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Induction of defenses and within-alga variation of palatability in two brown algae from the northern-central coast of Chile: Effects of mesograzers and UV radiation

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Abstract

Macroalgae possess different defense mechanisms in response to herbivory. Some species produce anti-herbivore secondary metabolites, but production of these substances can be costly. Therefore, algae may produce defensive metabolites only in response to herbivory (inducible defense) or defend particular parts of the alga differentially (within-alga variation). In the present study, we examined whether two species of brown algae from the SE-Pacific show evidence of inducible chemical defense (non-polar compounds) or within-alga variation of defense, which we estimated in form of palatability of differently treated algae to amphipod grazers (with live algae and agar-based food containing non-polar algal extracts). In *Glossophora kunthii* (C. Agardh) J. Agardh, we observed an increase in palatability after algae were acclimated for 12 days without grazers. Subsequent addition of grazers for 12 days then resulted in a reduction of palatability indicating the existence of inducible defense. After removal of grazers for 12 days, these induced effects again disappeared. The reaction of *G. kunthii* was triggered even by the mere presence of grazers, which suggests that this alga can respond to waterborne cues by reducing palatability. Effects were only found for agar-based food containing non-polar extracts, but not for live algae, suggesting that some parts of the algae are undefended. Our second experiment on within-alga variation confirmed that only apical (growth region) and basal parts (near the holdfast region) of *G. kunthii* are defended against herbivores. For the second species, *Macrocystis integrifolia* Bory, the first experiment revealed no induction of defense, while the second experiment on within-alga variation showed that amphipods avoided basal parts and in particular stipes of *M. integrifolia* but only in live algae. Although both studied algal species differed substantially in their defensive strategies, their reaction was independent of the presence or absence of UV

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radiation. Thus, it appears that UV effects play only a minor role in anti-herbivore defense, which is in accordance with most previous studies.

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1. Introduction

Herbivory influences marine benthic community structure (Lubchenco and Gaines, 1981) and can be intense in both tropical (Carpenter, 1986; Hay, 1997) and temperate habitats (Breen and Mann, 1976; Scheibling et al., 1999; Gagnon et al., 2004). In response to herbivory, macroalgae have evolved a variety of mechanisms including escape in time or space (Lubchenco and Gaines, 1981), tolerating (Hay and Fenical, 1988) or deterring herbivores (e.g., Hay and Fenical, 1988). Several modes are known by which algae deter consumers (Cronin, 2001), of which morphological and chemical traits in anti-herbivore defenses are the most commonly employed mechanisms (Hay, 1996; Cronin, 2001).

Although difficult to assess, the production of defenses might be costly (Hay and Fenical, 1988; Cronin, 2001) if defenses use resources that could have been allocated to growth or reproduction (Cronin and Hay, 1996a; Hay, 1997; Duffy and Hay, 2001). In this sense, constitutive, i.e., permanent, defenses require expenditure of resources even when consumers are absent and the benefits of protection are not realized (Cronin and Hay, 1996a). In contrast, inducible defenses allow costs of defenses to be deferred until enemies have been detected, at which time the costs can be offset by the benefits of protection (Harvell, 1990; Cronin and Hay, 1996a).

Besides temporal variation in defense levels, defensive traits are assumed to vary within plants (Zangerl and Rutledge, 1996). According to optimal defense theory, valuable tissues (e.g., growth meristems) at high risk of grazer attacks should be protected by strong constitutive defenses, while tissues that are less valuable or less likely to be attacked should have lower levels of constitutive defenses and/or be capable of inducing defenses following attack. First evidence of within-plant variation in concentrations of secondary metabolites came from terrestrial plants (McKey,

1979). Differential distribution of defensive compounds has also been reported for some kelp species (Steinberg, 1984; Tugwell and Branch, 1989), siphonous green seaweeds (Hay et al., 1988; Paul and Van Alstyne, 1988), antarctic species of *Desmarestia* (Fairhead et al., 2005) and rockweeds (Tuomi et al., 1989; Pavia et al., 2002; Toth et al., 2005). Cronin and Hay (1996b) reported differences in palatability between different parts of the brown algae *Dictyota ciliolata*. Within-alga variation should be expected to be most pronounced in species with different degrees of differentiation and translocation of nutrients. Large kelps such as, for example, *Macrocystis* spp. have internal transport systems (Raven, 2003) and high degree of tissue differentiation with structural tissues such as the holdfast and stipes, on one hand and, on the other hand, blade regions that are mainly active in nutrient acquisition and photosynthesis (North, 1994). While blades of these large kelps are readily consumed by grazers and are considered to be largely undefended (Steinberg, 1985; Winter and Estes, 1992), there exists evidence that structural tissues contain higher concentrations of chemical compounds than other tissues (Tugwell and Branch, 1989; Van Alstyne et al., 1999). Fairhead et al. (2005) also revealed recently that the primary stem of the brown algae *Desmarestia anceps* resisted amphipod grazing by toughness and deterrent chemistry.

At present, inducible chemical defenses have been documented for over 100 terrestrial plant species (Karban and Baldwin, 1997), but there are only few reports on herbivore-induced defenses in seaweeds (e.g., Van Alstyne, 1988; Cronin and Hay, 1996a; Pavia and Toth, 2000; Sotka et al., 2002; Rohde et al., 2004; Weidner et al., 2004). In a suite of studies, algae were artificially wounded ('clipping') instead of using natural herbivores (e.g., Pavia et al., 1997). Lack of herbivore-specific cues, e.g., grazer-associated microorganisms, are seen as one explanation for missing effects in assessing the induction of defenses in most

clipping experiments (Hay, 1996 and references therein). Experiments exposing natural grazers to algae revealed exclusively inducible defenses for brown seaweeds. Furthermore, waterborne cues released by directly grazed *Ascophyllum nodosum* individuals induced chemical defenses in ungrazed adjacent conspecifics (Toth and Pavia, 2000). However, it remains unclear whether waterborne chemicals originated from the grazer, the algae, or both.

Algal secondary metabolites also have other functions besides deterrence of herbivores. These compounds can inhibit settlement or development of fouling organisms (Schmitt et al., 1995, 1998), microbial films (Hay, 1996) and furthermore provide protection against damaging effects of UV radiation (Pavia et al., 1997; Stachowicz and Lindquist, 1997; Swanson and Druehl, 2002). Pavia et al. (1997) found that the brown seaweed *Ascophyllum nodosum* increased the concentration of phlorotannins under enhanced UV-B levels. On the other hand, Cronin and Hay (1996c) suggested that in *Dictyota ciliolata*, UV-stress may reduce chemical defenses and increase algal susceptibility to herbivores. Thus, UV radiation may also affect alga–herbivore interactions by influencing concentrations of secondary metabolites, and given globally changing UV-regimes, it appears important to include this factor in studies testing inducible defenses of shallow-water algae.

Finally, most studies on inducible defenses focused only on factors that initiate anti-herbivore responses. However, if induction of anti-herbivore defenses is a mechanism to reduce costs, then defenses should also decrease when grazing pressure diminishes. Nevertheless, to date, experimental evidence on testing the decrease of anti-herbivore defense in absence (or at low levels) of grazing is not available (for exceptions, see Hemmi et al., 2004; Rohde et al., 2004; Weidner et al., 2004; Ceh et al., in press).

