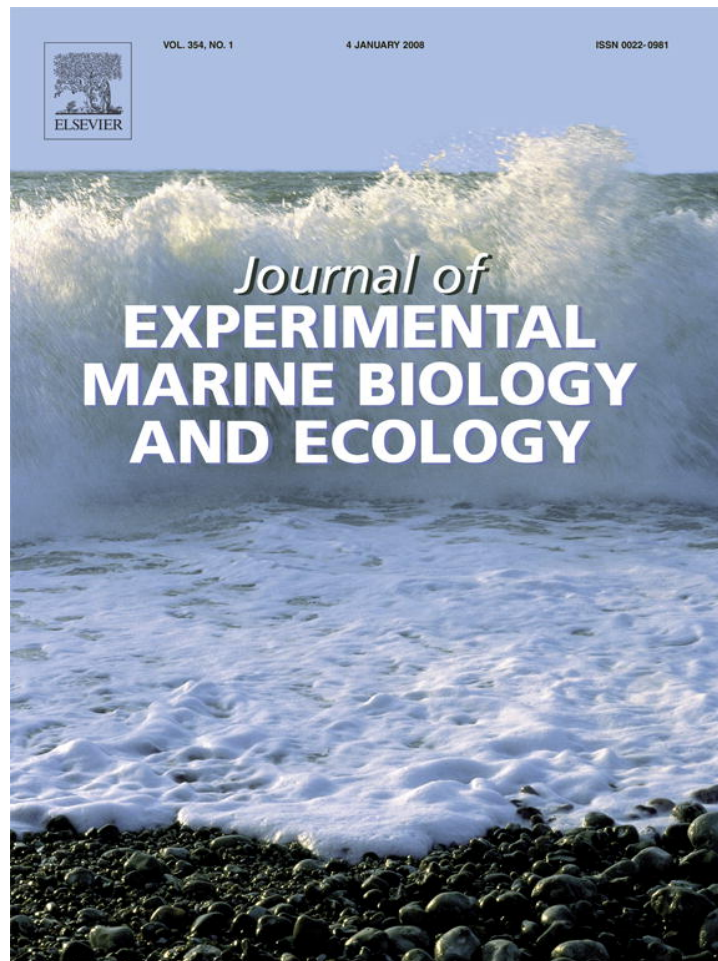


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In situ tests on inducible defenses in *Dictyota kunthii* and *Macrocystis integrifolia* (Phaeophyceae) from the Chilean coast

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Abstract

Numerous experimental studies have reported inducible defenses in macroalgae, but most of them have been conducted in laboratory environments where algae were maintained detached from the substratum and in artificial flow regimes. The results of those experiments might not reflect the natural situation, which can only be studied *in situ*. We examined whether the brown macroalgae *Dictyota kunthii* (C. Agardh) Greville and *Macrocystis integrifolia* (Bory) show inducible defenses following exposure to different grazing levels (direct, water-borne cues from nearby grazed conspecifics, presence of a non-grazing herbivore and natural grazing) in field experiments, striving to maintain natural conditions as much as possible. We measured palatability of algae after exposure to different grazing levels by using live algae and agar-based food containing non-polar extracts. *M. integrifolia* showed no induction of defenses (at least not of non-polar compounds), suggesting constitutive defenses, absence of defenses (tolerance) or use of another strategy to avoid herbivory. These results are similar to those from previous laboratory experiments. In *D. kunthii*, defense was induced after two weeks of direct grazing by amphipods under field conditions. Water-borne cues from nearby grazed conspecifics, presence of a non-grazing herbivore and natural grazing did not induce defenses. Induction of defense in response to direct grazing agrees with results from a previous laboratory study, but while indirect cues induced defenses in the laboratory, there was no measurable induced defense in the field. Probably chemical cues from grazers are diluted quickly in the field, not reaching concentrations that cause induction of defenses. This might be the reason why in some algae induction by direct grazing is a more important defensive strategy than induction by water-borne cues. The results from our study also suggest that laboratory experiments showing induced defenses in response to grazed neighbours or mere grazer presence need to be interpreted with caution.

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Keywords: Chile; *Dictyota kunthii*; Inducible defenses; *Macrocystis integrifolia*; Mesograzers; *Parhyalella ruffoi*

1. Introduction

Macroalgae have evolved different strategies to defend themselves against herbivores, with chemical and morphological defenses being most common (Cronin, 2001). Defenses can be expressed constitutively, or can be induced in response to herbivore-specific cues (Amsler, 2001). Inducible responses have

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the benefits of metabolic cost savings (Fairhead et al., 2006), are advantageous over constitutive defenses due to a lower risk of autotoxicity, and furthermore might prevent herbivores adapting to plant defenses (see Agrawal and Karban, 1999). Various methods of experimental induction of defenses have been used in marine macroalgae, including artificial damage (simulated herbivory) (e.g. Van Alstyne, 1988; Borell et al., 2004; Lüder and Clayton, 2004), direct grazing (e.g. Martinez, 1996; Pavia and Toth, 2000; Yun et al., 2007), water-borne cues from nearby grazed conspecifics (e.g. Toth and Pavia, 2000, 2002; Toth, 2007) and mere grazer presence (e.g. Weidner et al., 2004; Diaz et al., 2006; Molis et al., 2006). Most of these studies are based on laboratory experiments, either in outdoor or indoor tanks where algae were kept detached from the primary substratum (e.g. Toth and Pavia, 2000; Rohde et al., 2004). Recent experimental evidence, though, suggests that attachment status of algae might be important when studying the induction of defenses (Rothhäusler and Thiel, 2006). Stressed algae often show a limited potential for chemical defense (Renaud et al., 1990; Cronin and Hay, 1996b), and detached individuals might have few extra resources available for defense (Rothhäusler and Thiel, 2006). Thus, results from laboratory induction experiments might not necessarily represent the natural condition where algae are growing attached to the substratum. Foster (1992) had already emphasized that field experiments are necessary to determine whether certain algal chemicals indeed reduce consumption by grazers in natural field settings.

Field manipulations studying inducible defenses usually employ methods such as artificial damage of algae (e.g. Van Alstyne, 1988; Martinez, 1996; Peckol et al., 1996) or transplanting algae to sites with “high” or “low” densities of herbivores (e.g. Steinberg, 1984; Van Alstyne, 1988). Hammerstrom et al. (1998) examined induction of phlorotannins in several kelp species, based on algae manipulated (mechanical wounding) on racks suspended from floating docks. Subsequently, Pavia & Toth (2000) suggested that artificial damage in brown algae should not be used to evaluate herbivory-induced changes in secondary metabolites, because this simulation might lack important chemical or biological cues necessary to stimulate production of chemical defenses. Recently Coleman et al. (2007) demonstrated inducible defense in *Ascophyllum nodosum* by application of a digestive enzyme found in snail saliva. In controlled field experiments, Cronin and Hay (1996a) used detached algae entwined in plastic ropes attached to the bottom, and after manipulating amphipod presence, demonstrated induction of defenses. Compared to

undamaged controls, algae that were grazed by amphipods contained higher concentrations of secondary metabolites and were less palatable to amphipods.

