories concerning the evolution of chemical defences in plants is that herbivores are important selective agents for defensive traits (Karban and Baldwin 1997). Herbivores can have large negative effects on plant fitness through increased mortality, biomass losses, and/or decreased reproductive output in both terrestrial (e.g., Karban and Baldwin 1997) and marine (Hay 1996) plant communities. However, the mode of grazing, and thus the ecological and evolutionary consequences of herbivory, may be very different depending on the specific herbivore species involved. Individual plants are commonly challenged with many different types of herbivores, ranging from large grazing mammals to small arthropods with different feeding modes (e.g., Karban and Baldwin 1997). In marine habitats, large generalist herbivores

may remove a significant portion of the primary production and thus have a structuring effect on marine communities in tropical (Carpenter 1986) as well as temperate (e.g., Lubchenco and Gaines 1981; Hawkins and Hartnoll 1983; Estes and Steinberg 1988; Sala and Graham 2002; Jenkins et al. 2005; Coleman et al. 2006) areas. Small (<2.5 cm) herbivorous gastropods and crustaceans (mesoherbivores) can also change the compositions of algal assemblages through either consumption of macroalgal juvenile stages (Worm and Chapman 1998) or selective grazing on fast-growing filamentous algal species (Duffy and Hay 2000; Heck and Valentine 2006). Still, due to their low feeding rate in comparison to larger herbivores, mesoherbivores have commonly not been considered to be important selective agents for chemical defences in habitat-forming, perennial seaweeds such as fucoids and kelps. Many mesoherbivores can, however, occur in high densities and reside on individual seaweeds for long timeperiods, which also makes them a potential threat to perennial algal hosts (Duffy and Hay 1991; Brawley 1992). There is presently very little experimental data available about the effects of mesoherbivore grazing on the individual fitness of macroalgae in natural populations (but see Dethier et al. 2005).

Both terrestrial plants and marine seaweeds can produce a variety of mechanical and/or chemical characters that increase plant resistance towards grazing (e.g., Karban and Baldwin 1997; Hay 1996). Apart from the constitutively produced traits that affect plant resistance, a wide range of terrestrial plant species have been shown to induce an increased production of defensive metabolites in response to herbivore grazing (e.g., Karban and Baldwin 1997). Van Alstyne (1988) provided the first study that also demonstrated the occurrence of induced chemical resistance in seaweeds. This was followed by an almost identical investigation by Yates and Peckol (1993). These studies showed that artificial clipping of the fucoid seaweeds Fucus distichus and F. vesiculosus in natural populations resulted in increased levels of polyphenolic secondary metabolites (phlorotannins) that were negatively correlated with the feeding preference of the gastropods Littorina sitkana and L. littorea (Van Alstyne 1988; Yates and Peckol 1993). Artificial clipping was also used in several other early studies that failed to find induced production of secondary metabolites in seaweeds (e.g., Paul and Van Alstyne 1992; Pfister 1992; Steinberg 1994). However, studies on vascular plants imply that artificial clipping procedures are often poor mimics of natural herbivory (Baldwin 1990). More ecologically relevant data were presented in a pioneering study by Cronin and Hay (1996), where transplanted plants of the brown seaweed Dictyota menstrualis were exposed to natural levels of herbivory by the amphipod Amphitoe longimana in the field. The seaweeds responded by increasing their content of diterpene alcohols, which deterred further amphipod feeding (Cronin and Hay 1996). Over the last decade, a number of short-term laboratory experiments have reported herbivore-induced chemical resistance in seaweeds (e.g., Pavia and Toth 2000; Toth and Pavia 2000; Sotka et al. 2002; Taylor et al. 2002; Borell et al. 2004; Hemmi et al. 2004; Rohde et al. 2004; Weidner et al. 2004; Ceh et al. 2005; Macaya et al. 2005; Toth et al. 2005; Molis et al. 2006). Like these recent laboratory experiments, the study by Cronin and Hay (1996) was performed on a short time scale (10-16 days), but it remains the only published field study testing for induced resistance in seaweeds in response to natural herbivory. Because many mesoherbivore species are rather sedentary and generally stay on their host plants for a long period of time, long-term (i.e., several weeks or months rather than a few days or weeks) manipulative experimental field studies are needed in order to evaluate the importance of mesoherbivores on natural variation in seaweed chemical defences and resistance.

