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PLANT-ANIMAL INTERACTIONS - ORIGINAL PAPER

Compensation and resistance to herbivory in seagrasses: induced responses to simulated consumption by fish

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Abstract Herbivory can induce changes in plant traits that may involve both tolerance mechanisms that compensate for biomass loss and resistance traits that reduce herbivore preference. Seagrasses are marine vascular plants that possess many attributes that may favour tolerance and compensatory growth, and they are also defended with mechanisms of resistance such as toughness and secondary metabolites. We quantified phenotypic changes induced by herbivore damage on the temperate seagrass *Posidonia oceanica* in order to identify specific compensatory and resistance mechanisms in this plant, and to assess any potential trade-offs between these two strategies of defence. We simulated three natural levels of fish herbivory by repeatedly clipping seagrass leaves during the summer period of maximum herbivory. Compensatory responses were determined by measuring shoot-specific growth, photosynthetic rate, and the concentration of nitrogen and carbon resources in leaves and rhizomes. Induced resistance was determined by measuring the concentration of phenolic secondary metabolites and by assessing the long-term effects of continued clipping on herbivore feeding preferences using bioassays. Plants showed a significant ability to compensate for low and moderate losses of leaf biomass by increasing aboveground growth of damaged shoots, but this was not supported by an increase in photosynthetic capacity. Low levels of herbivory induced compensatory growth without any measurable effects on stored resources. In contrast, nitrogen reserves in the rhizomes played a crucial role in the plant's ability to compensate and survive herbivore damage under moderate and high levels of herbivory, respectively. We found no evidence of inducibility of long-term resistance traits in response to herbivory. The concentration of phenolics decreased with increasing compensatory growth despite all treatments having similar carbon leaf content, suggesting reallocation of these compounds towards primary functions such as cell-wall construction.

Keywords Compensatory growth · Tolerance · Resistance · Defence · Phenolic compounds · Induced responses · Plant–herbivore interactions · Mediterranean sea

Introduction

Herbivory strongly influences the organisation and functioning of ecosystems through the control of plant community structure and composition (McNaughton et al. 1989; Hulme 1996). Plants that do not succeed in escaping herbivores in either space or time are defended against consumers by a wide variety of interacting traits that entail either resistance or tolerance to herbivory (Lubchenco and Gaines 1981). Resistance strategies are based on nutritional, chemical, or structural plant adaptations that lower plant quality and palatability and reduce herbivore preference or performance (Fritz and Simms 1992). Tolerance refers to the ability of plants to withstand and survive herbivory, and is a property of genotypes defined as the extent to which plant fitness is affected by consumer damage relative to fitness in

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the undamaged state (Stowe et al. 2000). The degree of tolerance may be observed through phenotypic responses such as compensation, which is quantified by measuring growth and/ or reproduction of damaged plants relative to undamaged plants (Strauss and Agrawal 1999).

Changes in plants that occur as a result of herbivore damage are known as induced responses, and these responses can involve both tolerance and resistance traits. For instance, an increase in the relative growth rate of plants as a response to herbivore damage is a particularly widespread compensatory mechanism of plant tolerance in terrestrial systems (Ferraro and Oesterheld 2002). The internal plant traits that facilitate such an increase in growth of damaged plants include the presence of storage organs, the number and distribution of meristems, the ability to reallocate energy and resources from undamaged to damaged tissues, and the ability to increase photosynthetic rates in residual tissues (Belsky et al. 1993). On the other hand, herbivore damage can also increase the resistance of the plant to further herbivore attack by inducing the synthesis of herbivore-deterrent metabolites (Karban and Baldwin 1997). Although tolerance and resistance are not mutually exclusive responses of plants to herbivory (see review by Leimu and Koricheva 2006), resource limitation and competition with defensive functions can limit the ability of plants to compensate for herbivory (Fineblum and Rausher 1995; Stowe 1998).