Numerous studies have also reported defensive responses of terrestrial plants to grazing of their neighbours (the so-called “talking trees” phenomenon) (e.g. Karban et al., 2000, 2004; Karban and Maron, 2002). Similar to terrestrial plants, aquatic algae that are able to assess and respond to water-borne signals might have a selective advantage. In aquatic systems, water-borne cues could originate from grazers or from neighbouring grazed algae, providing a warning of imminent herbivore attack, and these signals could also be used to induce defense (Sotka et al., 2002). In marine systems little attention has been paid to the possibility of a grazed alga releasing water-borne cues that stimulate an increase in the production of secondary metabolites in an ungrazed neighbouring alga (Diaz et al., 2006). Few examples of water-borne cues from neighbouring grazed individuals are known for marine algae (Toth and Pavia, 2000; Rohde et al., 2004; Diaz et al., 2006; Toth, 2007). These studies used laboratory conditions, where water flows might not be representative of those in the natural habitat of the algae (e.g. intertidal conditions). In the brown alga *Dictyota kunthii*, chemical defenses were recently shown to be induced by mere grazer presence (Macaya et al., 2005), but since this and other algae commonly grow in high-flow environments (Stewart, 2004), it is considered possible that water-borne chemical cues might not induce defenses under field conditions.

Here we examine inducible defenses *in situ* in two brown algae, *Macrocystis integrifolia* and *D. kunthii*, both common species along the outer coast of Chile. In testing for the chemical basis of induction, we focused on non-polar compounds, because the strong emphasis in linking some polar compounds (e.g. phlorotannins) with chemical defense in algae seems unjustified by currently available evidence (see review by Amsler and Fairhead, 2006). The deterrent role of phlorotannins against herbivores has been increasingly questioned and seems to be highly dependant on both algae and herbivore species (Amsler and Fairhead, 2006). This is underlined by the finding that non-polar extracts reduced feeding by the sea urchin *Echinometra lucunter*, whereas polar extracts (including phlorotannins) stimulated feeding (Eriksson et al., 2006). Recent studies have reported deterrent effects of non-polar compounds from different brown algae on mesograzers: Taylor et al. (2002) in *Sargassum filipendula*, Fairhead et al. (2005) in *Desmarestia anceps*, and Ceh et al. (2005) in *Sargassum asperifolium* and *Cystoseira myrica*. More importantly, *Macrocystis* is known to be poor in phenolic

(polar) compounds (Steinberg, 1985; Winter and Estes, 1992) and *D. kunthii* contains several non-polar compounds such as pachydictyol A and dictyodial (de Nys et al., 1993) that have been shown to be deterrents to amphipods, sea urchins and fishes (e.g. Hay et al., 1987; Cronin and Hay, 1996a; Pereira et al., 2000; Barbosa et al., 2004).

The specific aims of this study were to test (1) whether direct grazing by amphipods or natural grazing (algae exposed to ambient herbivory) can induce defenses in both species of macroalgae, and (2) whether water-borne cues from nearby grazed conspecifics (hereafter termed “indirect grazing”) or mere presence of amphipods can induce defenses under field conditions. Furthermore, in *D. kunthii* we tested induction of defenses in basal and medium parts, because previous outdoor laboratory experiments had shown that defense is limited to specific parts of this alga (Macaya et al., 2005).

2. Materials and methods

2.1. Study site and organisms

The collection and experimental sites are located on the northern-central coast of Chile between 28°S and

30°S. The experiment with *M. integrifolia* was carried out *in situ* at Playa Blanca (28°10'S, 71°10'W) in a subtidal kelp forest. Giant kelp *M. integrifolia* is present along the Peruvian and northern Chilean coast (6–32° S), and inhabits bays at a depth of 0–15 m in wave-protected and semi-protected areas (Buschmann et al., 2004). Due to the large size of *M. integrifolia*, we used only blades from the middle section of the algae for induction experiments (see Fig. 1A), because these are usually the best developed blades and a previous laboratory study with fresh food and agar-based food from non-polar extracts revealed no distinct preferences of grazers for blades from different sections of *M. integrifolia* (Macaya et al., 2005).

Attached individuals of *D. kunthii* were collected in boulder fields from the low intertidal zone of Totoralillo (30° 03'S, 71°28'W), but the field experiment was conducted in the low intertidal zone of Bahía La Herradura (BLH: 29°58'S; 71°21'W), about 10 km north of the collection site. The experiment was conducted in BLH in order to ensure that the experiment could be surveyed and terminated at the desired time intervals. The population of *D. kunthii* in Totoralillo is only accessible during extreme spring low tides and very good sea conditions. Small boulders (diameter 30–40 cm)

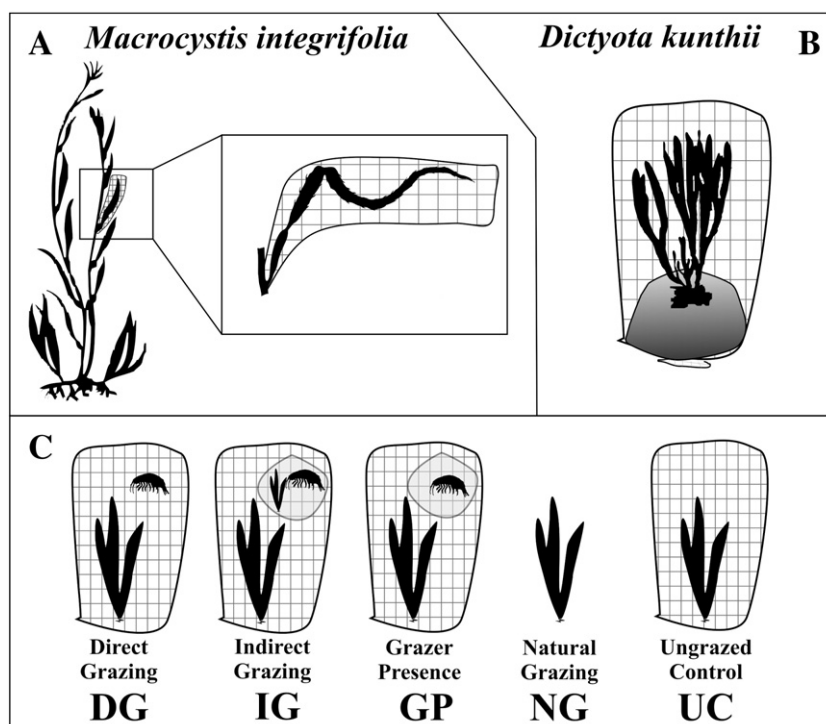


Fig. 1. Experimental set-up. A) *Macrocyctis integrifolia*, a medium blade was enclosed using a plastic mesh-bag. B) Whole individuals of *Dictyota kunthii* including alga and boulder substrate were enclosed using similar plastic mesh-bag. C) The five treatments of grazing levels used in the present experiments.

with fully developed algae were transferred from Totoralillo to BLH. Preliminary experiments had demonstrated that adult *D. kunthii* transferred in this way (with their boulder substratum) grow well in the low intertidal zone in BLH. This alga grows in the low intertidal and shallow subtidal zone down to 8 m depth. Individuals consist of two parts: one formed by prostrate, overlapping, vegetative fronds, attached to rocks on the ventral surface by rhizoids, and the other by erect dichotomously branched thalli about 25 cm long (Malbran and Hoffmann, 1990). Numerous small ligulae, 3–4 mm long, cover the blade surface (Hoffmann, 1988). All induction experiments were conducted with entire individuals in the natural environment.