Apart from the influence of herbivory, the production of defensive chemicals can be affected by resources such as the nutrient availability (e.g. Herms 2002). Nitrogen is generally considered to be the limiting nutrient in both terrestrial and marine environments (e.g., Hanisak 1983; Herms 2002), and increases in tissue nitrogen have been shown to decrease the constitutive production of phenolic secondary metabolites in both vascular plants and seaweeds (e.g., Yates and Peckol 1993; Koricheva et al. 1998; Herms 2002). Furthermore, increased nutrient availability usually results in an increased nutritional value of both plants (Herms 2002) and seaweeds (e.g., Pavia and Brock 2000). High nutrient status of food plants can have positive effects on herbivore preference and performance, which may counteract the effect of induced resistance (e.g., Herms 2002; Cruz-Rivera and Hay 2003). Fertilization and herbivory (natural or artificial) have been shown to produce weak or variable interactive effects on terrestrial plant defensive chemistry or herbivore resistance (e.g., Osier and Lindroth 2001, 2004; Herms 2002; Glynn et al. 2003; Hale et al. 2005; Stevens and Lindroth 2005). Similarly, the few studies that have investigated interactive effects of nutrient addition and damage on seaweeds have generally found no or weak evidence that nitrogen affects the ability of seaweeds to induce polyphenolic defensive compounds (Peckol et al. 1996; Pavia and Brock 2000; Hemmi et al. 2004, 2005). Even less is known about the interactive effects of nutrient availability and natural herbivory on the ability of seaweeds to induce resistance towards future consumption. In a

previous study on induced resistance where seaweeds were exposed simultaneously to fertilization and herbivores, nutrient addition was not found to affect induced resistance (Weidner et al. 2004). To our knowledge, there are no previous manipulative field studies of interactive effects of fertilization and damage on defensive chemistry or resistance in natural seaweed populations.

In this study, we investigate the interactive effects of gastropod herbivory and elevated nutrient levels on seaweed net biomass change (net growth) and resistance to herbivore consumption in a long-term (five-month) manipulative field experiment in natural populations of Ascophyllum nodosum (L.) Le Jol. A. nodosum is an intertidal fucoid seaweed species that grows on sheltered rocky shores in the northern Atlantic. A. nodosum functions as both habitat and food for a wide range of small crustacean and gastropod herbivores (Pavia et al. 1999). We focused on the gastropod herbivores, because previous aquarium experiments have shown induced resistance to gastropod herbivores in A. nodosum (e.g., Pavia and Toth 2000; Toth and Pavia 2000; Borell et al. 2004; Toth et al. 2005). The induced resistance is correlated with an increase in polyphenolic secondary metabolites (phlorotannins) that have a deterrent effect on gastropod herbivores (Gieselman and McConnell 1981; Pavia and Toth 2000). Furthermore, gastropod herbivores are less mobile than crustaceans and easier to manipulate in the field without constructing cages around the seaweeds and thereby creating an artificial environment. We specifically focused on gastropod herbivores of the genus Littorina (L. littorea, L. obtusata, and L. fabalis), because these are by far the most abundant herbivorous gastropods on A. nodosum in the study area (Pavia et al. 1999; Wikström et al. 2006). We hypothesized (1) that removal of gastropod herbivores and/or addition of nutrients would increase the net growth of seaweed individuals in the field, (2) that herbivores would consume less of the previously grazed experimental seaweeds and more of the experimental seaweeds exposed to elevated nutrient levels in subsequent feeding experiments, and (3) that seaweeds exposed to grazing by littorinid gastropods in the field would have higher phlorotannin contents compared to seaweeds that were relieved from grazing.