The present study assessed (i) the effect of grazing on inducible defenses and (ii) the palatability of different algal parts of two common brown macroalgae, *Glossophora kunthii* and *Macrocystis integrifolia*, from the northern-central coast of Chile. The specific aims of this study were to test (1) whether grazing by amphipods can induce defenses in both species of macroalgae, (2) whether waterborne cues from adjacent grazed conspecifics or from non-feeding amphipods can induce defenses, (3) whether defense is

reduced when grazing ceased, (4) whether UV radiation alters macroalgal responses in this context and (5) if there exists within-alga variation in palatability.

2. Materials and methods

2.1. Sampling sites of algae and grazers

We collected both algae in the vicinity of Coquimbo (Chile), *Glossophora kunthii* from the intertidal zone of Totalillo (30°03'S; 70°38'W) and *Macrocystis integrifolia* from subtidal habitats of Islas Damas (29°13'S; 71°31'W). For all experiments, the amphipod *Parhyalella ruffoi* (Lazo-Wasem and Gable) was used as grazer after confirming in preliminary studies that this amphipod consumed a wide variety of macroalgae, including *G. kunthii* and *M. integrifolia* (0.012 ± 0.005 g ind⁻¹ day⁻¹ and 0.041 ± 0.020 g ind⁻¹ day⁻¹, respectively). All amphipods were only used once, either in grazing treatments or in feeding assays.

2.2. Experiment I: effects of grazing, grazer presence and UVR on palatability

2.2.1. Experimental design and set-up

We designed two three-phased, two-factorial experiments to test the effects of amphipod grazing (4 levels, fixed), UV radiation (2 levels, fixed) and block (4 levels, random) on palatability levels of the brown algae *Glossophora kunthii* (entire individuals) and *Macrocystis integrifolia* (apical tissues), respectively. Using a randomized block design, each treatment combination of the fully crossed fixed factors was replicated 5 times, where each combination was represented once within each of the four blocks, and the fifth replicate was assigned randomly to one of these four blocks.

Induction experiments were set-up in an outdoor flow-through aquaria system. Filtered seawater (10 µm cotton cartridge) was pumped from the shallow subtidal of Bahía La Herradura (29°58' S; 71°21' W) into four plastic reservoirs (70 l), supplying each EU (=transparent plastic aquarium, 10 × 19 × 13 cm, 1.5-l volume) via flow-regulated hoses individually at a rate of 0.1 l h⁻¹ with seawater. The EUs were distributed among 4 tables (=blocks) that were covered

with plastic shading cloth to protect algae from direct sunlight. During the late afternoon, the average (\pm SD) water temperatures in the aquaria increased to 17.57 °C (\pm 0.48), slightly above ambient water temperature in Bahía La Herradura (16.72 °C \pm 0.67), but well within the normal growth range of *G. kunthii* (Hoffmann and Malbran, 1989) and *Macrocystis* (North, 1994).

To restrict movements of grazers (*P. ruffoi*), each EU was divided midway into an up- and a downstream compartment by a rigid green plastic mesh (1 mm mesh size). Prior to the experiments, all algae were carefully cleaned from grazers and epiphytes, dried with absorbent paper, and weighed. Between January and March 2003, we ran the first induction experiment using the tips (top 25 cm) of *M. integrifolia*, whereas entire individuals of *G. kunthii* were used in the second induction experiment, conducted between March and April 2003. Both experiments were separated into an acclimation, a treatment, and a recovery phase. Each phase lasted 12 or 14 days for *G. kunthii* and *M. integrifolia*, respectively. The objective of the acclimation phase was to reduce the probability of past consumption history to decrease variation among replicates. At the beginning of the acclimation phase, we stored five algal pieces at -40 °C to conserve their natural defense levels. Moreover, 4 algal pieces (=target algae, used later in feeding assays) were placed in the downstream compartment of each EU, to pass through the acclimation phase. This was also done with 5 additional algal pieces, placed separately in five aquaria, which were then used to assess the acclimated state of defense at the end of the acclimation phase after transferring them for 48 h to -40 °C. Subsequently, the palatability of agar-based food containing extracts of acclimated and non-acclimated algae was compared in feeding assays (see subsection Feeding assays). Both acclimated and non-acclimated algae were frozen prior to their use in feeding assays to avoid confounding effects of freezing non-acclimated algae alone. At the beginning of the following treatment phase, algae were exposed to different combinations of UV radiation and grazing treatments to allow them to produce anti-herbivore defenses. Grazing treatments tested the effects of (1) direct grazer exposure by adding 15 amphipods to the target algae in the downstream compartment, (2) waterborne cues from nearby grazed conspecifics by

adding 15 amphipods to the upstream compartment together with one non-target piece of the same species as the target algae, (3) waterborne cues from non-grazing amphipods by introducing 15 amphipods without algae in the upstream compartment, and (4) controls where neither amphipods nor non-target pieces of algae were introduced. At the end of the treatment phase, all amphipods and non-target algal pieces were removed from the aquaria to give algal pieces during the recovery phase a chance to lower any anti-herbivore defenses previously induced in the treatment phase. Using cut-off filters, we tested for UV radiation (UVR) effects by manipulating irradiance regimes during the treatment and the recovery phase. To block radiation <400 nm completely (PAR), half of all EUs were covered with 4 mm thick sheets of Makrolon (long life plus 293, Röhm, Germany), while the other half of the EUs were covered with 3 mm thick Perspex sheets (GS 2648 Röhm, Germany) allowing complete transmission of ambient solar irradiance (PAR+UV) (see Molis and Wahl, 2004 for details on optical properties of cut-off filters). Procedural controls from unfiltered EUs were compared with algae from the Perspex-covered EUs. In most comparisons, amphipod consumption rates were not significantly different between Perspex-filtered and unfiltered individuals of both brown algae, meaning that no filter artifacts were found herein, with the single exception of *Macrocystis integrifolia* in the treatment phase (paired *t*-test: $t=3.22$, $p=0.032$).

2.3. Experiment II: within-alga variation in palatability

2.3.1. Experimental design and set-up

Ten individuals of *G. kunthii* and eight of *M. integrifolia* were collected in October 2003 in order to examine whether amphipod grazers preferentially consume different parts of the algae. Apical, medium, and basal portions of *G. kunthii*, and apical, medium, basal blades, and stipe portions of *M. integrifolia* were offered to the amphipod *P. ruffoi*. Individuals of *G. kunthii* were dissected into the apical parts (the top 2–3 cm) with the large apical cell, the medium parts with a dense cover of ligulae (causing an irregular surface texture—Hoffman and Santelices, 1997), and the basal parts immediately above the small holdfasts. For the morphologically more differentiated alga *M.*

integrifolia, we dissected blades from the apical region (the top 3–5 cm), blades from the medium part (middle of the alga), basal blades without sporangial tissues (the blades that are located at the base of the alga) and the stipes directly above the holdfasts. Similar amounts of pieces from the respective algal regions were used in choice and no-choice feeding assays (see next subsection).