Herbivory in aquatic systems is three times higher than in terrestrial systems (Cyr and Pace 1993), and the production of toxic or deterrent secondary metabolites by marine and freshwater macrophytes is recognised as a particularly important strategy of defence against consumers (McClintock and Baker 2001; Pohnert 2004; Prusak et al. 2005). In contrast, tolerance to herbivory does not seem to be a common mechanism of defence among algae (e.g. Honkanen and Jormalainen 2002) with the exception of some coralline rhodophytes (Steneck 1982; Littler et al. 1995; Wai and Williams 2005). This is probably due to the simple morphology and functional organisation of algae, since they lack a root system for the storage of resources, and there are few or no lateral meristems that can be activated in response to apical disturbances. Seagrasses, on the other hand, are marine vascular plants that possess several of the attributes that favour tolerance and compensatory growth in terrestrial plants, such as the presence of largely inaccessible basal meristems, clonal propagation and physiological integration amongst ramets (Marbà et al. 2002, 2006). Additionally, in many species seagrass rhizomes function as storage organs (Dawes and Guiry 1992; Alcoverro et al. 2001b; Invers et al. 2004), and there is evidence that stored nutrients are translocated to leaves during growth (see review by Romero et al. 2006). Moreover, increases in productivity have been recorded in some seagrass species as a response to defoliation (Tomasko and Dawes 1989; Valentine et al. 1997; Moran and Bjorndal 2005).

A broad variety of organisms are known to feed directly on seagrasses and can consume large amounts of the above-ground biomass of some species (for an exhaustive review on seagrass herbivory see Valentine and Duffy 2006). Despite this, we know remarkably little about the mechanisms that regulate seagrass–herbivore interactions. There is evidence that seagrasses are chemically defended (Vergés et al. 2007b), but specific deterrent compounds have not been isolated yet in any species. However, seagrasses are a rich source of phenolics (Zapata and McMillan 1979; McMillan 1984), which are compounds with well-known anti-herbivore properties in terrestrial plants and algae (Bennett and Wallsgrove 1994; Amsler and Fairhead 2006), and these may also be acting as feeding deterrents in these marine plants. On the other hand, the persistence of seagrass meadows in conditions of high herbivory, both past and present, as well as the presence of several plant traits that can contribute to compensate for loss of biomass suggest that tolerance may be an important mechanism of defence in these macrophytes.

Posidonia oceanica (L.) Delile is the dominant seagrass in the Mediterranean Sea. Its meadows cover a substantial part of the coastal zones of this basin (Procaccini et al. 2003) and are considered key and endangered ecosystems. Recent studies have highlighted the importance of herbivory in this system and show that over half of the annual leaf production of this species is lost to herbivores in shallow meadows (Prado et al. 2007a). The main consumers of *P. oceanica* are the sparid fish *Sarpa salpa* Linné, which is responsible for ca. 70% of the total annual losses to herbivory, and the edible sea urchin *Paracentrotus lividus* Lamarck, which is responsible for the removal of the remaining 30% (Prado et al. 2007a). In addition to direct effects on the plant, removal of biomass by these macrograzers strongly influences the epiphytic community composition of this seagrass (Prado et al. 2007b).

P. oceanica meadows are known to persist despite remarkably high levels of herbivory (Tomas et al. 2005). Moreover, this seagrass possesses various traits that may facilitate mechanisms of tolerance to herbivory, such as the presence of particularly large underground rhizomes and the ability to translocate nutrients and carbon both from the rhizome and from senescent leaves (Alcoverro et al. 2000, 2001b). Additionally, *P. oceanica* is known to produce a wide variety of phenolic compounds (Cuny et al. 1995; Agostini et al. 1998), which are enclosed in distinctive tanniferous cells (Pellegrini and Pellegrini 1993), and leaf toughness and secondary metabolites are known mechanisms of resistance against consumers of this plant (Vergés et al. 2007a, b).

The aim of this study was to quantify the phenotypic changes in *P. oceanica* induced by herbivore damage, and to identify specific compensatory and resistance mechanisms in this plant. To this end, we mimicked three natural levels of fish consumption on a *P. oceanica* meadow by repeatedly clipping seagrass leaves during the summer months, when herbivory peaks in the Mediterranean Sea. The impacts of simulated herbivory were determined on three mechanisms of compensation: (1) shoot-specific growth, (2) leaf-saturated photosynthetic rate, and (3) utilisation of stored reserves. Additionally, we assessed the impacts of simulated herbivory on induced resistance by measuring: (1) the concentration of total phenolic compounds in leaves, and (2) the feeding preferences of the fish *S. salpa* and the sea urchin *Paracentrotus lividus* between undamaged control shoots and continuously clipped shoots, to determine the direct effects on consumers of long-term induced changes in plant traits.

Materials and methods

Study site

The study site, Port-Lligat (Girona; 42°17′30′′N; 3°17′16′′E), is a protected bay located in the Cap de Creus Marine Reserve in the northeastern coast of Spain. Enclosed within the bay is an extensive monospecific *P. oceanica* meadow that ranges from 0.5 to 16 m depth. Shoot density in the meadow is 300–1,000 shoots m^{-2} (Alcoverro et al. 1995). Fish densities and natural rates of herbivory are particularly low in this meadow, with consumption values that correspond to half the average annual consumption values in the Catalan coast (Prado et al. 2007a).