Materials and methods

Manipulative field experiment

The experiment was performed in the archipelago outside the Tjärnö Marine Biological Laboratory (TMBL) on the Swedish west coast over a period of five months (April to September) in 2004. At the start of the experiment, a total of 40 A. nodosum individuals were tagged by attaching labeled plastic cable ties around their holdfasts. All visible Littorina spp. snails were removed from half of the tagged seaweeds and from an area of $\sim 1 \text{ m}$ around the tagged plants to prevent recolonization. The rest of the tagged seaweeds were handled in the same way at the start of the experiment, i.e., the plants were searched for snails that were first removed but then replaced on the experimental seaweeds. The removal of snails was repeated once a week in order to keep the density of gastropod herbivores at a minimum on the seaweeds with low gastropod density. Recolonization of littorinid snails was low (only a few snails per plant and week), and therefore the seaweeds exposed to ambient gastropod densities were handled in the same way as the seaweeds with low gastropod densities, i.e., the plants were searched for snails, but the snails were not removed. The gastropod density was evaluated in April before the experiment started, and at the end of the experiment in September, by counting the number of Littorina spp. gastropods in 0.20×0.20 m frames (*n* = 6) placed haphazardly among the experimental A. nodosum individuals. The snails were identified to species, but because the different species were counted in the same quadrats, data on the abundance for separate species are dependent and the statistical analysis was performed for the total number of Littorina spp. individuals.

The ambient nutrient concentration in the water surrounding half of the high and half of the low gastropod density seaweeds (n = 10) was increased by attaching five mesh bags (mesh size 1 mm^2) containing $\sim 150 \text{ g}$ each of a commercial slow-release NPK-fertilizer (Plantacote Depot 6M, Urania Agrochem., Hamburg, Germany) consisting of pellets with a semipermeable polyurethane layer (for the kinetics of nutrient release in situ from this material, see Worm et al. 2000). The bags were attached to the holdfast of seaweed individuals growing ~ 10 cm beneath the tagged experimental seaweeds and were replaced once a month. The rest of the tagged seaweeds were kept as controls and did not receive these additional nutrients. In the middle of the experiment (August), a total of 24 water samples were collected close to the thallus of a balanced subsample of the tagged seaweeds (with and without herbivores/ addition of nutrients, n = 6) in order to evaluate the ammonium and phosphate concentrations in the water. The water samples were collected at high tide (i.e., the seaweeds and the bags with NPK fertilizer were fully submerged) under calm conditions. The samples were placed in coolers and immediately transported to the

laboratory. Ammonium and phosphate concentrations were analyzed according to the method described in Grasshoff et al. (1983).

Seaweed net growth and herbivore resistance

In order to quantify the seaweed biomass without detaching the experimental seaweeds, the length (L) and circumference (C) of each was determined with a measuring tape in the field, and seaweed biomass (B, g dry weight) was calculated using the formula $\log_e B = 0.7900 \log_e LC^2 - 2.8547$ for the spring measurements, and $\log_e B = 0.8605 \log_e LC^2 - 3.3769$ for the fall measurements, according to the log-linear relationship $(R^2 = 0.99, n = 499$ for spring and $R^2 = 0.99, n = 287$ for fall measurements) between *A. nodosum* biomass and volume in the study area, as described by Åberg (1990). The biomass of the experimental seaweeds was determined at the start and at the end of the experiment, and the net growth was expressed as the percentage increase over the initial dry weight.

The susceptibility of the experimental A. nodosum to herbivore consumption was evaluated in a no-choice feeding assay with the gastropod L. obtusata. One primary shoot with several secondary shoots was subsampled haphazardly in September from each of the tagged seaweeds in the field and brought back to the laboratory. The primary shoots were divided into smaller pieces $(1.25 \pm 0.25 \text{ g wet weight})$ that were pooled according to the treatment they had received in the field. About 20 algal pieces were haphazardly chosen from each pool and placed individually in aquaria (0.201) supplied with filtered (10, 100 µm) flowthrough seawater. Three adult (>1 cm) L. obtusata individuals were added to half of the aquaria and allowed to feed on the algal shoots for four days. The rest of the shoots were kept as controls in order to quantify autogenic wet weight changes during the experiment. The wet weight of each shoot was determined at the start and at the end of the experiment after carefully blotting the algal pieces between tissue paper. The change in wet weight for each shoot was calculated by subtracting the starting weight from the end weight, and was expressed as a percentage of the initial wet weight.