2.4. Feeding assays

The aim of feeding assays was to examine whether anti-herbivore defenses were induced and reduced in the induction experiments by assessing and comparing palatability levels separately for live algae and agar-based food containing non-polar algal extracts after each phase of the induction experiment. Significantly higher consumption rates of the amphipod *P. ruffoi* on live control algae compared to treated algae would indicate an increase in morphological and/or chemical defenses, while the same result for agar-based food confines anti-herbivore defenses to non-polar algal chemistry.

All feeding assays lasted 3 days and were conducted in a culture room under constant temperature ($15\text{ }^{\circ}\text{C} \pm 1^{\circ}$), using a 12-h photoperiod of $40 \pm 10\ \mu\text{mol m}^{-2}\ \text{s}^{-1}$ (fluorescent lamp, 40 W, Phillips, Brazil). Feeding assays with live algae were performed in transparent (volume: 1 l) plastic containers, while those with agar-based food containing non-polar algal extracts used Petri dishes (volume: 30 ml). In both types of assays, water was exchanged twice daily. Prior to feeding assays, amphipods were offered a diverse algal diet, because starvation may alter the feeding behavior of some herbivores (Cronin and Hay, 1996c).

After the acclimation phase, one piece of agar-based food containing non-polar extracts of acclimated and one piece of agar-based food from non-acclimated algae were simultaneously incubated with 6 amphipods in choice feeding assays. Miniscule incisions were cut into food items to distinguish treatments. At the end of both, the treatment and the recovery phase, two pieces of algae were removed from each aquarium and used in no-choice feeding assays of either live algae (exposure to 30 amphipods) or agar-based food containing non-polar algal extracts (exposure to 4 amphipods). No-choice assays were

used in the first experiment, because it is considered unlikely that amphipods in their natural habitat chose among several algal individuals before starting to feed. This will in particular be true for *P. ruffoi*, which lives in association with drift algae (personal observation). However, *P. ruffoi* probably chose among different tissue types within one algal individual before a meal, which is why in the second experiment, we conducted choice assays in addition to no-choice assays when assessing within-alga variation of palatability. Choice and no-choice feeding assays were conducted in Petri dishes (30 ml) using 6 and 4 amphipods per assay, respectively.

As response variable for assessment of palatability, we used for live algae wet mass of consumed algal tissue [$\text{g}\ 3\ \text{day}^{-1}$] and the number of consumed squares for agar-based food (see next subsection for details). Wet mass of each alga was determined to the nearest milligrams, using an analytical balance (Denver Instrument 100 A $\pm 0.2\ \text{mg}$), after blotting the alga for 30 s with absorbent paper to remove excess water. In order to account for non-grazing related changes in live algae, we calculated real consumption according to Cronin and Hay (1996a) as $C_{\text{real}} = T_i (C_i/C_f) - T_f$ where T_i and T_f correspond to initial and final wet mass of the alga used in the feeding assay, respectively, and C_i and C_f to initial and final mass of a control algae not used in the feeding assay, respectively.

2.5. Production of agar-based food containing non-polar extracts

Single algal pieces, stemming from the induction experiments, were placed for 48 h in dichloromethane (DCM) after blotting the alga dry. We used 2 ml DCM per 1 g algal wet mass. DCM extracts the non-polar algal chemistry, which had been shown for several algae species to possess effective anti-herbivore compounds (Steinberg and Van Altena, 1992; Paul et al., 2001; Steinberg et al., 2001). Herein, we focused on the non-polar compounds because *Macrocyctis* is known to be poor in phenolic compounds (Steinberg, 1985), and the strong emphasis in linking some polar compounds (e.g., phlorotannin) with chemical defense in algae seems unjustified by present experimental evidence (Jormalainen et al., 2001, 2003, 2005; Deal et al., 2003; Hemmi et al., 2004; Kubanek et al., 2004). Recently, Taylor et al. (2002) revealed that

non-polar extracts of stipes in the brown alga *Sargassum filipendula* had a deterrent effect on the amphipod *Amphitoe longimana*, and similar results were found by Fairhead et al. (2005) in *Desmarestia anceps*. In experiments similar to ours, Ceh et al. (in press) recently demonstrated induction of chemical defense with non-polar compounds in two species of brown algae. For *G. kunthii*, several non-polar compounds such as pachydictyol A and dictyodal had been described previously (see Rivera et al., 1987; de Nys et al., 1993), which are known to be deterrents to amphipods, sea urchins and fishes (Hay et al., 1987; Pereira et al., 1994; Cronin and Hay, 1996a; Cronin et al., 1997; Barbosa et al., 2004).

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 of *U. lactuca* (3:1) and mixed extracts with an amount of dry *U. lactuca* powder representing one-third of algal wet mass from which extracts were made. Following the methodology of (Hay et al., 1994), blended *U. lactuca* powder was incorporated into agar, poured into a mould lying over a fly mesh (mesh size 1 mm²), and allowed to harden prior to excision of a 200 mm² quadratic section, which was offered as agar-based food containing non-polar algal extracts to amphipods in feeding assays. Consumption rates of these food items were determined by counting emptied 1 mm² fly mesh squares with a stereomicroscope.

2.6. Statistical analysis

Choice feeding assays after the acclimation phase (induction experiment) and from within-algal palatability experiments were evaluated with paired *t*-tests. Consumption rates from no-choice feeding assays from within-alga palatability experiments were analyzed with a 1-way ANOVA. Consumption rates from no-choice feeding assays after the treatment and recovery phases (induction experiment) were analyzed by a mixed model 3-way ANOVA. Since no block effects (main and interactions) were found at $\alpha=0.25$, data from all blocks were pooled and reanalyzed by a 2-way ANOVA. Tukey HSD was used for post-hoc comparisons of significant ANOVA results. Homogeneity of variances was confirmed with Cochran's test for all data.

3. Results

3.1. Experiment I: effects of grazing, grazer presence and UVR on palatability

No significant block effects were detected in *Glossophora kunthii* after the treatment phase with live algae and agar-based food (ANOVA: $F=3.19$, $p=0.084$ and $F=0.93$, $p=0.471$, respectively) and after the recovery phase (ANOVA: $F=0.51$, $p=0.683$ and $F=1.37$, $p=0.320$, respectively). Similar results were found for *M. integrifolia* after the treatment phase with live algae and agar-based food (ANOVA: $F=0.96$, $p=0.458$ and $F=0.10$, $p=0.958$, respectively) and after the recovery phase (ANOVA: $F=3.06$, $p=0.090$ and $F=0.76$, $p=0.545$, respectively).

3.1.1. *Glossophora kunthii*

Consumption rates of *P. ruffoi* on agar-based food containing non-polar extracts of acclimated algae were higher than on extracts of non-acclimated algae (paired *t*-test: $t=4.80$, $p=0.001$, Fig. 1I). There was no significant UVR \times grazing interaction for live algae at the end of the treatment and the recovery phase (Table 1), indicating that effects of grazing treatments were independent from UVR-regimes throughout the experiment. There were no significant UVR-effects on consumption rates neither after the treatment phase nor after the recovery phase (Table 1). No grazing effects were found in feeding assays with live algae, neither after the treatment phase nor after the recovery phase (Table 1). However, using agar-based food, strong grazing effects were detected at the end of the treatment phase. Amphipod consumption rates on agar-based food containing non-polar extracts of control algae were significantly higher than on extracts from *G. kunthii* individuals exposed to direct grazing, nearby grazed conspecifics, and non-grazing consumers (Table 1, Fig. 1II(B)). At the end of the recovery phase, neither live algae nor agar-based food was significantly different among grazing treatments (Table 1, Fig. 1III(A) and III(B)). There was no significant interaction between UVR and grazing treatment effects (Table 1).