Simulated herbivory experiment

This experiment was designed to mimic grazing by the fish *Sarpa salpa*, since this is the most important consumer of *P. oceanica* in shallow meadows (Prado et al. 2007a). We established 12 plots (1.5 m^2) at 5 m depth in the seagrass bed. These plots were placed at a distance of at least 3 m from each other and corresponded to four herbivory treatments (control, low, moderate and high herbivory). The 5 m experimental depth was chosen because this is where fish herbivory is greatest (Tomas et al. 2005). The size of the plots was chosen so as to simulate the tendency of *S. salpa* to maintain distinct mowed patches of about 1–5 m^2 in size (Tomas et al. 2005), and the levels of simulated herbivory all fall within the natural range of herbivory experienced by this seagrass at this depth (Tomas et al. 2005; Prado et al. 2007a). We removed any sea urchins present inside the plots at the beginning of the experiment to avoid any potential confounding effects, and no urchins were found in the plots in successive visits.

Three plots were randomly allocated to each herbivory treatment at the start of the experiment. In the high herbivory plots, leaves were cut to 15 cm height, which corresponds to removal of about 70% of leaf biomass. In the moderate herbivory plots leaves were cut to 30 cm height, corresponding to removal of about 40% of leaf biomass. In the low herbivory plots only the tips were cut, corresponding to a leaf biomass removal of less than 5%. In the control plots, there was no clipping of the leaves. Since the particular meadow where we performed the experiment has naturally low levels of fish herbivory (see Study site), no herbivore exclusion cages were established in the undamaged plots, thus avoiding potential cage artefacts. The apex of control shoots were examined at the end of the experimental period and no marks of recent fish grazing were observed.

In order to simulate fish grazing as closely as possible, the experimental clipping period was May to September, coinciding with the period of maximum fish herbivory in *P. oceanica* meadows (Prado et al. 2007a). This also includes the period of maximum growth of the plant (June; Alcoverro et al 1995) and the period of maximum carbon fixation and storage of carbohydrate reserves (July–August; Alcoverro et al. 2001b). Clipped plots were maintained from May to September 2004 by clipping all the leaves within each plot every 2–3 weeks. After clipping of the leaves, all blades were removed from the area, as if ingested by the fish. Nutrient input from fish faeces was not simulated, since fish faeces may not necessarily be deposited in grazed plots.

Induced compensatory responses

In order to determine the ability of *P. oceanica* to compensate for herbivory, we measured leaf growth and the number of leaves per shoot across the four herbivory treatments and we then measured those specific plant traits known to facilitate an increase in regrowth in damaged plants, i.e. photosynthetic capacity and mobilisation of stored reserves. All shoots were collected from within the central part of each plot at the end of the experimental period (September). Leaf growth was measured in ten shoots that were individually tagged in situ by punching two parallel holes just above the ligule of the outermost leaf using a hypodermic needle (Romero 1989). Fourteen days later we collected all tagged shoots and we determined growth by measuring the displacement of holes relative to the ligule of the oldest non-growing leaf. We converted leaf elongation values to production of new biomass in dry weight (DW) per shoot and per day (mg DW $(\text{shoot} \times \text{day})^{-1}$ by drying the leaves of different ages to a constant weight at 70°C.

We used these same samples to measure the number of leaves per shoot, leaf length and leaf width.

We collected three shoots per plot to measure the photosynthetic capacity (Pmax) of young leaves as their light-saturated photosynthetic rate. Shoots were transported in abundant seawater to the laboratory to measure the *P*max in temperature, stirring and light-controlled incubators. Once in the laboratory, *P*max was measured as the net oxygen released by leaf segments 6 cm long from the second youngest leaf from each shoot, following Alcoverro et al. (1998). Each leaf segment was incubated for 1 h in glass bottles (115 ml) filled with seawater collected at the sample site, with constant stirring at ca. 200 rpm and at a temperature of 23°C, roughly the same as that recorded in the field. We kept irradiances at ca. 800 µmol quanta $m^{-2}\,s^{-1}$, clearly exceeding the levels of saturating irradiances described for this species (Alcoverro et al. 1998). At the end of the incubation period, water oxygen concentration was measured using an oximeter (WTW Oximeter, OXI196), and compared to that of control bottles without plant ($n_c = 5$). The incubated tissues were dried at 70°C for 24 h and weighed, and the results were expressed as mg $O_2\,g^{-1}$ DW h^{-1}. Leaf nitrogen and carbon concentration were measured on four shoots. The second youngest leaf from each shoot was oven dried and ground, and carbon and nitrogen concentration was determined with a Carlo-Erba elemental auto-analyser (Serveis Científico-Tècnics, Universitat de Barcelona).