Chemical and statistical analyses

Tissue samples for phlorotannin and nitrogen analyses were collected by pooling several apical tips (<1 cm) from each of the primary shoots collected from the tagged seaweeds in the field. The tissue samples were immediately frozen, freeze-dried and homogenized. Phlorotannins were extracted in aqueous acetone (60 vol%), and the phlorotannin content was quantified with the Folin–Ciocalteus method (Van Alstyne 1995) using phloroglucinol as a standard. Tissue nitrogen content was determined using an elemental analyzer (Model: EA 1108 CHNS-O, Fision, Italy) using 2,5-bis-[5-tert-butyl-bensoaxzol-2-yl]-thiophen (BBOT) as a standard.

Data were tested for homogeneity of variances prior to all statistical analyses using Cochran's test (Underwood 1997). Data on the density of gastropod herbivores were transformed (log [x + 1]) to fulfil the assumption of homogeneity; otherwise all variances were homogeneous and data were not transformed. Data on the density of gastropod herbivores, the ammonium and phosphate concentrations in the water samples, as well as the net seaweed growth and the tissue phlorotannin and nitrogen contents, were statistically analyzed using two-way analysis of variance (ANOVA) with gastropod density and nutrients as fixed, orthogonal two-level factors. Data on the changes in the wet weights of the seaweed shoots offered to L. obtusata in the no-choice feeding assay were analyzed using a three-way ANOVA with gastropod density, nutrients and grazer treatment as fixed, orthogonal two-level factors. Statistically significant differences between mean values were explored using the Student-Newman-Keul's multiple comparisons test (SNK-test, Underwood 1997).

Results

Snail densities and nutrient concentrations

The total density of *Littorina* spp. on and around the experimental *A. nodosum* plants was on average 460 ± 190 individuals m⁻² (mean \pm SD) at the start of the experiment (122 ± 87 *L. fabalis* m⁻², 163 ± 145 *L. littorea* m⁻² and 206 ± 110 *L. obtusata* m⁻², mean \pm SD). There was a statistically significant effect of the gastropod removal treatment on the total density of *Littorina* spp. individuals at the end of the manipulative field experiment, as shown by the significant main factor gastropod density [Table 1(part a)]. There were significantly more *Littorina* spp. individuals on the seaweeds exposed to the ambient gastropod density treatment compared to those on seaweeds exposed to the gastropod removal treatment (Fig. 1).

The overall background concentrations of ammonium and phosphate detected in the water surrounding the experimental *A. nodosum* plants was low (Fig. 2), which is in accordance with previous measurements **Table 1** Analysis of variance of (a) the total density of *Littorina* spp. gastropods (log [x + 1] individuals m⁻²), and the concentrations of (b) ammonium (μ M), and (c) phosphate (μ M) around

experimental seaweeds exposed to different gastropod densities (D) and nutrient levels (N) in the field

Source of variance	df	(a) Gastropod density			(b) Ammonium			(c) Phosphate		
		MS	F	Р	MS	F	Р	MS	F	Р
Gastropod density (D)	1	11.036	23.247	< 0.001	0.006	0.631	0.436	0.003	7.613	0.012
Nutrients (N)	1	0.079	0.167	0.687	0.191	20.408	< 0.001	0.011	31.732	< 0.001
$D \times N$	1	0.051	0.108	0.746	0.016	1.729	0.203	0.002	5.199	0.034
Residual	20	0.475			0.009			< 0.001		

Data on mean values and standard errors are presented in Fig. 1 for gastropod density, and in Fig. 2 for ammonium and phosphate concentrations