Both algae species harbour high abundances of amphipod and isopod mesograzers (Dayton, 1985; Tegner and Dayton, 1987; North, 1994; Palma and Ojeda, 2002), which principally live or feed on blades. For all experiments, the amphipod *Parhyalella ruffoi* (Lazo-Wasem and Gable) was used as grazer after preliminary studies confirmed that this amphipod consumed a wide variety of macroalgae, including *D. kunthii* and *M. integrifolia* (Macaya et al., 2005; Rothäusler et al., 2005). *P. ruffoi* has a wide distribution along the coast of northern-central Chile and Peru (Lazo-Wasem and Gable, 2001), where it occurs, often in very high abundances, in a wide variety of shallow subtidal habitats. The duration of the field experiment was 14 days, based on induction times revealed in previous studies with these and other algal species (Pavia and Toth, 2000; Rohde et al., 2004; Macaya et al., 2005; Rothäusler et al., 2005).

2.2. Manipulation of amphipod grazing levels

To test the direct and indirect effects of grazing by the amphipod *P. ruffoi*, we manipulated grazing conditions on *M. integrifolia* and *D. kunthii*. Five different treatments were used: (1) DG, direct grazing by amphipods; (2) IG, indirect grazing (algae receiving cues from nearby grazed conspecifics); (3) GP, grazer presence (algae receiving water-borne cues from amphipods that were kept without direct access to algae); (4) NG, natural grazing (algae under ambient conditions of grazing); and (5) UC, ungrazed controls where the algae were maintained without amphipods. For *M. integrifolia*, 30 replicates of each treatment were placed in the natural environment. For *D. kunthii* 10 replicates were used. For *M. integrifolia*, the manipulative treatments were done by enclosing a mid-blade piece of the alga in plastic mesh-bags (mesh size: 1 mm). These bags included the whole blade and the

pneumatocyst, and were fastened to the stipe using cable ties with soft foam plastic surrounding the base of the blade in order to minimize damage to the blade (see Fig. 1A). Shading by one layer of mesh does not affect chemical defenses in these algae (C. Pansch, O. Cerda, M. Thiel et al., unpublished data).

During the experiment (14 days) many individuals of *M. integrifolia* were lost from the study site at Playa Blanca due to unfavorable sea conditions. However, six replicates of each treatment were recovered.

For *D. kunthii*, whole individuals were enclosed in plastic mesh-bags (mesh size: 1 mm). Boulders with *D. kunthii* were collected in Totoralillo and transported to large tanks (2000 l) at Universidad Católica del Norte. Here the algae and boulders were carefully cleaned of epibionts and then transferred to a nearby intertidal boulder field in BLH. Whole algae and boulders were completely enclosed in a mesh-bag that was closed below the boulder using plastic cable ties (see Fig. 1B). Five replicates of each treatment of *D. kunthii* were recovered at the end of the experiment (14 days).

Direct grazing treatments had 20 or 30 amphipods inside each bag for *M. integrifolia* and *D. kunthii*, respectively. These densities were chosen based on data from natural populations of epifauna on these and other algae (Gelcich, 1999; E.C. Macaya personal observation) and are sufficient to produce damage as was visually confirmed for both algae species. Indirect grazing had 20 amphipods enclosed in a small plastic mesh-bag with (treatment 2—IG) or without (treatment 3—GP) a piece of algae from the same thallus inside the large plastic mesh-bags that enclosed the *M. integrifolia* blade, or the entire *D. kunthii* thallus. Ungrazed controls were kept in large mesh-bags without amphipods (see Fig. 1C).

2.3. Food-preference assays

After the experimental induction a series of no-choice and choice assays were run to determine feeding preferences of *P. ruffoi*. Amphipods were isolated with a single piece of tissue (i.e. a no-choice assay) to assess feeding rates on that tissue when alternative foods are unavailable (Taylor et al., 2002). These data can then be contrasted with data from choice assays (i.e. where two food types are simultaneously available to each consumer) to evaluate the importance of compensatory feeding and diet mixing (Cruz-Rivera & Hay, 2000; Taylor et al., 2002).

The feeding assays were conducted with live algae to assess chemical and/or structural defenses, and with agar-based food (containing non-polar algal extracts) to

assess defenses based only on chemical components. All feeding assays lasted 3d and were conducted in a culture room under constant temperature (15 ± 1 °C), using a 12 h dark/light photoperiod of $40 \pm 10 \mu\text{mol m}^{-2} \text{s}^{-1}$. In assays conducted with algal pieces, two different parts (basal and medium) of *D. kunthii* were used, because previous experiments had shown a differential defense with induction in basal parts (Macaya et al., 2005). For logistic reasons we initially conducted no-choice assays with *D. kunthii*, and conducted choice assays only when no-choice assays showed significant differences.

In no-choice assays with live algae, two paired pieces (~ 0.25 g) were removed from each alga and wet mass was determined to the nearest mg, using an analytical balance (Denver Instrument 100 $A \pm 0.2$ mg), after blotting the alga for 30 s with absorbent paper to remove excess water. The two paired pieces were placed in separate Petri dishes ($n=6$ and $n=5$ pairs, for *M. integrifolia* and *D. kunthii*, respectively) containing seawater. Six individuals of *P. ruffoi* were added to one of the two paired dishes, while the other was left free of grazers to control for autogenic changes in plant mass (Cronin & Hay, 1996a). Algal pieces were reweighed after 3d. The amount of algal biomass consumed (AC) in each replicate was calculated as: $AC = T_i(C_f/C_i) - T_f$ where T_i and T_f are initial and final wet mass of the alga

used in the feeding assay, and C_i and C_f are initial and final mass of control algae not used in the feeding assay (according to Cronin and Hay, 1996a,b).

In choice assays with live algae, amphipods were given pair-wise choices of all ten possible combinations of the five treatments. Assays were conducted in a similar way as in the no-choice assays, except that two pieces from different treatments were offered simultaneously to 8 amphipods (miniscule incisions were made in algal pieces to distinguish treatments).