Rhizome nitrogen and non-structural carbohydrates (NSC) were analysed on five additional shoots. The top 2 cm from each rhizome were dried at 70°C and ground into a fine powder. Rhizome nitrogen concentration was measured using a Carlo-Erba elemental auto-analyser (Serveis Científico-Tècnics, Universitat de Barcelona). Rhizome soluble carbohydrates (sugars) and starch were measured using a modified method from Alcoverro et al (1999). Ground rhizome samples were dissolved in 96% (v/v) ethanol, sonicated for 5 min and heated at 80°C during 15 min to extract sugars, and this process was repeated three times. Starch was extracted from the ethanol-insoluble remaining pellet by dissolving it in 0.1 N NaOH and incubating it for 24 h at room temperature. Sugar and starch content were determined spectrophotometrically using an anthrone assay with sucrose as a standard. Sugar and starch content were combined as non-structural carbohydrate content (NSC).

Induced resistance responses

In order to measure any induced resistance caused by herbivore damage, we measured the concentration of phenolic secondary metabolites in leaves. Total phenolics were quantified using a modified Folin-Ciocalteu assay (Bolser et al. 1998) on the same dried leaves used to measure *P*max ($n = 3$). Although the Folin-Ciocalteu method does not discriminate among the different classes of phenolics and has other important limitations, this assay is considered adequate for relative comparisons (Amsler and Fairhead 2006 and references therein). Subsequently, we determined whether induced changes in plant traits had an effect on the feeding preferences of herbivores using feeding assays.

Herbivore feeding preferences between shoots that had been exposed to high levels of simulated herbivory and undamaged shoots were determined for *Sarpa salpa* and *Paracentrotus lividus*, the two main consumers of *P. oceanica* (Prado et al., in press). We performed paired feeding preference assays at the end of the simulated herbivory experimental period (September). Preliminary trials indicated that shoot leaf length influenced fish feeding preferences, as these herbivores significantly preferred longer to shorter shoots (paired *t* test; $t = 4.22$; $df = 14$; $P < 0.001$). Sea urchins are also influenced by leaf age, as they strongly prefer the distal tips of older leaves to the basal parts of younger leaves (Boudouresque and Verlaque 2001). Additionally, epiphytes are also known to influence the feeding preferences of both *S. salpa* (A. Vergés, unpublished data) and *Paracentrotus lividus* (Boudouresque and Verlaque 2001). We therefore standardised all replicates and treatments on the starting day of the experiment by removing all epiphytes using a blunt glass blade to minimise damage and by cutting shoots from both treatments at the apex to a length of about 13 cm. Consequently, high herbivory and control treatments were damaged equally before the experiment. This approach prevented us from testing short-term induced resistance effects such as those that may occur within hours and days, but allowed us to test the long-term effects of continuous herbivory on consumer behaviour.

The fish feeding assays were performed in the Medes Islands Marine Reserve (42°2′50′′N; 3°13′18′′E), where *S. salpa* densities are remarkably high (Macpherson et al. 2002; Tomas et al. 2005). We determined fish feeding preferences by offering high herbivory and undamaged tethered shoots side by side as pairs ($n = 20$) in the field. The experiment was set up in sandy patches at least 5 m away from seagrass meadows and rocky reefs, in order to target the herbivore fish *Sarpa salpa* and prevent consumption by the urchin *Paracentrotus lividus*. When feeding, *S. salpa* leaves a characteristic bite mark on the seagrass leaves (Boudouresque and Meinesz 1982). Consequently, we could confidently attribute all feeding observed during the experiment to this herbivore. Replica tethered pairs were at least 3 m apart from each other. Five control replicate pairs were individually protected from herbivores with 1 cm plastic mesh cages. No changes in shoot leaf area were observed throughout the experiment in the controls. We calculated consumption as the leaf area lost to herbivores, and we quantified preference through proportional consumption (i.e. amount of one food consumed divided by total consumption; Lockwood 1998). The experiment lasted 5 days.