Fig. 1 Gastropod density. The density of *Littorina* spp. individuals (m^{-2}) when the experiment was terminated (September). Gastropod density was manipulated by removing (*low gastropod density*) or removing and replacing (*ambient gastropod density*) all visible *Littorina* spp. gastropods on and around the experimental *A. nodosum* plants. *Error bars* show +SE (n = 6)

performed during the summer in the study area $([NH^{4+}] = 0.5 \ \mu M, \ [PO^{4-}] < 0.1 \ \mu M, \ Axe \ et al. \ 2004).$ The addition of nutrients resulted in a large increase in both the ammonium and the phosphate concentrations in water samples, as shown by the statistically significant factor "nutrients" for ammonium [Table 1(b)] and the interaction between the factors "gastropod density" and "nutrients" for phosphate [Table 1(c)]. On average, the ammonium concentration in the water where nutrients were added was 484% higher than background levels (Fig. 2a). Furthermore, the SNK test showed that the phosphate concentration in the water where nutrients were added increased significantly compared to background levels under both ambient and low gastropod densities (1,525 and 1,600%, respectively), and that the phosphate concentration was significantly (P > 0.05) higher under ambient than low gastropod density (Fig. 2b). The ammonium and phosphate concentrations in the water where nutrients were added approximately corresponded to previous measurements performed during winter in the study area $([NH^{4+}] = 1 \ \mu M, [PO^{4-}] = 0.6 \ \mu M, Axe et al. 2004).$



Fig. 2a–b Nutrient concentrations. The concentrations of **a** ammonium (μ M) and **b** phosphate (μ M) in water samples collected near the thallus of experimental *A. nodosum* plants where the gastropod densities and/or nutrient levels were manipulated in the field. *Capital letters* above bars indicate significant differences between mean values based on the Student–Newman–Keul's multiple comparisons test (SNK, *P* < 0.05). *Error bars* show +SE (*n* = 6)

Seaweed net growth and herbivore resistance

The experimental *A. nodosum* plants showed a large positive biomass change during the five-month experiment (Fig. 3). However, natural densities of littorinid gastropod herbivores decreased the net growth of



Fig. 3 *A. nodosum* net growth. The net growth (% of initial dry weight) of experimental *A. nodosum* plants exposed to different gastropod densities and/or nutrient levels in the field. *Capital letters* above bars indicate significant differences between mean values based on the Student–Newman–Keul's multiple comparisons test (SNK, P < 0.05). *Error bars* show SEM (n = 10)

A. nodosum, as shown by the statistically significant effect of the factor "gastropod density" on seaweed biomass change [Table 2(a)]. The seaweeds exposed to low grazing pressure doubled their biomass in five months, while the biomass of seaweeds exposed to natural gastropod densities increased by an average of 55% of the initial dry weight (Fig. 3). There was no statistically significant effect of nutrient enrichment on the seaweed net growth in natural *A. nodosum* populations [Table 2(a); Fig. 3].

Ascophyllum nodosum that had been exposed to natural gastropod herbivory in the field were consumed less by *L. obtusata* compared to seaweeds where grazing pressure had been decreased, as shown by the statistically significant interaction between the factors gastropod density and grazing (Table 3, Fig. 4a). The snails readily consumed *A. nodosum* shoots from experimental plants that had been relieved of littorinid herbivores in the field, as shown by the negative change in wet weight of the shoots offered to *L. obtusata* in the no-choice consumption bioassays (Fig. 4a). In contrast,

Table 3 Analysis of variance of the change in wet weight (% of initial wet weight) of experimental *A. nodosum* shoots exposed to different gastropod densities (*D*) and nutrient levels (*N*) in the field, which were subsequently offered to *L. obtusata* snails in laboratory no-choice feeding bioassays (grazing, *G*)

Source of variance	df	MS	F	Р	
Gastropod density (D)	1	23.67	3.61	0.061	
Nutrients (N)	1	6.15	0.94	0.336	
Grazing (G)	1	60.70	9.27	0.003	
$D \times N$	1	4.26	0.65	0.423	
$D \times G$	1	40.14	6.13	0.016	
$N \times G$	1	0.01	0.002	0.968	
$D \times N \times G$	1	13.31	2.03	0.158	
Residual	72	6.55			

Data on mean values and standard errors for the changes in wet weights of the shoots are presented in Fig. 4

there was no statistically significant herbivore consumption of shoots from experimental plants previously exposed to grazing in the field (Fig. 4a). Furthermore, there was no statistically significant effect of previous nutrient enrichment on subsequent herbivore consumption (Table 3, Fig. 4b).