The second assay used agar-based food, and single algal pieces were placed for 48 h in dichloromethane (DCM) after blotting the alga dry. We used 2 ml DCM per 1 g algal wet mass. In order to obtain comparable concentrations of extracted chemistry between the original alga and agar-based food items, we used the ratio wet mass:dry mass (freeze-dried powder) of *Ulva lactuca* (3:1) (a green alga palatable to *P. ruffoi*) and mixed extracts with an amount of dry *U. lactuca* powder representing one third of algal wet mass from which extracts were made. Using a pipette the DCM extracts were slowly dropped onto the *Ulva* powder and evaporated under the fume hood, leaving extracted compounds behind in the *Ulva* powder. Following the methodology of Hay et al. (1994), this extract-treated *Ulva* powder was then incorporated into agar, poured

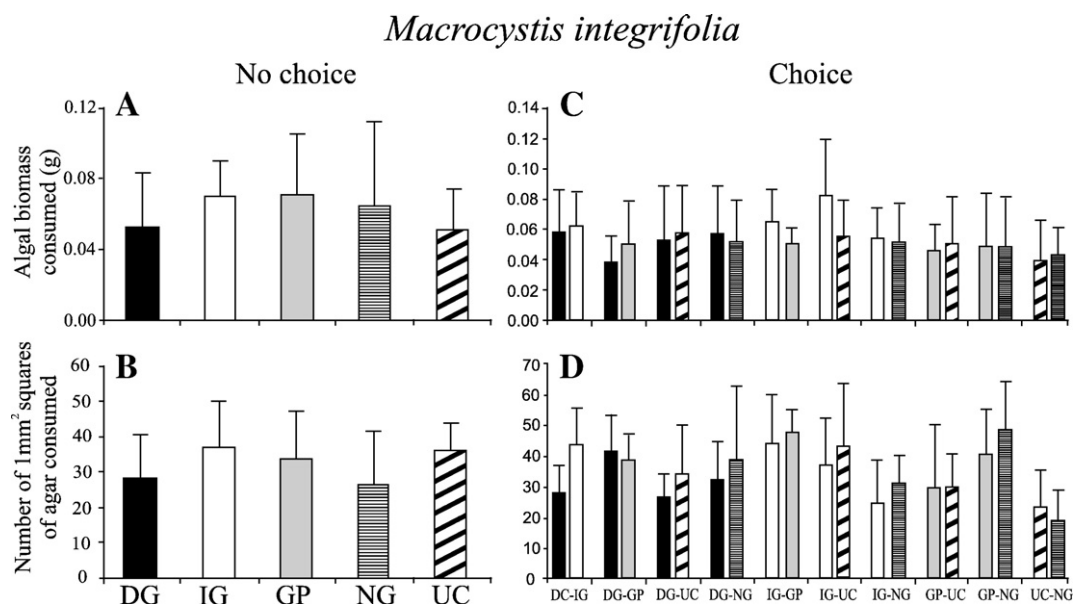


Fig. 2. Mean consumption (total algal biomass consumed in 3 days) of *Macrocyctis integrifolia* by the amphipod *Parhyalella ruffoi* after the field induction experiment. No-choice feeding assays with (A) live algal pieces, and (B) with agar-based food containing non-polar algal extracts. Paired choice feeding assays with (C) live algal pieces, and (D) with agar-based food containing non-polar algal extracts. Error bars represent +1 S.D. ($n=6$ replicates for each treatment). DG = direct grazing, IG = indirect grazing, GP = grazer presence, UC = ungrazed controls, NG = natural grazing. For further details see Materials and methods.

Table 1

Results of paired *t*-tests, comparing amphipod consumption rates from choice feeding assays between different grazing levels in *Macrocystis integrifolia* and basal parts of *Dictyota kunthii*. Extracts = agar-based food containing non-polar algal extracts, Live=intact algal pieces

	<i>Macrocystis integrifolia</i>				<i>Dictyota kunthii</i>			
	Live		Extracts		Live		Extracts	
	<i>t</i>	<i>p</i>	<i>t</i>	<i>p</i>	<i>t</i>	<i>p</i>	<i>t</i>	<i>p</i>
DG vs. IG	-0.19	0.857	-2.22	0.078	-5.81	0.004	-7.06	0.001
DG vs. GP	-0.85	0.435	0.39	0.716	-4.43	0.011	-5.08	0.007
DG vs. UC	-0.23	0.830	-0.98	0.371	-5.34	0.006	-7.83	0.001
DG vs. NG	0.40	0.708	-0.63	0.557	-1.09	0.338	-3.55	0.280
IG vs. GP	1.16	0.297	-0.75	0.487	-0.30	0.777	1.60	0.185
IG vs. UC	1.62	0.165	-0.80	0.461	1.47	0.215	-2.60	0.06
IG vs. NG	0.23	0.830	-0.83	0.446	-2.09	0.105	-1.70	0.164
GP vs. UC	-0.33	0.754	0.04	0.966	0.56	0.608	0.43	0.690
GP vs. NG	0.03	0.978	0.50	0.640	0.28	0.797	0.60	0.579
UC vs. NG	0.03	0.974	-0.84	0.437	0.98	0.382	1.14	0.318

DG = direct grazing, IG = indirect grazing, GP = grazer presence, UC = ungrazed controls, NG = natural grazing.

into a mould lying over a fly mesh (mesh size 1 mm²), and a pellet of 200 mesh squares was cut out after hardening. These pellets were offered to amphipods as agar-based food containing non-polar algal extracts.

In no-choice assays one piece of agar-based food was placed in Petri dishes containing seawater with 4 amphipods. In choice assays, two pieces of agar-based food of all ten possible combinations of the five treatments were simultaneously offered to 6 amphipods (small incisions allowed us to distinguish treatments). Consumption of these food items was determined by counting the number of empty 1 mm² fly mesh squares with a stereomicroscope after completing the assays.

2.4. Statistical analysis

Results from no-choice feeding assays were analyzed by one-way ANOVA, and Tukey HSD was used for post-hoc comparisons of significant ANOVA results. Choice feeding assays were analyzed with paired *t*-tests. Prior to both analyses homogeneity of variance was confirmed using Cochran's test.

3. Results

The blades of the individuals of *M. integrifolia* that were recovered after the two week long experiment were in good condition, with all amphipods inside the mesh-bags, depending on the treatment. Directly grazed blades showed scars of grazing by the amphipods. Blades exposed to the different grazer treatments

showed no significant differences in palatability compared to controls. This was confirmed in no-choice feeding assays with both live algae (one-way ANOVA; $F=0.4757$, $p=0.7531$; Fig. 2A) and agar-based food (one-way ANOVA; $F=0.7517$, $p=0.5663$; Fig. 2B). Choice assays produced similar results with live algae (Fig. 2C, Table 1) and agar-based food (Fig. 2D, Table 1).

Some of the *D. kunthii* individuals without mesh-bags (natural grazing) disappeared completely during the two weeks of the field experiment, possibly due to grazing by large herbivores (e.g. sea urchins, fishes and snails e.g. *Tegula* spp.) or due to boulder movements. Many algae from natural grazing treatment lost more than 50–60% of their tissue during the experiment while algae in the direct grazing treatment only lost 5–10% of their biomass. Naturally and directly grazed individuals showed signs of grazing activity, principally in the medium parts of the algae.