3.1.2. *Macrocystis integrifolia*

After the acclimation phase, amphipods *P. ruffoi* showed no significant feeding preferences for either

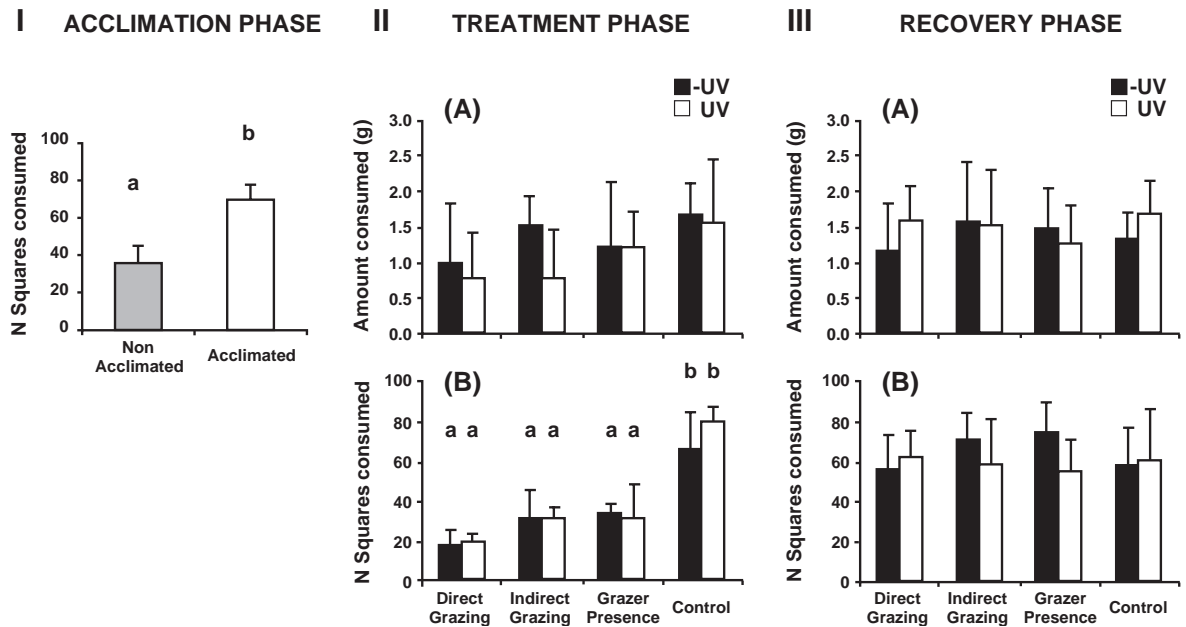


Fig. 1. Mean consumption of *Glossophora kunthii* by the amphipod *Parhyalella ruffoi* after the three experimental phases I–III. (A) Feeding assays with intact algal pieces, (B) feeding assays with agar-based food containing non-polar algal extracts; different letters above bars indicate significant differences in consumption, absence of letter above the bars indicates no significant differences. Error bars represent +1 S.D. ($n=5$ replicates for each treatment).

acclimated or non-acclimated food items (paired t -test: $t=-0.80$, $p>0.05$, Fig. 2I). Regardless of the UVR-regime, grazing treatments were not significantly different at the end of both, the treatment and the recovery phase in feeding assays using live

M. integrifolia as well as agar-based food containing the non-polar extracts of this species (Table 1, Fig. 2II and III). UVR-effects as well as UVR \times grazing interactions of amphipod consumption rates on *M. integrifolia* were missing, both after the treatment

Table 1

Results of 2-way ANOVAs, testing for the combined and interactive effects of UVR and grazing, on amphipod consumption rates, obtained in 3-day long no-choice feeding assays at the end of the treatment and the recovery phase of the induction experiment

	<i>Glossophora kunthii</i>					<i>Macrocystis integrifolia</i>			
	Live			Extracts		Live		Extracts	
	<i>df</i>	<i>F</i>	<i>p</i>	<i>F</i>	<i>p</i>	<i>F</i>	<i>p</i>	<i>F</i>	<i>p</i>
Treatment phase									
Grazing (G)	3	1.87	0.155	42.68	<0.001	1.49	0.236	2.09	0.121
UV	1	1.38	0.250	0.70	0.497	0.66	0.425	0.1	0.757
G*UV	3	0.57	0.634	0.81	0.408	0.14	0.936	1.95	0.141
Residual	32								
Recovery phase									
Grazing (G)	3	0.44	0.728	0.48	0.699	0.21	0.89	0.83	0.487
UV	1	4.58	0.050	1.19	0.285	0.66	0.423	1.12	0.299
G*UV	3	0.78	0.513	1.12	0.357	1.01	0.403	1.18	0.332
Residual	32								

Analyses of pooled data after confirming lack of block effects at $\alpha=0.25$ with 3-way mixed model ANOVA (results not presented). Extracts—agar-based food containing non-polar algal extracts (6 amphipods per assay), live—intact algal pieces (30 amphipods per assay).

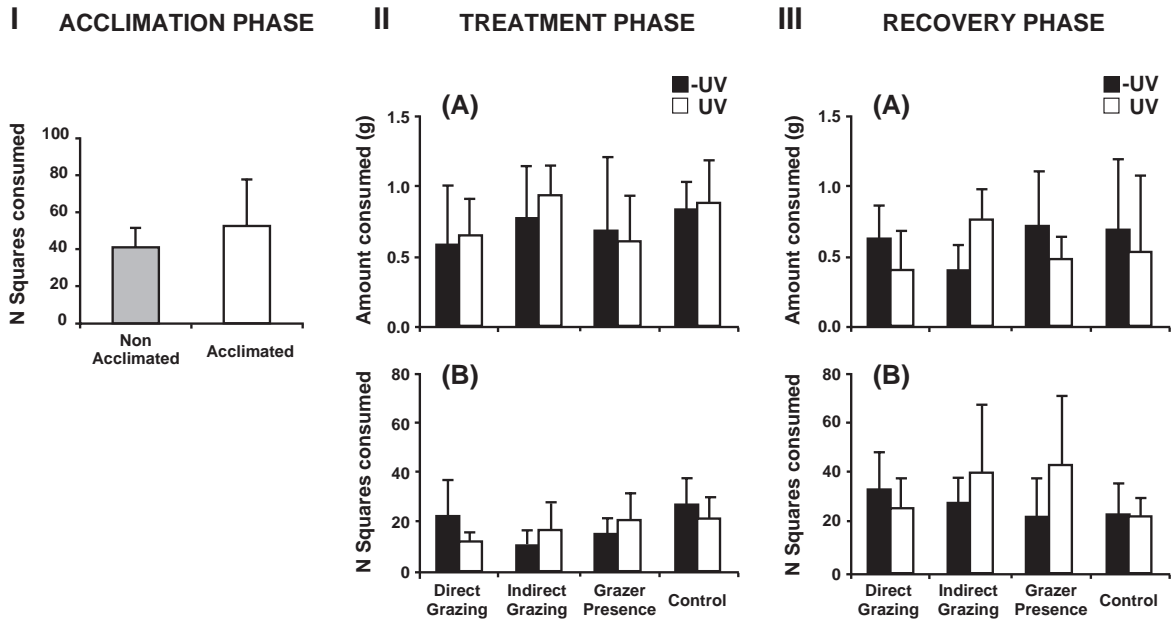


Fig. 2. Mean consumption of *Macrocyctis integrifolia* by the amphipod *Parhyalella ruffoi* after the three experimental phases I–III. (A) Feeding assays with intact algal pieces, (B) feeding assays with agar-based food containing non-polar algal extracts; different letters above bars indicate significant differences in consumption, absence of letter above the bars indicates no significant differences. Error bars represent +1 S.D. ($n=5$ replicates for each treatment).

and the recovery phase (Table 1, Fig. 2II(B) and Fig. 2III(B)).