Ascophyllum nodosum tissue constituents

The *A. nodosum* plants that were exposed to natural gastropod herbivory in the field had higher phlorotannin levels than seaweeds where herbivorous snails were removed, as shown by the statistically significant effect of the factor "gastropod density" on phlorotannin content [Table 2(b), Fig. 5a]. Seaweeds exposed to natural littorinid densities had 15% higher phlorotannin content (on average) than seaweeds where the gastropods had been removed. No statistically significant effect of nutrient enrichment (Table 2b) was detected for the phlorotannin content in the experimental seaweeds (Fig. 5a).

The tissue nitrogen content of the *A. nodosum* in the field experiment increased by a significant average of 17% when seaweeds were exposed to elevated nutrient

Table 2 Analysis of variance of the (a) net growth (% of initial dry weight), (b) tissue phlorotannin content (% of dry weight), and (c) tissue nitrogen content (% of dry weight) in experimental

A. nodosum seaweed exposed to different gastropod densities (D) and nutrient levels (N) in the field

Source of variance	df	(a) Net growth			(b) Phlorotannin content			(c) Nitrogen content		
		MS	F	Р	MS	F	Р	MS	F	Р
Gastropod density (D)	1	19,503.5	4.727	0.036	5.491	8.779	0.005	0.007	0.074	0.788
Nutrients (N)	1	0.8	< 0.001	0.989	1.208	1.931	0.173	0.573	5.914	0.020
$D \times N$	1	255.4	0.062	0.805	0.001	0.002	0.965	0.096	0.992	0.326
Residual	36	4,126.4			0.625			0.097		

Data on mean values and standard error are presented in Fig. 3 for biomass change of A. nodosum, and in Fig. 5 for tissue phlorotannin and nitrogen contents



Fig. 4a–b No-choice consumption experiment. Changes in wet weights (% of initial wet weight) of *A. nodosum* shoots from plants previously exposed to **a** different gastropod densities and **b** different nutrient levels in the field. The shoots were offered to herbivorous snails (*Littorina obtusata*) in no-choice feeding bioassays or kept as controls without herbivores to measure autogenic weight changes (*controls*) in the laboratory. *Letters above* bars indicate significant differences between mean values based on the Student–Newman–Keul's multiple comparisons test (SNK, P < 0.05). *Error bars* show SEM (n = 10)

levels, as shown by the statistically significant effect of the factor nutrient enrichment [Table 2(c), Fig. 5b]. There was no statistically significant effect of the factor "gastropod density" [Table 2(c), Fig. 5b] on the tissue nitrogen content of the experimental *A. nodosum*.

Discussion

Removal of littorinid gastropods from *A. nodosum* in the field greatly increased the net growth of adult seaweed individuals, showing that the presence of gastropods can have a significant negative impact on natural seaweed populations. The individual seaweed size is strongly correlated to individual fitness in *A. nodosum* (Pavia et al. 2002), and therefore the littorinid gastropods