None of the different grazing treatments induced any change in palatability of medium parts of *D. kunthii* after

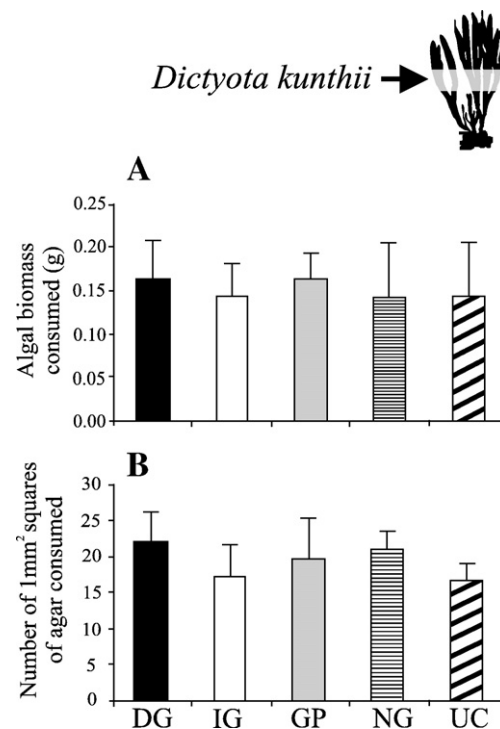


Fig. 3. Mean consumption (total algal biomass consumed in 3 days) of medium parts of *Dictyota kunthii* by the amphipod *Parhyalella ruffoi* after the field induction experiment. No-choice feeding assays with (A) live pieces, and (B) with agar-based food containing non-polar algal extracts. Error bars represent +1 S.D. ($n=5$ replicates for each treatment). DG = direct grazing, IG = indirect grazing, GP = grazer presence, UC = ungrazed controls, NG = natural grazing. For further details see Materials and methods.

Table 2

ANOVA results, testing for the effects of different grazing levels, on amphipod consumption rates of different thallus parts of *Dictyota kunthii*, obtained in 3 days long no-choice feeding assays at the end of the field induction experiment

		Basal parts				Medium parts			
		Live		Extracts		Live		Extracts	
Treatment	df	F	p	F	p	F	p	F	p
Grazing	4	14.92	<0.001	7.40	<0.001	1.42	0.216	1.60	0.212

Extracts = agar-based food containing non-polar algal extracts, Live = intact algal pieces.

two weeks. In no-choice feeding assays the grazers *P. ruffoi* showed no preference for pieces from all treatments tested, neither in live algae assays (Fig. 3A, Table 2) nor in agar-based food assays (Fig. 3B, Table 2).

In contrast to the medium parts of *D. kunthii*, defense was induced in basal parts. At the end of the field experiment, directly grazed basal parts of *D. kunthii* were significantly less palatable than controls both for live algae and agar-based food. These results were confirmed in no-choice (Fig. 4A and B, Table 2) and choice (Fig. 4C and D, Table 1) assays with live algae and agar-based food. Indirect grazing, grazer presence

and natural grazing did not reduce palatability compared to controls (Fig. 4, Tables 1 and 2). Although algae exposed to natural grazing showed a tendency to be less palatable than other grazing treatments for live algae and agar-based food, no significant differences were detected for any of the tissue comparisons.

4. Discussion

Our results showed a clear reduction in palatability in basal parts of *D. kunthii* after two weeks of experimental grazing by amphipods, providing evidence for induced

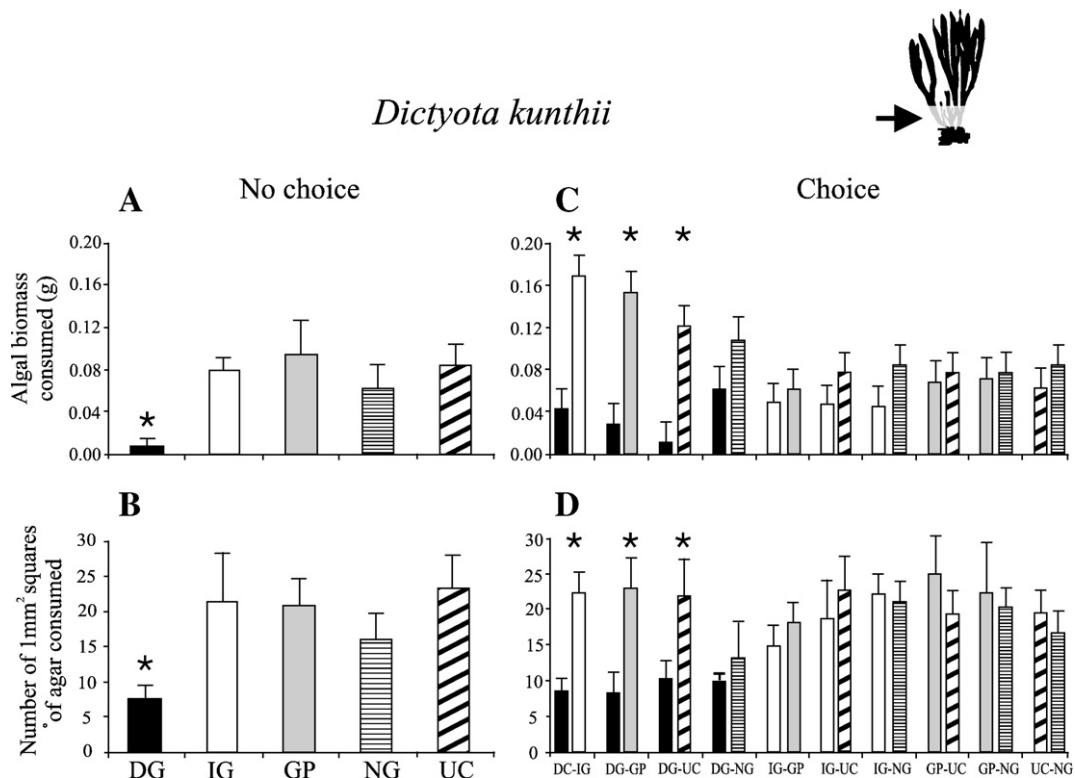


Fig. 4. Mean consumption (total algal biomass consumed in 3 days) of basal parts *Dictyota kunthii* by the amphipod *Parhyalella ruffoi* after the field induction experiment. No-choice feeding assays with (A) live algal pieces, and (B) with agar-based food containing non-polar algal extracts. Paired choice feeding assays with (C) live algal pieces, and (D) with agar-based food containing non-polar algal extracts. Error bars represent +1 S.D. ($n=5$ replicates for each treatment). DG = direct grazing, IG = indirect grazing, GP = grazer presence, UC = ungrazed controls, NG = natural grazing. *Treatments that are significantly different. For further details see Materials and methods.

defenses. In contrast, field experiments with blades of *M. integrifolia* showed no changes of palatability after exposure to different grazing treatments. Interestingly, the results for *D. kunthii* in this field experiment deviate from those of a previous laboratory study, which had shown that defense is induced in this alga when neighbouring individuals are consumed, and even to the mere presence of grazers (Macaya et al., 2005). This suggests that laboratory results might not be entirely representative of the outcome of herbivore–alga interactions in the natural environment.