3.2. Within-alga variation in palatability of *G. kunthii* and *M. integrifolia*

Feeding assays with different parts of *G. kunthii* indicated a clear preference of amphipods for medium parts of the algae. Using live algae, amphipods consumed medium parts 8.4 and 27.3 times more than

basal and apical parts, respectively, while no significant difference was detected between consumption rates of basal and apical parts of live *G. kunthii* (Table 2, Fig. 3(A)). The same pattern with smaller effects was observed in feeding assays using agar-based food containing non-polar algal extracts. Medium parts were preferred over apical and basal parts, respectively (Table 2, Fig. 3(B)). This tendency was similar in no-choice assays, where amphipods consumed significantly more of the medium parts, both

Table 2

Results of paired *t*-tests, comparing amphipod consumption rates from choice feeding assays between three (*Glossophora kunthii*) and four (*Macrocyctis integrifolia*) different tissue types

	<i>Glossophora kunthii</i>				<i>Macrocyctis integrifolia</i>			
	Live		Extracts		Live		Extracts	
	t_9	<i>p</i>	t_9	<i>p</i>	t_7	<i>p</i>	t_7	<i>p</i>
Apical vs. medium	-5.72	<0.001	-7.71	<0.001	3.37	<0.001	2.13	0.059
Apical vs. stipe					11.62	<0.001	0.99	0.339
Apical vs. basal	-2.06	0.069	1.64	0.136	2.44	0.054	2.03	0.061
Medium vs. stipe					14.93	<0.001	2.29	0.056
Medium vs. basal	-11.19	<0.001	-9.13	<0.001	2.36	0.051	0.73	0.423
Basal vs. stipe					-6.89	<0.001	0.64	0.540

Extracts—agar-based food containing non-polar algal extracts, live—intact algal pieces, subscript—degrees of freedom.

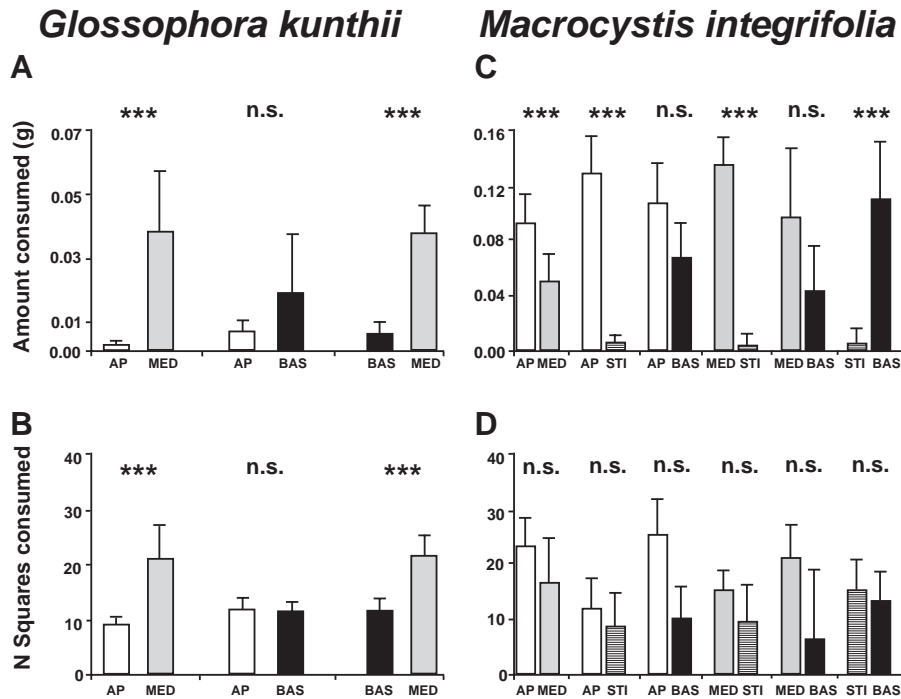


Fig. 3. Mean consumption of different parts of *Glossophora kunthii* and *Macrocyctis integrifolia* by the amphipod *Parhyalella ruffoi*. (A–C) Feeding assay with intact algal pieces, (B–D) feeding assay with agar-based food containing non-polar algal extracts. *** $P < 0.001$ (two-tailed paired t -tests). n.s.—No significant differences, AP—apical; MED—medium; STI—stipe; BAS—basal. Error bars represent +1 S.D. ($n = 10$ for *Glossophora kunthii* and $n = 8$ for *Macrocyctis integrifolia*).

in the feeding assays with live intact algae (1-way ANOVA; $F = 23.41$, $p < 0.001$) as well as in those with agar-based food (1-way ANOVA; $F = 5.82$, $p < 0.01$).

As a general trend, palatability levels in live *M. integrifolia* declined from top parts of the algae to the bottom parts (Fig. 3(C)). The live apical parts were preferred over medium, basal, and stipe portions (Fig. 3(C)), with significant differences between apical vs. medium and apical vs. stipe parts (Table 2). Furthermore, there was a very strong difference in consumption rates between medium parts and stipes, but no significant difference in consumption rates between medium and basal portions of *M. integrifolia* (Table 2). Basal parts of this brown seaweed were significantly preferred over stipes (Table 2, Fig. 3(C)). The general pattern of tissue palatability in live *M. integrifolia* individuals was confirmed when using agar-based food containing its non-polar extracts (Fig. 3(D)), but no significant differences were detected

for any of the tissue comparisons (Table 2). Similar results were obtained in the no-choice assays with live algae, where consumption of the stipe parts was significantly lower than that of other parts (1-way ANOVA; $F = 7.99$, $p < 0.001$). Amphipods also consumed less of the agar-based food made with extracts from the lower region of the alga (1-way ANOVA; $F = 18.28$, $p < 0.001$).

4. Discussion

We observed strong effects of amphipod grazing (at least in *Glossophora kunthii*) but no UVR-effects on algal palatability. Grazing effects were dependent on algal species, but independent of UVR levels and diminished within 14 days of amphipod removal. We observed strong differences in palatability levels among tissues in field-grown individuals of *G. kunthii* and *Macrocyctis integrifolia*.