Fig. 5a–b *A. nodosum* tissue constituents. The **a** tissue phlorotannin contents (% initial dry weight), and **b** tissue nitrogen contents (% initial dry weight) of experimental *A. nodosum* plants exposed to different gastropod densities and/or different nutrient levels in the field. *Letters above* bars indicate significant differences between mean values based on the Student–Newman– Keul's multiple comparisons test (SNK, P < 0.05). *Error bars* show SEM (n = 10)

most likely exert a considerable selection pressure on A. nodosum plants in natural seaweed populations. Mesoherbivores have often been considered to have a minor effect on seaweed fitness in the field compared to larger herbivores such as sea urchins and fish (e.g., Hay and Steinberg 1992), despite the fact that they commonly are found in very high numbers on their seaweed hosts (Brawley 1992). To our knowledge, only one manipulative study investigating the effect of grazing by natural densities of mesoherbivores on habitat-forming, perennial seaweeds in the field has been published previously (Dethier et al. 2005). The individual biomass of the intertidal brown seaweed Fucus gardneri was not affected by long-term (five months) limpet removal in natural populations, but it increased when disruptive stress was decreased by using awnings made from window screening (Dethier et al. 2005). However, the authors noted that the number of littorinid snails (Littorina scutulata) was less abundant under the awnings,

and that this could be an alternative explanation for the higher individual dry mass of seaweed that was kept under awnings (Dethier et al. 2005). Both L. scutulata and L. obtusata have radulae with shovel-like cusps (Reid 1996) that make them capable of excavating tissue from the surface of large leathery seaweeds such as fucoids (Steneck and Wating 1982; Voltolina and Sacchi 1990), which weakens the seaweed shoots and makes them more prone to breakage (cf. Viejo and Åberg 2001). Mesoherbivores are a large and heterogeneous group of animals, and their effects on seaweed fitness are probably highly dependent on the mode of herbivore feeding. Grazing damage that greatly increases the probability of loss of whole shoots may have large negative effects on seaweed fitness, especially in combination with factors such as drought and wind-induced tearing of the seaweed thallus (cf. Lowell et al. 1991; Toth and Pavia 2006). Therefore, it is important that possible effects of herbivory on seaweed fitness are evaluated in natural field settings, and not just in aquarium or mesocosm experiments, where the stress from physical factors are greatly reduced.

The results from the present study showed that A. nodosum growing in natural field populations responds to grazing by littorinid snails by inducing resistance to further herbivore consumption. Furthermore, the induced resistance was correlated to increased levels of phlorotannins in the seaweed tissues. The results are in accordance with earlier aquarium experiments on this study system, where an induced production of A. nodosum phlorotannins in response to L. obtusata grazing or waterborne cues has been shown to be correlated to reduced herbivore preference (Pavia and Toth 2000; Toth and Pavia 2000) and decreased herbivore performance (Toth et al. 2005), and to cause an increased dispersal of herbivore damage (Borell et al. 2004). The difference in phlorotannin content between grazed and control seaweeds in the present study was relatively small ($\approx 15\%$) compared to some of the previous studies (e.g., $\approx 100\%$ in Pavia and Toth 2000 and in Borell et al. 2004). A 20% increase in phlorotannin content in F. distichus in response to artificial clipping reduced subsequent grazing by L. sitkana by $\approx 50\%$ (Van Alstyne 1988), indicating that the lower consumption of previously grazed shoots found in the present study may have been caused by the increased phlorotannin content. However, it is important to note that no direct test of the deterrent effect of phlorotannins was performed in these studies, and changes in other primary or secondary metabolites may also be responsible for the observed resistance in damaged seaweeds. Previous investigations of induced chemical resistance in seaweeds have almost exclusively been conducted through laboratory experiments where seaweeds are confined to aquariums under more or less artificial conditions and exposed to either artificial clipping or to grazing by one herbivore species (e.g., Pavia and Toth 2000; Toth and Pavia 2000; Sotka et al. 2002; Taylor et al. 2002; Borell et al. 2004; Hemmi et al. 2004; Rhode et al. 2004; Weidner et al. 2004; Ceh et al. 2005; Macaya et al. 2005; Toth et al. 2005; Molis et al. 2006). However, in natural populations, seaweeds interact with a number of different organisms (e.g., other herbivores, seaweeds, fouling organisms, pathogens and parasites) as well as different environmental factors, which may affect their response to specific seaweed-herbivore interactions. To our knowledge, this is the first study where previous results from laboratory experiments on induced chemical resistance in seaweeds have been corroborated with those from manipulative field experiments.