4.1. Lack of induced defense in *M. integrifolia*

After two weeks, the different grazing levels applied *in situ* had no effect on palatability of blades from the medium part tissue of *M. integrifolia*, i.e. no induction of defenses (of non-polar compounds) was detected, confirming results from previous outdoor laboratory experiments (Macaya et al., 2005). These results are similar to those of a previous field experiment at the same site with identical treatments (in which we could only recover 3 replicates of each treatment due to wave-induced losses, Macaya & Thiel, unpublished data). Increased shading or reduced water flow in the mesh-bag does not seem to affect chemical defenses of the blades, since consumption rates on natural grazing controls were similar to those of the other treatments. Another recent study confirmed that shading has only minor effects on chemical defenses in *M. integrifolia* (C. Pansch, O. Cerda, M. Thiel et al., unpublished data).

Steinberg (1995), who enclosed the kelp *Ecklonia radiata* with different numbers and sizes of the sea urchin *Holopneustes purpureus* in field experiments, also found no elevated levels of defense metabolites after 4 weeks. Similarly, Martínez (1996) found no inducible response in the kelp *Lessonia nigrescens* after mechanical injury of blades from thalli transplanted from intertidal to shallow subtidal hard-bottoms. Toth & Pavia (2002) attributed a lack of inducible response after direct grazing by snails on the large brown alga *Laminaria hyperborea* to the artificial conditions in their outdoor laboratory experiments.

The lack of induction for these large kelp species can be attributed to (1) no anti-herbivore effects of non-polar compounds, or (2) low levels of chemical defenses. Recent experiments suggested that the defensive level of blades of *M. integrifolia* is low (Rothäusler & Thiel, 2006). In laboratory experiments, Macaya et al. (2005) also revealed absence of inducible defense in *M. integrifolia*. They suggested that this alga shows either

(a) constitutional defenses or (b) absence of defenses (tolerance), since *M. integrifolia* is consumed by a wide variety of vertebrate and invertebrate grazers (North, 1994). Furthermore, in laboratory experiments Irelan and Horn (1991) did not find deterrents by polar or non-polar extracts from *M. integrifolia* in feeding experiments with the herbivorous fish *Cebidichthys violaceus*. More studies on the chemical ecology of giant kelp should be conducted in order to determine whether and how these large kelp are defended against mesograzers. Several authors referred to *Macrocystis* as palatable kelp (e.g. Hay and Fenical, 1988), and this (and other large kelps) might possess other mechanisms to avoid the damaging effects of herbivory, for example rapid growth (Duffy and Hay, 1990).

4.2. Induction in *D. kunthii*

This species can respond with inducible defense. Our experiments confirmed that palatability of basal parts is reduced after direct grazing by amphipods under field conditions. Feeding assays with live algae and agar-based food produced similar results suggesting that chemical, rather than structural, mechanisms are involved. Similar results of inducible defenses after direct grazing by mesoherbivores were found for different brown (e.g. Cronin and Hay, 1996a; Sotka et al., 2002; Ceh et al., 2005) and red algae (Diaz et al., 2006).

Macaya et al. (2005) found that indirect grazing and mere presence of grazers also led to reduced palatability in detached individuals of *D. kunthii*, but these results were obtained in laboratory experiments where water flow was very slow compared to the natural environment of *D. kunthii*. Possibly, in field situations the dilution rates of chemical cues are much higher than in laboratory conditions. Water movement in the low intertidal zone is high, and concentrations of chemical cues from neighbouring grazed algae or amphipod grazers might have been too low to induce defense in the experimental algae. Results from another laboratory study with *D. kunthii* also suggested that water flow might affect induction of chemical defenses in this alga (C. Pansch, O. Cerda, M. Thiel et al., unpublished data). The mesh-bags used in our present study might also have affected water flow around the algae. However, the fact that (i) there were no differences between direct grazing DG and natural grazing NG (i.e. without mesh-bag) and (ii) DG was significantly different from all other treatments with mesh-bags suggests that direct grazing does indeed induce chemical defenses in *D. kunthii*. Shading artifacts in the mesh-bags can also be

excluded, because the reduction in light levels produced by one layer of mesh (as in the mesh-bags) does not affect the chemical defenses of this alga (C. Pansch, O. Cerda, M. Thiel et al., unpublished data). We conclude that the effects observed here, namely that direct grazing induces chemical defenses in *D. kunthii*, reflect the reaction of this alga to herbivore attack under natural conditions.

Sotka et al. (2002) suggested that induction by direct grazing is a more important defensive strategy than induction by water-borne cues, because it tends to induce a stronger response (Van Alstyne, 1988; Cronin and Hay, 1996a,b; Toth and Pavia, 2000). It is likely that the presence of herbivores on nearby algae is not a good predictor of imminent attack, in which case there might be no selective advantage in recognizing and responding to water-borne grazing cues (Sotka et al., 2002). Natural grazing did not induce defenses, neither in medium nor in basal parts of *D. kunthii*, probably because these individuals were grazed by large herbivores, indicated by the high amount of algal biomass removed (Macaya E.C. personal observation). In our field experiments, the algae from the natural grazing treatment might represent some transition between induction and non-induction, because palatability of algae exposed to natural grazing (by large grazers) was not significantly different from all other treatments, but DG algae (by mesograzers) were slightly less grazed than NG algae. Hay (1997) emphasized that induction of defense in algae rarely occurs in response to artificial damage or grazing by larger herbivores. It has been suggested that induction of defenses in marine macroalgae depends to a high degree on grazer specificity (Pavia and Toth, 2000).

Our results on induction of defense in *D. kunthii* triggered by direct grazing of mesograzers is in accordance with other studies (e.g. Van Alstyne, 1988; Pavia and Toth, 2000; Taylor et al., 2002; Rohde et al., 2004; Ceh et al., 2005). In temperate areas, mesograzers can significantly impact macroalgal abundance (Brawley, 1992) and species composition (Tegner and Dayton, 1987; Duffy and Hay, 2001), but they do this over days, weeks, or months providing an opportunity for macroalgae to respond by inducing defenses over ecologically appropriate scales of time (Hay, 1997). Additionally, because these herbivores can function on small spatial scales, they might selectively prefer small regions of the algal tissues with low levels of secondary chemicals that are unavailable to large herbivores (Poore, 1994). A differential defense in *D. kunthii* is an optimal strategy to avoid damage from these herbivores. Similar differential induction has been reported for *S. filipendula* (Taylor et al., 2002) and *A. nodosum* (Toth et al., 2005).

5. Conclusion

Under natural conditions, *D. kunthii* responded with inducible defenses against direct grazing by amphipods. In contrast, no inducible defense was found in the giant kelp *M. integrifolia*, suggesting constitutive defenses or another mechanism (e.g. tolerance and high growth rates) to avoid the damaging effects of herbivory. In accordance with most previous studies the grazers responsible for the induction are mesograzers, but in contrast to recent laboratory experiments only direct grazing elicited a response in *D. kunthii*. Based on these results, we suggest that laboratory experiments (with reduced exchange of water in holding tanks) showing induced defenses in response to grazed neighbours or mere grazer presence need to be interpreted with caution. Anti-herbivory responses to water-borne cues from nearby grazed conspecifics or herbivores might be more likely in aquatic systems with calm water conditions (e.g. lakes, lagoons or tidepools) rather than on exposed rocky shores with high-flow regimes.