4.1. Inducible defense in *Glossophora kunthii*

The feeding assays for *G. kunthii* revealed inducible defense in response to direct grazing, indirect grazing, and even non-grazing amphipods (*Parhyalella ruffoi*). However, effects were only significant with agar-based food, which suggests that defense is based on non-polar algal compounds. The discrepancy between the results with live algae and those with agar-based food appears inconsistent at first view, because if chemical defense is ecologically significant, then one should expect similar results for both live algae and for agar-based food. The results from the second experiment offer an explanation for this apparent discrepancy. Medium parts of the algae are consumed significantly more than apical and basal parts suggesting that chemical defense (constitutive and/or inductive) is absent or low in these medium parts of the alga. In the feeding assays with live algae, grazers probably consumed the undefended medium parts of the thallus, and consequently, we did not find significant differences between the grazing treatments and the control. Thus, the induction effect that we observed in *G. kunthii* was limited to the defense of apical and basal tissues. Possibly, the agar-based food contained a blend of non-polar extracts from induced and non-induced tissues, which in sum could have been effective to deter amphipods. Poore (1994) also found within-alga variation in secondary chemistry of *Zonaria angustata* (Dyctotales) occurring over a very small spatial scale (a few millimeters). Their small size and ability to feed selectively at similar scales may allow amphipods to exploit small regions of the algal tissues with low levels of secondary chemicals that are unavailable to large herbivores (Poore, 1994).

Anti-herbivore defense of tissues that are responsible for the production of new cells has also been reasoned as a useful strategy to allocate resources for defending tissues, which are of superior importance for algal fitness in the brown alga *Sargassum filipendula* (Taylor et al., 2002). Similarly, the green alga *Halimeda macroloba*, particularly defended new segments that are non-calcified (Paul and Van Alstyne, 1988), and Tuomi et al. (1989) reported phenols accumulating especially in vegetative apical parts of *Fucus vesiculosus*. Basal parts also were defended (result from live intact algae and agar-based food) in *Sargassum filipendula* (Taylor et al., 2002) and *Asco-*

phyllum nodosum (Pavia et al., 2002; Toth et al., 2005).

Inducible defense in response to direct grazing had been reported for several species of brown macroalgae. For example, Cronin and Hay (1996a) reported that *Dictyota menstrualis* responded with increased concentrations of diterpenoid compounds to direct grazing. Several other studies also demonstrated enhanced secondary metabolite production of macroalgae in response to direct grazing (Pavia and Brock, 2000; Pavia and Toth, 2000; Sotka et al., 2002; Rohde et al., 2004; Weidner et al., 2004; Ceh et al., in press). Furthermore, grazing on adjacent conspecifics induced anti-herbivore defense in *G. kunthii*, which deterred further feeding by the amphipod *P. ruffoi*. Palatability of the brown alga *Ascophyllum nodosum* (Toth and Pavia, 2000) and *Fucus vesiculosus* (Rohde et al., 2004) also decreased in response to waterborne signals from neighboring grazed algae. The decline of palatability due to waterborne cues could be caused either by chemicals released from amphipods, wounded conspecific algae, or both. The fact that reduced consumption was also found in feeding assays with *G. kunthii* maintained in mere presence of grazers suggests that waterborne signals are released from the consumers themselves. Algae that are able to assess and respond to waterborne cues indicating an imminent risk of herbivore attack may have a selective advantage (Toth and Pavia, 2000). *Glossophora kunthii* grows on cold-temperate shores and the dense monospecific stands of this alga are often inhabited by amphipods (Gelcich, 1999; Palma and Ojeda, 2002). These grazers usually remove modest amounts of algal biomass (Carpenter, 1986), which has been viewed as an intrinsic element of inducing defenses in seaweeds (Karbon et al., 1999; Cronin, 2001). Consumption by grazers with patchy spatial and/or variable temporal distribution patterns, whose individuals remove only modest amounts of algal biomass, e.g., amphipods, may represent reliable cues for the induction of anti-herbivore defense (Hay, 1996; Pavia and Toth, 2000).

4.2. No inducible defense in *Macrocystis integrifolia*

For the giant kelp *M. integrifolia*, no significant differences were detected in palatability between control and grazer-treated algae pieces, neither with agar-based food nor with live apical tips. This absence of

differences, and hence of inducible defense, could be due to the fact that this alga possesses (a) constitutive defense, or (b) no defense. Since *M. integrifolia* is usually exposed to a wide variety of vertebrate and invertebrate grazers (North, 1994; Godoy, 2000), this alga probably has constitutive defense. Recent experiments suggest that the defensive level of blades of *M. integrifolia* is low (Rothäusler and Thiel, in press). Since phenolic levels are also low in blades of *Macrocystis* (Steinberg, 1985; Winter and Estes, 1992), chemical defense may only be of minor importance in the upper parts of this large kelp. At least in our feeding assays with intact algae, no induction was detected, and these pieces contained both non-polar and polar compounds. However, at present and based on our experiments, we cannot completely discard lack of induction—Toth and Pavia (2002) recently attributed negative inducible response after direct grazing by snails in *Laminaria hyperborea* to the artificial conditions in outdoor laboratory experiments.

Six fish (omnivorous and herbivorous) species are known to be associated with *M. integrifolia* close to our collection site (Godoy, 2000) as well as sea urchins (personal observation). These fish probably feed primarily on the apical and medium parts of the alga, while sea urchins mainly reach the lower parts. Individuals and tissues subjected to intense and/or frequent attacks (by large fish grazers) such as large kelp species likely benefit from constitutive defenses, while those subject to low, intermediate or highly variable attacks (by mesograzers) likely would benefit from inducible defenses (Karban and Baldwin, 1997).

Our results on within-alga variations of palatability to mesograzers and concentrations of secondary compounds are consistent with those from other studies (Steinberg, 1984; Tugwell and Branch, 1989; Fairhead et al., 2005). As in our study, in most other studies, it was revealed that stipes and reproductive blades were least palatable to mesograzers. Steinberg (1984) found that reproductive blades of the intertidal kelp *Alaria marginata* had higher phenolic concentrations than vegetative blades. Stipes, holdfasts, reproductive blades and growth meristems may be more important for long-term survival of kelps than vegetative blades (Hammerstrom et al., 1998; Van Alstyne et al., 1999; Taylor et al., 2002). In some large brown algae, defense in these tissues may also be inducible (e.g., Sotka et al., 2002), but this was not the case in

M. integrifolia, in which basal stipes seem to be primarily defended structurally.

4.3. UV effects

Besides the influence of grazing treatments, algal food quality was not affected by UVR treatments. To date, conflicting results of UVR-effects on algal palatability exist. While enhanced UVBR increased palatability of the brown algae *Ascophyllum nodosum* for the isopod *Idotea granulosa* (Pavia et al., 1997), UVR had detrimental effects on the physiology of *Dictyota ciliolata* from shallow-waters (<3 m), which may lower chemical defenses in this species and thus indirectly increase its susceptibility to herbivores (Cronin and Hay, 1996c). Past studies revealed that plants (e.g., Figueroa et al., 2003) and animals (e.g., Gleason and Wellington, 1995) respond to UVR-stress with the production of potential UVR-blocking substances, including phlorotannins (Pavia et al., 1997; Pavia and Brock, 2000; Swanson and Druehl, 2002; but see Henry and Van Alstyne, 2004). Depending on the identity of UVR-induced chemical compounds and the grazer species, UVR might indirectly increase (e.g., Pavia et al., 1997) or decrease (e.g., Cronin and Hay, 1996c) algal palatability for grazers. Interestingly, in the present study, grazing effects were independent of UVR-regimes. This suggests as a third alternative that UVR does not affect the induction of anti-herbivore defense and thus a decoupling of the production of grazer- and UVR-induced chemicals. Possibly, *Glossophora kunthii* does not produce UV-induced chemicals, because it inhabits the intertidal zone and may be well adapted to high UVR levels. Alternatively, this alga may employ UVR-blocking chemicals that have no effect on palatability. Thus, only grazing could have been responsible to induce chemical compounds with anti-herbivore function. The fact that no grazing \times UVR interaction was detected also indicates that UVR-effects might not always be as harmful in shallow-water animal–alga interaction as suggested in the past (e.g., Häder et al., 1995).