As far as we know, there is only one previous study where inducible chemical seaweed resistance in response to natural herbivores has been demonstrated in the field. Cronin and Hay (1996) attached plants of the brown seaweed D. menstrualis to nylon ropes that were transplanted to field sites, and the density of amphipod herbivores (Ampithoe longimana) was manipulated in cages surrounding the seaweeds. The seaweeds responded to grazing by increasing their production of diterpene alcohols, which increased the resistance of the plants to further herbivory (Cronin and Hay 1996). Furthermore, induction of phlorotannins, but not induced resistance, has been investigated in natural populations of fucoids (F. gardneri) exposed to grazing by gastropod herbivores (Dethier et al. 2005), and in kelps (Ecklonia radiata) exposed to grazing by sea urchins (Steinberg 1995), without detecting any significant correlations between grazer density and seaweed tissue phlorotannin concentrations. The results for F. gardneri are not surprising considering that the herbivore removal treatment only reduced the number of herbivores by an average of 25% compared to control populations (Dethier et al. 2005). Furthermore, induced (as opposed to constitutive or activated) chemical resistance is hypothesized to occur in response to mesoherbivores that use the seaweeds as both habitat and food (cf. Hay 1996; Pavia and Toth 2000) rather than in response to larger, more mobile herbivores such as the sea urchins used by Steinberg (1995). In summary, the only two manipulative field experiments (Cronin and Hay 1996; the present study) that have studied induced resistance in response to grazing by natural mesoherbivores have both found support for the hypothesis that seaweeds induce chemical resistance to decrease further herbivory.

Long-term nutrient addition resulted in increased ammonium and phosphate levels in the water, as well as increased net uptake of nitrogen by the A. nodosum in the manipulative field experiment. However, we did not detect any significant separate or interactive fertilization effects on the net seaweed biomass change, indicating that the growth of A. nodosum in the study area is limited by factors other than nutrients (e.g., light) during the main growth season (April to September). Furthermore, we did not detect any significant fertilization effects on the seaweed resistance (i.e., herbivore consumption) or phlorotannin content. These results are in accordance with previous laboratory studies where no significant interactive effects of natural herbivory (Weidner et al. 2004) or artificial damage (Hemmi et al. 2004) and nutrient addition on induced resistance in seaweeds have been detected. Moreover, nutrient availability and simulated or natural herbivory have been simultaneously manipulated without revealing significant interactive effects on the phlorotannin contents of A. nodosum (Pavia and Brock 2000) or F. vesiculosus (Hemmi et al. 2005; but see Peckol et al. 1996). The results from the present and previous studies on induced resistance in seaweeds (e.g., Hemmi et al. 2004; Weidner et al. 2004) indicate that nutrient availability may have minor effects on chemically mediated plant-herbivore interactions in habitat-forming intertidal seaweeds.

A striking difference between studies on herbivoreinduced resistance in plants in terrestrial and marine habitats is that field experiments are much more common in terrestrial studies (Karban and Baldwin 1997). This probably reflects the greater difficulty involved in manipulating grazer densities, especially mesoherbivore densities, in aquatic systems. The results from this study clearly show that it is important that the lack of experimental field data on marine mesoherbivory does not lead to rash conclusions about the lack of significant effects of these herbivores on seaweed performance (see Hay and Steinberg 1992). We found that gastropod mesherbivores alone can halve net algal growth, which strongly suggests that the gastropods exert a significant selection pressure on the evolution of defensive traits in the seaweeds. Accordingly, this study confirmed the findings of previous laboratory experiments (Pavia and Toth 2000; Toth et al. 2005) by showing that brown seaweeds can respond to attacks by natural densities of littorinid herbivores through increased chemical resistance to further grazing. Increased efforts to conduct future experimental studies of marine mesoherbivores in field settings, where other natural factors may interact with herbivory, are essential to further establish whether these herbivores can select for algal defences, as well as to connect mechanisms of induced resistance that can be observed in laboratory settings to consequences for natural populations.

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