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References

- Agrawal, A.A., Karban, R., 1999. Why induced defenses may be favored over constitutive strategies in plants. In: Tollrian, R., Harvell, C.D. (Eds.), The ecology and evolution of inducible defenses. University Press, Princeton, pp. 45–61.
- Amsler, C.D., 2001. Induced defenses in macroalgae: the herbivore makes a difference. *J. Phycol.* 37, 353–356.
- Amsler, C.D., Fairhead, V.A., 2006. Defensive and sensory chemical ecology of brown algae. *Adv. Bot. Res.* 43, 1–91.
- Barbosa, J.P., Teixeira, V.L., Pereira, R.C., 2004. A dolabellane diterpene from the brown alga *Dictyota pfaffii* as chemical defense against herbivores. *Bot. Mar.* 47, 147–151.
- Borell, E.M., Foggo, A., Coleman, R.A., 2004. Induced resistance in intertidal macroalgae modifies feeding behaviour of herbivorous snails. *Oecologia* 140, 328–334.

- Brawley, S.H., 1992. Mesoherbivores. In: John, D.M., Hawkins, S.J., Price, J.H. (Eds.), *Plant–animal interaction in the marine benthos*. Oxford University Press, Oxford, pp. 235–263.
- Buschmann, A.H., Vásquez, J., Osorio, P., Reyes, E., Filun, L., Hernandez-Gonzalez, M.C., Vega, A., 2004. The effect of water movement, temperature and salinity on abundance and reproductive patterns of *Macrocystis* spp. (Phaeophyta) at different latitudes in Chile. *Mar. Biol.* 145, 849–862.
- Ceh, J., Molis, M., Dzeha, T.M., Wahl, M., 2005. Induction and reduction of anti-herbivore defenses in brown and red macroalgae off the Kenyan Coast. *J. Phycol.* 41, 726–731.
- Coleman, R.A., Ramchunder, S.J., Moody, A.J., Foggo, A., 2007. An enzyme in snail saliva induces herbivore-resistance in a marine alga. *Funct. Ecol.* 21, 101–106.
- Cronin, G., 2001. Resource allocation in seaweeds and marine invertebrates: chemical defense patterns in relation to defense theories. In: McClintock, J.B., Baker, B.J. (Eds.), *Marine Chemical Ecology*. CRC Press, Boca Raton, pp. 325–353.
- Cronin, G., Hay, M.E., 1996a. Induction of seaweed chemical defenses by amphipod grazing. *Ecology* 77, 2287–2301.
- Cronin, G., Hay, M.E., 1996b. Susceptibility to herbivores depends on recent history of both the plant and animal. *Ecology* 77, 1531–1543.
- Cruz-Rivera, E., Hay, M.E., 2000. Can quantity replace quality? Food choice, compensatory feeding, and fitness of marine mesograzers. *Ecology* 81, 201–219.
- Dayton, P.K., 1985. The structure and regulation of some South American kelp communities. *Ecology* 55, 447–468.
- de Nys, R., Wright, J.T., König, G.M., Sticher, O., 1993. A diterpene from the marine alga *Glossophora kunthii*. *Phytochemistry* 32, 463–465.
- Diaz, E., Guldenzoph, C., Molis, M., McQuaid, C., Wahl, M., 2006. Variability in grazer-mediated defensive responses of green and red macroalgae on the south coast of South Africa. *Mar. Biol.* 149, 1301–1311.
- Duffy, J.E., Hay, M.E., 1990. Seaweed adaptations to herbivory: chemical, structural, and morphological defenses are often adjusted to spatial or temporal patterns of attack. *Bioscience* 40, 368–375.
- Duffy, J.E., Hay, M., 2001. The ecology and evolution of marine consumer–prey interactions. In: Bertness, M.D., Gaines, S.D., Hay, M.E. (Eds.), *Marine Community Ecology*. Sinauer Associates, Sunderland, MA, USA, pp. 131–159.
- Eriksson, A.A., Paul, V.J., Van Alstyne, K.L., Kwiatkowski, L.M., 2006. Palatability of macroalgae that use different types of chemical defenses. *J. Chem. Ecol.* 32, 1883–1895.
- Fairhead, V.A., Amsler, C.D., McClintock, J.B., Baker, B.J., 2005. Within-thallus variation in chemical and physical defences in two species of ecologically dominant brown macroalgae from the Antarctic Peninsula. *J. Exp. Mar. Biol. Ecol.* 322, 1–12.
- Fairhead, V.A., Amsler, C.D., McClintock, J.B., Baker, B.J., 2006. Lack of defense or phlorotannin induction by UV radiation or mesograzers in *Desmarestia anceps* and *D. menziesii* (Phaeophyceae). *J. Phycol.* 42, 1174–1183.
- Foster, M.S., 1992. How important is grazing to seaweed evolution and assemblage structure in the North-East Pacific? In: John, D.M., Hawkins, S.J., Price, J.H. (Eds.), *Plant–Animal Interactions in the Marine Benthos*. Oxford University Press, pp. 61–86.
- Gelcich, S., 1999. Dimension fractal de macroalgas submareales: su relación con la fauna epibionte. *Marine Biology Thesis*, Universidad Católica del Norte, 75 pp.
- Hammerstrom, K., Dethier, M.N., Duggins, D.O., 1998. Rapid phlorotannin induction and relaxation in five Washington kelps. *Mar. Ecol. Prog. Ser.* 165, 293–305.
- Hay, M.E., Fenical, W., 1988. Marine plant–herbivore interactions: the ecology of chemical defense. *Ann. Rev. Ecol. Syst.* 19, 111–145.
- Hay, M.E., Kappel, Q.E., Fenical, W., 1994. Synergisms in plant defenses against herbivores: interactions of chemistry, calcification, and plant-quality. *Ecology* 75, 1714–1726.
- Hay, M.E., Duffy, J.E., Pfister, C.A., Fenical, W., 1987. Chemical defenses against different marine herbivores: are the amphipods insect equivalents? *Ecology* 68, 1567–1580.
- Hoffmann, A.J., 1988. Daylength and light responses in growth and fertility of *Glossophora kunthii* (Phaeophyta, Dictyotales) from Pacific South-America. *J. Phycol.* 24, 203–208.
- Ireland, C.D., Horn, M.H., 1991. Effects of macrophyte secondary chemicals on food choice and digestive efficiency of *Cebidichthys violaceus* (Girard), an herbivorous fish of temperate marine waters. *J. Exp. Mar. Biol. Ecol.* 153, 179–194.
- Karban, R., Maron, J., 2002. The fitness consequences of interspecific eavesdropping between plants. *Ecology* 83, 1209–1213.
- Karban, R., Baldwin, I.T., Baxter, K.J., Laue, G., Felton, G.W., 2000. Communication between plants: induced resistance in wild tobacco plants following clipping of neighboring sagebrush. *Oecologia* 125, 66–71.
- Karban, R., Huntzinger, M., McCall, A.C., 2004. The specificity of eavesdropping on sagebrush by other plants. *Ecology* 85, 1846–1852.
- Lazo-Wasem, E., Gable, M.F., 2001. A revision of *Parhyalella* Kunkel (Crustacea: Amphipoda: Gammaridea). *Bulletin of the Peabody Museum of Natural History, Yale University*, vol. 46, pp. 1–80.
- Lüder, U.H., Clayton, M.N., 2004. Induction of phlorotannins in the brown macroalga *Ecklonia radiata* (Laminariales, Phaeophyta) in response to simulated herbivory—the first microscopic study. *Planta* 218, 928–937.
- Macaya, E., Rothäusler, E., Thiel, M., Molis, M., Wahl, M., 2005. Induction of defences and within-alga variation on palatability in two brown algae from the northern-central coast of Chile: effects of mesograzers and UV radiation. *J. Exp. Mar. Biol. Ecol.* 325, 214–227.
- Malbran, M.E., Hoffmann, A.J., 1990. Seasonal cycles of growth and tetraspore formation in *Glossophora kunthii* (Phaeophyta, Dictyotales) from Pacific South-America. *Field and laboratory studies*. *Bot. Mar.* 33, 219–223.
- Martinez, E.A., 1996. Micropopulation differentiation in phenol content and susceptibility to herbivory in the Chilean kelp *Lessonia nigrescens* (Phaeophyta, Laminariales). *Hydrobiologia* 327, 205–211.
- Molis, M., Korner, J., Ko, Y.W., Kim, J.H., Wahl, M., 2006. Inducible responses in the brown seaweed *Ecklonia cava*: the role of grazer identity and season. *J. Ecol.* 94, 243–249.
- North, W.J., 1994. *Macrocystis*. In: Akatsuka (Ed.), *Biology of Economic Algae*. SPB, Academic Publishing, The Hague, Netherlands, pp. 407–446.
- Palma, A., Ojeda, P., 2002. Abundance, distribution and feeding patterns of a temperate reef fish in subtidal environments of the Chilean coast: the importance of understory algal turf. *Rev. Chil. Hist. Nat.* 75, 189–200.
- Pavia, H., Toth, G.B., 2000. Inducible chemical resistance to herbivory in the brown seaweed *Ascophyllum nodosum*. *Ecology* 81, 3212–3225.
- Peckol, P., Krane, J.M., Yates, J.L., 1996. Interactive effects of inducible defense and resource availability on phlorotannins in the North Atlantic brown alga *Fucus vesiculosus*. *Mar. Ecol. Prog. Ser.* 138, 209–217.
- Pereira, R.C., Cavalcanti, D.N., Teixeira, V.L., 2000. Effects of secondary metabolites from the tropical Brazilian brown alga