The sea urchin feeding assays were performed under controlled aquaria conditions. Sea urchins were collected from the Port-Lligat shallow meadow and transported in aerated seawater tanks to the aquaria facilities, where the animals were acclimated and fed the palatable green alga *Ulva* spp. for 2 days prior to the start of the assays. Each urchin was offered a choice between high herbivory and undamaged shoots, which were attached side by side onto the bottom of individual containers ($n = 20$). Consumption and preference for each food type were quantified as described for the fish feeding assays. The experiment lasted 3 days. We discarded any replicate pairs where herbivores failed to feed or where all food available was consumed, since they offered no information on relative feeding preferences.

Statistical analyses

For each individual variable, differences between treatments were analysed using a two-way nested univariate analysis of variance (ANOVA), considering herbivory treatment (four levels: control, low, moderate and high) as a fixed effect and plot (three levels) as a random effect nested within treatment. To test for between-treatment differences for each variable, we used Tukey's HSD post hoc tests. All variables were individually checked for normality, homogeneity of variance and outliers using exploratory data analysis procedures, and where necessary data were log transformed (Quinn and Keough 2002). Differences in feeding preferences between high herbivory and control shoots were analysed using paired *t* tests in both the fish and urchin experiments. Statistical analyses were performed using Systat v.10.

Results

Compensatory effects of simulated herbivory

Simulated herbivory induced structural and physiological changes in all the plant traits measured except on leaf carbon concentration and rhizome carbohydrate content (Table 1). Blade width did not differ between treatments ($df = 3$; $F = 0.995$; $P = 0.443$), but the number of leaves significantly increased with herbivory (Table 1; Fig. 1a). Low, moderate and high herbivory treatments had a significantly larger number of leaves than control shoots (Fig. 1a). Simulated herbivory significantly increased growth in the low and moderate herbivory treatments, but not under high herbivory (Table 1; Fig. 1b). Growth in shoots subjected to the low herbivory treatment was 15% higher than in control treatments, while growth in shoots from the moderate herbivory treatment was 60% higher than controls (Fig. 1b). A similar but opposite effect was detected for photosynthetic capacity (Table 1; Fig. 1c). Control shoots had significantly higher photosynthetic capacity than shoots exposed to moderate herbivory, and there were no differences between control and either low or high herbivory shoots (Fig. 1c). Shoots subjected to moderate herbivory had significantly less nitrogen than any other treatments (Table 1; Fig. 1d). Differences in leaf carbon concentration between treatments were not significant (Table 1; Fig. 1e).

Rhizome nitrogen concentration was affected by moderate and high herbivory treatments only, but not by low herbivory (Table 1; Fig. 1f). Rhizome nitrogen decreased gradually, with rhizomes from plots subjected to moderate and high herbivory having ca. 30 and 40% less nitrogen than controls, respectively (Fig. 1f). We did not detect any significant differences between herbivory treatments in the carbohydrate reserves (NSC) (Table 1; Fig. 1g). Significant

Table 1 Results of the nested ANOVAs assessing the differences in leaf and rhizome plant traits between herbivory treatments

	Number of leaves				Growth[a]				Photosynthetic capacity[a]				Phenolics leaves			
Source of variation	*df*	MS	*F*	*P*	*df*	MS	*F*	*P*	*df*	MS	*F*	*P*	*df*	MS	*F*	*P*
Treatment	3	3.782	9.311	**0.005**	3	0.280	4.799	**0.034**	3	0.095	5.178	**0.028**	3	8.634	5.205	**0.028**
Plot (Treatment)	8	0.406	0.722	0.672	8	0.058	5.702	**<0.001**	8	0.018	1.114	0.389	8	1.659	3.582	**0.007**
Error	93	0.563			89	0.563			24	0.016			24	0.436		

	Nitrogen leaves				Carbon leaves				Nitrogen rhizomes[a]				NSC rhizomes[a]			
Source of variation	*df*	MS	*F*	*P*	*df*	MS	*F*	*P*	*df*	MS	*F*	*P*	*df*	MS	*F*	*P*
Treatment	3	1.376	11.896	**0.003**	3	14.656	3.898	0.055	3	0.161	12.517	**0.002**	3	0.070	0.776	0.540
Plot (Treatment)	8	0.116	3.638	**0.003**	8	3.760	6.117	**<0.001**	8	0.013	2.294	**0.036**	8	0.090	2.461	**0.028**
Error	36	0.032			36	0.615			48	0.006			42	0.037		