5. Conclusions

Herein, we found inducible defense in *Glossophora kunthii* at different grazing levels. It was

shown that *G. kunthii* can perceive and respond adequately to the presence of actively-feeding amphipods as well as to their mere presence. However, induction was limited to crucial parts of the alga, namely, the basal region and the growing zone. Our results suggest that the defense strategy of *G. kunthii* includes both temporal as well as spatial defense allocation, by (a) only reacting in response to grazer presence (b) limiting defense to specific algal parts. In contrast, no temporal allocation of chemical defense was found in *M. integrifolia*. Thus, the two studied algal species differ in their defense strategies. At present, it appears too early to determine whether these differences in defense strategy between the two examined algal species are due to habitat-specific or due to species-specific characteristics. However, it appears clear that UV effects play only a minor role in anti-herbivore defense of the two studied algal species, which is in accordance with most previous studies.

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References

- Barbosa, J.P., Teixeira, V.L., Pereira, R.C., 2004. A dolabellane diterpene from the brown alga *Dictyota paffii* as chemical defense against herbivores. *Bot. Mar.* 47, 147–151.
- Breen, P.A., Mann, K.H., 1976. Destructive grazing of kelp by sea urchins in eastern Canada. *J. Fish. Res. Board Can.* 33, 1278–1283.
- Carpenter, R.C., 1986. Partitioning herbivory and its effects on coral reef algal communities. *Ecol. Monogr.* 56, 345–364.
- Ceh, J., Molis, M., Dzeha, T.M., Wahl, M., in press. Induction and reduction of anti-herbivore defenses in brown and red macroalgae off the Kenyan Coast. *J. Phycol.*
- 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. Within plant variation in seaweed palatability and chemical defenses: optimal defense theory versus the growth differentiation balance hypothesis. *Oecologia* 105, 361–368.
- Cronin, G., Hay, M.E., 1996c. Susceptibility to herbivores depends on recent history of both the plant and animal. *Ecology* 77, 1531–1543.
- Cronin, G., Paul, V.J., Hay, M.E., Fenical, W., 1997. Are tropical herbivores more resistant than temperate herbivores to seaweed chemical defenses? Diterpenoid metabolites from *Dictyota acutiloba* as feeding deterrents for tropical versus temperate fishes and urchins. *J. Chem. Ecol.* 23, 289–302.
- Deal, M.S., Hay, M.E., Wilson, D., Fenical, W., 2003. Galactolipids rather than phlorotannins as herbivore deterrents in the brown seaweed *Fucus vesiculosus*. *Oecologia* 136, 107–114.
- de Nys, R., Wright, J.T., König, G.M., Sticher, O., 1993. A diterpene from the marine alga *Glossophora kunthii*. *Phytochemistry* 32, 463–465.
- 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.
- 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.
- Figueroa, F.L., Escassi, L., Perez-Rodriguez, E., Korbee, N., Giles, A.D., Johnsen, G., 2003. Effects of short-term irradiation on photoinhibition and accumulation of mycosporine-like amino acids in sun and shade species of the red algal genus *Porphyra*. *Photochem. Photobiol.* 69, 21–30.
- Gagnon, P., Himmelman, J.H., Johnson, L.E., 2004. Temporal variation in community interfaces: kelp bed boundary dynamics adjacent to persistent urchin barrens. *Mar. Biol.* 144, 1191–1203.
- Gelcich, S., 1999. Dimension fractal de macroalgas submareales: su relación con la fauna epibionte, Marine Biology thesis. Universidad Católica del Norte, Coquimbo, p. 75.
- Gleason, D.F., Wellington, F.M., 1995. Variation in UVB sensitivity of planula larvae of the coral *Agaricites agaricites* along a depth gradient. *Mar. Biol.* 123, 693–703.
- Godoy, N., 2000. *Macrocystis integrifolia* (Laminariales, Phaeophyta) en el norte de Chile: Distribución espacio temporal y fauna asociada, Marine Biology thesis. Universidad Católica del Norte, Coquimbo, p. 63.
- Häder, D.D., Worrest, R.C., Kumar, H.D., Smith, R.C., 1995. Effects of increased solar ultraviolet radiation on aquatic ecosystems. *Ambio* 24, 174–180.
- 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.