- Dictyota menstrualis* on the amphipod *Parhyale hawaiiensis*. Mar. Ecol. Prog. Ser. 205, 95–100.
- Poore, A.G.B., 1994. Selective herbivory by amphipods inhabiting the brown alga *Zonaria angustata*. Mar. Ecol. Prog. Ser. 107, 113–123.
- Renaud, P.E., Hay, M.E., Schmitt, T.M., 1990. Interactions of plant stress and herbivory—intraspecific variation in the susceptibility of a palatable versus an unpalatable seaweed to sea-urchin grazing. Oecologia 82, 217–226.
- Rohde, S., Molis, M., Wahl, M., 2004. Regulation of anti-herbivore defence by *Fucus vesiculosus* in response to various cues. J. Ecol. 92, 1011–1018.
- Rothäusler, E., Thiel, M., 2006. Effect of detachment on the palatability of two kelp species. J. Appl. Phycol. 18, 423–435.
- Rothäusler, E., Macaya, E.C., Molis, M., Wahl, M., Thiel, M., 2005. Laboratory experiments examining inducible defense show variable responses of temperate brown and red macroalgae. Rev. Chil. Hist. Nat. 78, 603–614.
- Sotka, E.E., Taylor, R.B., Hay, M.E., 2002. Tissue-specific induction of resistance to herbivores in a brown seaweed: the importance of direct grazing versus waterborne signals from grazed neighbors. J. Exp. Mar. Biol. Ecol. 277, 1–12.
- Steinberg, P.D., 1984. Algal chemical defense against herbivores: allocation of phenolic compounds in the kelp *Alaria marginata*. Science 223, 405–406.
- Steinberg, P.D., 1985. Feeding preferences of *Tegula funebris* and chemical defenses of marine brown algae. Ecol. Monogr. 53, 333–349.
- Stewart, H.L., 2004. Hydrodynamic consequences of maintaining an upright posture by different magnitudes of stiffness and buoyancy in the tropical alga *Turbinaria ornata*. J. Mar. Syst. 49, 157–167.
- Taylor, R.B., Sotka, E., Hay, M.E., 2002. Tissue-specific induction of herbivore resistance: seaweed response to amphipod grazing. Oecologia 132, 68–76.
- Tegner, M.J., Dayton, P.K., 1987. El Niño effects on southern California kelp forest communities. Adv. Ecol. Res. 17, 243–279.
- Toth, G.B., 2007. Screening for induced herbivore resistance in Swedish intertidal seaweeds. Mar. Biol. 151, 1597–1604.
- Toth, G.B., Pavia, H., 2000. Water-borne cues induce chemical defense in a marine alga (*Ascophyllum nodosum*). Proc. Natl. Acad. Sci. U. S. A. 97, 14418–14420.
- Toth, G.B., Pavia, H., 2002. Lack of phlorotannin induction in the kelp *Laminaria hyperborea* in response to grazing by two gastropod herbivores. Mar. Biol. 140, 403–409.
- Toth, G.B., Langhamer, O., Pavia, H., 2005. Inducible and constitutive defenses of valuable seaweed tissues: consequences for herbivore fitness. Ecology 86, 612–618.
- Van Alstyne, K.L., 1988. Herbivore grazing increases polyphenolic defenses in the brown alga *Fucus distichus*. Mar. Ecol. Prog. Ser. 69, 655–663.
- Weidner, K., Lages, B.G., da Gama, B.A.P., Molis, M., Wahl, M., Pereira, R.C., 2004. Effect of mesograzers and nutrient levels on induction of defenses in several Brazilian macroalgae. Mar. Ecol. Prog. Ser. 283, 113–125.
- Winter, F.C., Estes, J.A., 1992. Experimental evidence for the effects of polyphenolic compounds from *Dictyoneurum californicum* (Phaeophyta; Laminariales) on feeding rate and growth in the red abalone (*Haliotis rufescens*). J. Exp. Mar. Biol. Ecol. 155, 263–277.
- Yun, H., Cruz, J., Treitschke, M., Wahl, M., Molis, M., 2007. Testing for the induction of anti-herbivory defences in four Portuguese macroalgae by direct and water-borne cues of grazing amphipods. Helgol. Mar. Res. 61, 203–209.