Significant values at $P < 0.05$ are in bold. *NSC* non-structural carbohydrates

[a] Data log-transformed to satisfy parametric test assumptions

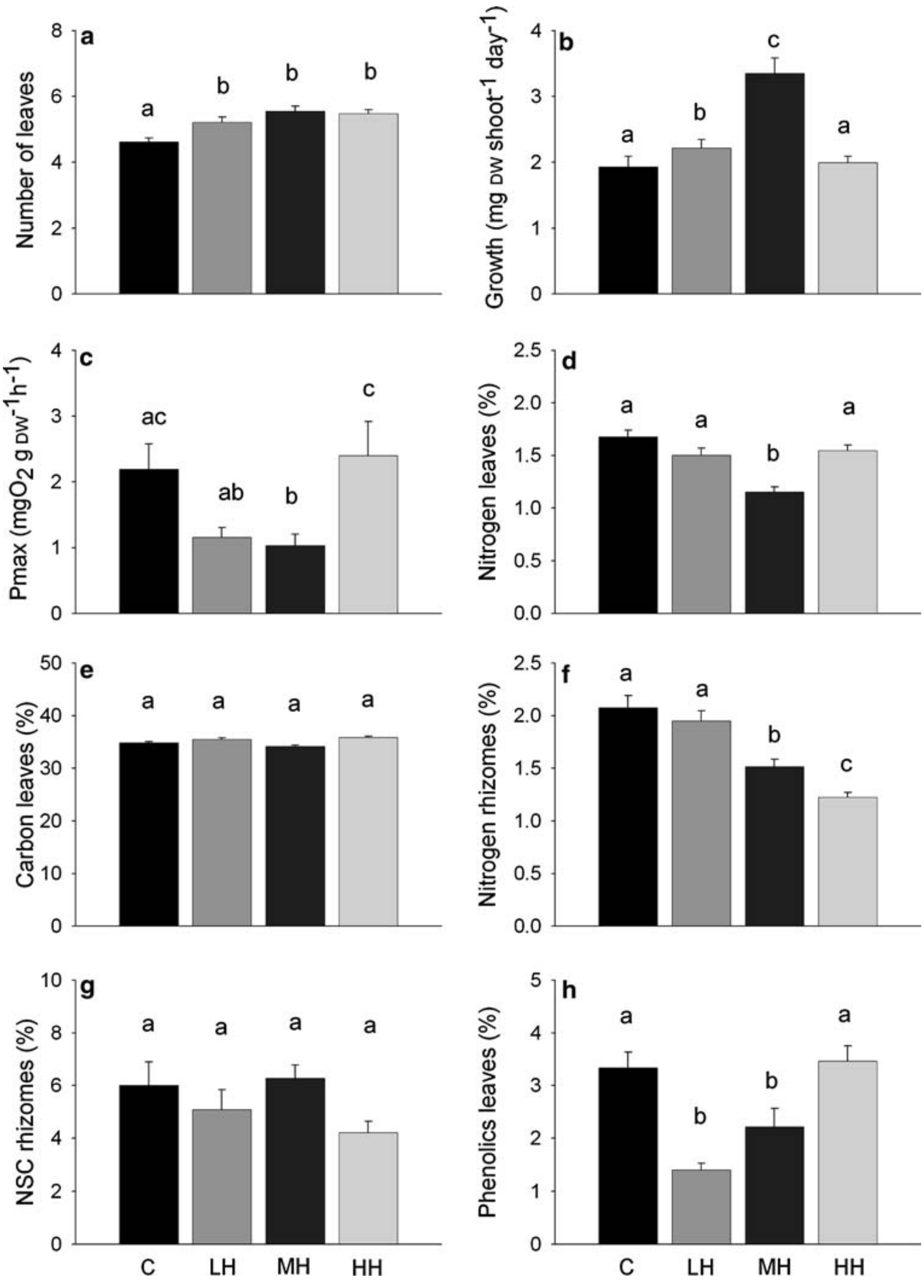

Fig. 1 Leaf and rhizome plant traits from control and treatment plots at the end of four months of simulated clipping at different intensities: *C* control, *LH* low herbivory, *MH* moderate herbivory, *HH* high herbivory. NSC refers to non-structural carbohydrates. *Bars* labelled with the same *lower case letter* do not differ significantly according to Tukey HSD test ($P \leq 0.05$)

between-plot differences were found for growth, leaf nitrogen, carbon and phenolics and for rhizome nitrogen and carbohydrate reserve (NSC) content, indicating important spatial variability of most variables considered.