- Harvell, C.D., 1990. The ecology and evolution of inducible defenses. *Q. Rev. Biol.* 65, 323–340.
- Hay, M.E., 1996. Marine chemical ecology: what's known and what's next? *J. Exp. Mar. Biol. Ecol.* 200, 103–134.
- Hay, M.E., 1997. The ecology and evolution of seaweed–herbivore interactions on coral reefs. *Coral Reefs* 16, S67–S76.
- 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., Duffy, J.E., Pfister, C.A., Fenical, W., 1987. Chemicals defenses against different marine herbivores: are the amphipods insect equivalents? *Ecology* 68, 1567–1580.
- Hay, M.E., Paul, V.J., Lewis, S.M., Gustafson, K., Tucker, J., Trindell, R.N., 1988. Can tropical seaweeds reduce herbivory by growing at night-diel patterns of growth, nitrogen content, herbivory, and chemical versus morphological defenses. *Oecologia* 75, 233–245.
- 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.
- Hemmi, A., Honkanen, T., Jormalainen, V., 2004. Inducible resistance to herbivory in *Fucus vesiculosus*—duration, spreading and variation with nutrient availability. *Mar. Ecol. Prog. Ser.* 273, 109–120.
- Henry, B.E., Van Alstyne, K.L., 2004. Effects of UV radiation on growth and phlorotannins in *Fucus gardneri* (Phaeophyceae) juveniles and embryos. *J. Phycol.* 40, 527–533.
- Hoffmann, A.J., Malbran, M.E., 1989. Temperature, photoperiod and light interactions on growth and fertility of *Glossophora kunthii* (Phaeophyta, Dictyotales) from Central Chile. *J. Phycol.* 25, 129–133.
- Hoffman, A., Santelices, B., 1997. *Flora Marina de Chile Central*. Ediciones Universidad Católica de Chile, Santiago. 434 pp.
- Jormalainen, V., Honkanen, T., Heikkilä, N., 2001. Feeding preferences and performance of a marine isopod on seaweed hosts: cost of habitat specialization. *Mar. Ecol. Prog. Ser.* 220, 219–230.
- Jormalainen, V., Honkanen, T., Koivikko, R., Eranen, J., 2003. Induction of phlorotannin production in a brown alga: defense or resource dynamics? *Oikos* 103, 640–650.
- Jormalainen, V., Honkanen, T., Vesakoski, O., Koivikko, R., 2005. Polar extracts of the brown alga *Fucus vesiculosus* (L.) reduce assimilation efficiency but do not deter the herbivorous isopod *Idotea baltica* (Pallas). *J. Exp. Mar. Biol. Ecol.* 317, 143–157.
- Karban, R., Baldwin, I.T., 1997. *Induced Responses to Herbivory*. University of Chicago Press, Chicago, IL, USA.
- Karban, R., Agrawal, A.A., Thaler, J.S., Adler, L.S., 1999. Induced plant responses and information content about risk of herbivory. *Trends Ecol. Evol.* 14, 443–447.
- Kubanek, J., Lester, S.E., Fenical, W., Hay, M.E., 2004. Ambiguous role of phlorotannins as chemical defenses in the brown alga *Fucus vesiculosus*. *Mar. Ecol. Prog. Ser.* 277, 79–93.
- Lubchenco, J., Gaines, S.D., 1981. A unified approach to marine plant–herbivore interactions: I. Populations and communities. *Ann. Rev. Ecol. Syst.* 12, 405–437.
- McKey, D., 1979. The distribution of secondary compounds within plants. In: Rosenthal, G.A., Janzen, D.H. (Eds.), *Herbivores: Their Interactions With Secondary Plant Metabolites*. Academic Press, San Diego, pp. 55–133.
- Molis, M., Wahl, M., 2004. Transient effects of solar ultraviolet radiation on the diversity and structure of a field-grown epibenthic community at Luderitz, Namibia. *J. Exp. Mar. Biol. Ecol.* 302, 51–62.
- North, W.J., 1994. Macrocytistis. In: Akatsuka, W.J. (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.
- Paul, V.J., Van Alstyne, K.L., 1988. Chemical defense and chemical variability in some tropical Pacific species of *Halimeda* (Halimedaecae; Chlorophyta). *Coral Reefs* 6, 263–269.
- Paul, V.J., Cruz-Rivera, E., Thacker, R.W., 2001. Chemical mediation of macroalgal–herbivore interactions: ecological and evolutionary perspectives. In: McClintock, J.B., Baker, B.J. (Eds.), *Marine Chemical Ecology*. CRC Press, Boca Raton, pp. 227–265.
- Pavia, H., Brock, E., 2000. Extrinsic factors influencing phlorotannin production in the brown alga *Ascophyllum nodosum*. *Mar. Ecol. Prog. Ser.* 193, 285–294.
- Pavia, H., Toth, G.B., 2000. Inducible chemical resistance to herbivory in the brown seaweed *Ascophyllum nodosum*. *Ecology* 81, 3212–3225.
- Pavia, H., Cervin, G., Lindgren, A., Aberg, P., 1997. Effects of UV-B radiation and simulated herbivory on phlorotannins in the brown alga *Ascophyllum nodosum*. *Mar. Ecol. Prog. Ser.* 157, 139–146.
- Pavia, H., Toth, G.B., Aberg, P., 2002. Optimal defense theory: elasticity analysis as a tool to predict intraplant variation in defenses. *Ecology* 83, 891–897.
- Pereira, R.C., Teixeira, V.L., Kelecom, A., 1994. Chemical defenses against herbivores in marine algae: 1. The brown alga *Dyctiota dichotoma* (Hudson) Lamaroux from Brazil. *Ann. Acad. Bras. Cienc.* 66, 230–235.
- Poore, A.G.B., 1994. Selective herbivory by amphipods inhabiting the brown alga *Zonaria angustata*. *Mar. Ecol. Prog. Ser.* 107, 113–123.
- Raven, J.A., 2003. Long-distance transport in non-vascular plants. *Plant Cell Environ.* 26, 73–85.
- Rivera, P.A., Astudillo, L.A., Gonzalez, A.G., Manta, E., Cataldo, F., 1987. Two new bicyclic diterpenoids from the brown alga *Glossophora kunthii*. *J. Nat. Prod.* 50, 965–967.
- 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., in press. Effect of detachment on the palatability of two kelp species. *J. Appl. Phycol.*
- Scheibling, R.E., Hennigar, A.W., Balch, T., 1999. Destructive grazing, epiphytism, and disease: the dynamics of sea urchin–kelp interactions in Nova Scotia. *Can. J. Fish. Aquat. Sci.* 56, 2300–2314.

- Schmitt, T.M., Hay, M.E., Lindquist, N., 1995. Constraints on chemically mediated coevolution: multiple functions for seaweed secondary metabolites. *Ecology* 76, 107–123.
- Schmitt, T.M., Lindquist, N., Hay, M.E., 1998. Seaweed secondary metabolites as antifoulants: effects of *Dictyota* spp. diterpenes on survivorship, settlement, and development of invertebrate larvae. *Chemoecology* 8, 125–131.
- 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.
- Stachowicz, J.J., Lindquist, N., 1997. Chemical defense among hydroids on pelagic *Sargassum*: predator deterrence and absorption of solar UV radiation by secondary metabolites. *Mar. Ecol. Prog. Ser.* 155, 115–126.
- 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 funebralis* and chemical defenses of marine brown algae. *Ecol. Monogr.* 53, 333–349.
- Steinberg, P.D., Van Alena, I., 1992. Tolerance of marine invertebrate herbivores to brown algal phlorotannins in temperate Australasia. *Ecol. Monogr.* 62, 189–222.
- Steinberg, P.D., de Nys, R., Kjelleberg, S., 2001. Chemical mediation of surface colonization. In: McClintock, J.B., Baker, B.J. (Eds.), *Marine Chemical Ecology*. CRC Press, Boca Raton, pp. 355–387.
- Swanson, A.K., Druehl, L.D., 2002. Induction, exudation and the UV protective role of kelp phlorotannins. *Aquat. Bot.* 73, 241–253.
- Taylor, R.B., Sotka, E., Hay, M.E., 2002. Tissue-specific induction of herbivore resistance: seaweed response to amphipod grazing. *Oecologia* 132, 68–76.
- 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.
- Tugwell, S., Branch, G.M., 1989. Differential polyphenolic distribution among tissues in the kelps *Ecklonia maxima*, *Laminaria pallida* and *Macrocystis angustifolia* in relation to plant defence theory. *J. Exp. Mar. Biol. Ecol.* 129, 219–230.
- Tuomi, J., Ilvessalo, H., Niemela, P., Siren, S., Jormalainen, V., 1989. Within-plant variation in phenolic content and toughness of the brown alga *Fucus vesiculosus*. *Bot. Mar.* 32, 505–509.
- Van Alstyne, K.L., 1988. Herbivore grazing increases polyphenolic defenses in the brown alga *Fucus distichus*. *Mar. Ecol. Prog. Ser.* 69, 655–663.
- Van Alstyne, K.L., McCarthy, J.J., Hustead, C.L., Kearns, L.J., 1999. Phlorotannin allocation among tissues of northeastern pacific kelps and rockweeds. *J. Phycol.* 35, 483–492.
- 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.
- Zangerl, A.R., Rutledge, C.E., 1996. The probability of attack and patterns of constitutive and induced defense: a test of optimal defense theory. *Am. Nat.* 147, 599–608.