Resistance effects of simulated herbivory

Leaf phenolic concentration changed significantly in response to simulated herbivory (Table 1; Fig. 1h). Control and high herbivory shoots had similar phenolic content, but both low and moderate herbivory shoots had lower phenolic concentrations than control shoots (Fig. 1h). High herbivory had no effect on the palatability of seagrass leaves, and both herbivores fed equally on shoots that had suffered long-term high herbivory than on control shoots. The fish *Sarpa salpa* consumed 55.94 ± 4.76% of control shoots, and 44.06 ± 4.76% of clipped shoots (proportional consumption ± SE; $t = 1.627$, $df = 18$, $P = 0.121$). The urchin *Paracentrotus lividus* consumed 47.88 ± 10.02% of control shoots, and 52.12 ± 10.02% of clipped shoots (proportional consumption ± SE; $t = -0.211$, $df = 14$, $P = 0.836$).

Discussion

Our results show that *P. oceanica* is able to compensate for low and moderate losses of leaf biomass by increasing leaf growth of damaged shoots. This compensatory growth is not supported by an increase in photosynthetic capacity or light availability, but rather by the reallocation and translocation of leaf and rhizome resources. Under high herbivory conditions, plants are not able to compensate for loss of biomass but manage to maintain the same levels of growth and photosynthesis as undamaged plants by translocating larger amounts of nitrogen from the rhizomes. In contrast, we found no evidence of inducibility of resistance traits following herbivore damage. Simulated herbivory had a negative or neutral effect on the concentration of phenolic secondary metabolites, and herbivores fed equally on shoots that had been exposed to continued high levels of simulated herbivory and on control undamaged shoots.

Plant growth results from this study are consistent with those of Tomas et al. (2005), who found no differences in growth rate between *P. oceanica* shoots from naturally occurring heavily mowed patches (with approximately 50% biomass removed) and shoots from herbivore exclusion cages. In contrast, Cebrian et al (1998) found contradictory effects of clipping on *P. oceanica* that varied with site. However, the latter study mimicked herbivory by clipping isolated single shoots, thereby allowing for instant translocation between neighbouring seagrass ramets, an important mechanism of resource allocation in this species (Marbà et al. 2002). While simulating herbivory on single shoots may resemble the action of urchins, the fish *Sarpa salpa* (responsible for 70% of the annual consumption of *P. oceanica*) is known to create heavily grazed distinct patches of dimensions that clearly exceed nutrient translocation transport distances between ramets (Tomas et al. 2005). Indeed, the effects of herbivory on seagrasses vary widely depending on the grazing behaviour of herbivores. Consumer effects range from that of limpets that remove the epidermal layer of leaves and may have devastating effects (Zimmerman et al. 1996), to those of turtles or dugongs that enhance the nutrient content of leaves by repeatedly cropping the above-ground biomass or by removing the entire plant (Aragones et al. 2006). Other studies to date on seagrass responses to herbivory have focused on the Caribbean turtlegrass *Thalassia testudinum*, and have yielded somewhat contradictory results. While some have reported an increase in productivity in response to grazing or defoliation (Valentine et al. 1997, 2000; Moran and Bjorndal 2005), others have found a decrease in productivity (Williams 1988; Heck and Valentine 1995). However, differences in the duration, timing, clipping area and intensity of grazing among these studies could potentially explain some of the apparent contradictions and reflect the need to assess the effects of ecologically relevant patterns of herbivory (summarised by Moran and Bjorndal 2005).

The decrease in photosynthetic capacity observed in plants subjected to moderate herbivory is in stark contrast with the findings from terrestrial ecosystems, where increased leaf photosynthetic activity is one of the most cited mechanisms of tolerance (Strauss and Agrawal 1999; Tiffin 2000). Since a large fraction of the total tissue nitrogen in plants is associated with the photosynthetic apparatus (i.e. pigments and associated enzymes), the lower capacity to photosynthesise in moderately damaged plants may have been caused by the decrease in leaf nitrogen content. Indeed, moderately clipped plants had a lower nitrogen concentration than controls, and the photosynthetic capacity of *P. oceanica* has been shown to depend on tissue nitrogen when the concentration of this nutrient falls below critical limits (Alcoverro et al. 2001a).

Mobilisation of nitrogen from within the plant emerges as an important mechanism facilitating compensatory growth in *P. oceanica*. Plants responded to both small and moderate losses of biomass by increasing the number of leaves per shoot and stimulating leaf growth. However, while low herbivory resulted in a ‘dilution’ of nitrogen leaf content per unit area with no effects on rhizome reserves, the enhanced growth of shoots subjected to moderate herbivory required the translocation of nitrogen from rhizomes to leaves. Similarly, plants subjected to high herbivory incremented the nitrogen demand from rhizomes even more and lost over 40% of the stored reserves of this nutrient, but this was insufficient to enhance growth. As a long-lived seagrass adapted to the oligotrophic waters of the Mediterranean, translocation of nutrients is a particularly important strategy of resource conservation for *P. oceanica* (Alcoverro et al. 2000). Indeed, rhizomes are known to play a major role in the nitrogen metabolism of *P. oceanica*, supplying up to 33% of the shoot nitrogen requirements during the maximum growth period from May to July (Invers et al. 2002). Our findings demonstrate that enhanced growth induced by loss of biomass to herbivores also results in substantial translocation of this nutrient. The immediate consequences of this loss of stored nitrogen are unknown, but since water nutrient availability increases in the autumn this may allow the plants to replenish their nitrogen reserves before the next period of leaf growth commences the following spring. However, any long-term loss of stored nitrogen will increase the dependence of the plant on external nutrients, making it more vulnerable to fluctuations of this resource in the environment.

In contrast to the crucial role of nitrogen rhizome reserves in supporting growth of damaged plants, simulated herbivory had no measurable effect on rhizome carbohydrate reserves. In *P. oceanica*, carbon fixation is highest during the summer period of optimal light conditions

(June–September), and this allows the plant to store enough reserves in the rhizomes to overwinter. Our results show that plants subjected to all levels of herbivory during that period had similar concentrations of total carbon in their leaves and were able to accumulate similar amounts of carbohydrates as undamaged plants. Moreover, simulated herbivory induced seagrass leaf growth even in those treatments where only the tips of leaves were cut and less than 5% of leaf biomass was lost, indicating that increased growth was not the result of an increase in light availability (decrease in self-shading). The question that then arises is: what are the mechanisms that allow damaged plants to increase their growth without either an enhancement in photosynthetic activity or a reduction in stored carbohydrate reserves? Since the concentration of leaf phenolic compounds strongly decreased in those plants that exhibited compensatory growth despite having similar carbon levels, we suggest that enhanced growth was at least partly supported by reallocation of these carbon-based compounds. A negative relationship between phenolic content and growth is often interpreted as a resource-based trade-off between compensation and defence (Vandermeijden et al. 1988; Boege 2005). However, phenolic compounds do not only function as defensive secondary metabolites, but are also involved in primary functions of plants and play a particularly crucial role in cell wall expansion during plant growth (Abdulrazzak et al. 2006). It is therefore likely that, as growth was stimulated in low and moderate herbivory treatments, some of the phenolics present in tanniferous cells were used in cell wall construction. Our findings are thus consistent with a model proposed for brown algal phlorotannins by Arnold and Targett (2003) that predicts that rates of accumulation of phenolics are not driven by the benefits of chemical defence but by the need for these compounds in cell wall construction. Once in the cell wall, phenolics are oxidatively coupled and crosslinked to polysaccharide chains (Fry 2000) and no longer extractable or detectable by the colorimetric assays used in this study, which explains the lower concentration of reactive phenolics in plant tissues undergoing rapid growth.

The importance of phenolics in the primary metabolic processes of *P. oceanica* does not rule out their putative function as secondary defensive metabolites, since these two functions may not be realised simultaneously. However, our results show that the synthesis of these compounds is not induced by herbivory. In fact, we found a clear lack of inducibility of any long-term resistance traits as a response to high levels of simulated herbivory. Plants subjected to continued intense clipping and undamaged plants were equally consumed by the sea urchin *Paracentrotus lividus* and the fish *Sarpa salpa*. Nevertheless, simulated herbivory does not always trigger the same effects in plants as natural herbivory, especially with regards to chemical responses (Baldwin 1990). In vascular plants and some algae, chemical or biological cues such as herbivore saliva induce a cascade of chemical responses that can result in synthesis of induced defences (Baldwin 1990; Pavia and Toth 2000). Further bioassays using plants damaged by different levels of actual herbivory against undamaged plants are needed to clarify this point.

In summary, *P. oceanica* can exhibit substantial phenotypic plasticity in response to herbivore stress. Low and moderate herbivory can induce substantial compensatory responses, such as enhanced growth through mobilisation of internal resources, in this marine plant. This is the first study to simultaneously examine compensation and resistance traits on seagrasses, and our findings show that there is no trade-off between compensatory growth and a cost for phenolic production rates, since increased growth was not mediated by de novo synthesis of metabolites but was instead driven by reallocation of existing resources within the plant.

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