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ORIGINAL PAPER

Species-specific defense strategies of vegetative versus reproductive blades of the Pacific kelps *Lessonia nigrescens* and *Macrocystis integrifolia*

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Abstract Chemical defense is assumed to be costly and therefore algae should allocate defense investments in a way to reduce costs and optimize their overall fitness. Thus, lifetime expectation of particular tissues and their contribution to the fitness of the alga may affect defense allocation. Two brown algae common to the SE Pacific coasts, *Lessonia nigrescens* Bory and *Macrocystis integrifolia* Bory, feature important ontogenetic differences in the development of reproductive structures; in *L. nigrescens* blade tissues pass from a vegetative stage to a reproductive stage, while in *M. integrifolia* reproductive and vegetative functions are spatially separated on different blades. We hypothesized that vegetative blades of *L. nigrescens* with important future functions are more (or equally) defended than reproductive blades, whereas in *M. integrifolia* defense should be mainly allocated to reproductive blades (sporophylls), which are considered to make a higher contribution to fitness. Herein, within-plant variation in susceptibility of reproductive and vegetative tissues to herbivory and in allocation of phlorotannins (phenolics) and N-compounds was compared. The results show that phlorotannin and N-concentrations were higher in reproductive blade tissues for both investigated algae. However, preferences by amphipod grazers (*Parhyalella penai*) for either tissue type differed between the two algal species. Fresh reproductive tissue of *L. nigrescens* was more consumed than vegetative tissue, while the reverse was found in *M. integrifolia*, thus confirming the original hypothesis. This suggests that future fitness function might indeed be a useful predictor of anti-herbivore defense in large, perennial kelps. Results from feeding assays with artificial pellets that were made with air-dried material and extract-treated *Ulva* powder indicated that defenses in live algae are probably not based on chemicals that can be extracted or remain intact after air-drying and grinding up algal tissues. Instead, anti-herbivore defense against amphipod mesograzers seems to depend on structural traits of living algae.

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Introduction

Herbivores can influence the structure of benthic algal communities through the consumption of large amounts of algal biomass (Lubchenco and Gaines 1981; Carpenter 1986; Vinueza et al. 2006; Jormalainen and Honkanen 2008). In response to this herbivore pressure, macroalgae have developed different strategies, one of which is the defense of tissues in ways that makes them less palatable for potential consumers (reviewed in Duffy and Hay 1990; Cronin 2001). To reduce tissue palatability, algae use defense strategies known as: (1) structural or morphological defense,

e.g., the calcification of tissue (Littler and Littler 1980), (2) chemical defense, e.g., synthesis and accumulation of unpalatable compounds (Amsler and Fairhead 2006), and (3) nutritional defense, e.g., algae are less palatable due to their low nutritional quality (Lubchenco and Gaines 1981; Duffy and Paul 1992).

Many studies have been conducted on algal chemical defense. Deterrent compounds are usually produced via the secondary metabolic pathway (Maschek and Baker 2008) and numerous examples have confirmed that a variety of substances can efficiently deter different grazers (reviewed in Cronin 2001; Amsler and Fairhead 2006). For example, diterpenes and phlorotannins, the most investigated groups of metabolites isolated from brown algae (Maschek and Baker 2008) were identified to act both as anti-herbivory and anti-fouling substances (summarized in Amsler and Fairhead 2006). Phlorotannins have also been invoked to serve or contribute to other vital functions such as protection against UV-radiation, cell-wall formation, and cytokinesis (Pavia et al. 1997; Schoenwaelder and Clayton 1999; Schoenwaelder 2002). On the other hand, herbivores are considered to be mainly N-limited, and therefore selective in foraging for N-rich algal tissue (Mattson 1980; Duffy and Paul 1992; Cruz-Rivera and Hay 2000). Consequently, chemical defense as well as nutritional status of algae will influence the feeding preferences of herbivores (Cruz-Rivera and Hay 2003).

It is widely assumed that the production, maintenance, and translocation of deterring metabolites are associated with metabolic costs because defenses use resources that could have been allocated to growth or reproduction (Hay and Fenical 1988; Fagerström 1989). Many hypotheses on chemical defenses (summarized in Cronin 2001; Pavia and Toth 2008) seek to explain the allocation of overall resources and defense metabolites in macroalgae. The growth-differentiation balance hypothesis (GDBH) predicts that actively growing and reproductive tissues are less defended because of lacking cell differentiation when compared to differentiated vegetative tissue (Herms and Mattson 1992), which has been discussed in former studies (Cronin and Hay 1996; Van Alstyne et al. 1999). Nevertheless, highly differentiated large brown algae (e.g., Laminariales, Fucales) may translocate low molecular weight compounds (e.g., precursors of phlorotannins) among functionally different tissues (Raven 2003), which makes predictions from the GDBH difficult to test (see discussion in Cronin and Hay 1996). The most widely accepted hypothesis, the optimal defense theory (ODT), predicts that chemical compounds for defense are allocated within the algae in a way that optimizes the overall fitness of the organism (Cronin 2001; Pavia and Toth 2008). Thus, algal parts with high fitness values that are susceptible to grazers should be most intensely defended, resulting in a within-plant variation in defense allocation (reviewed in Jormalainen and Honkanen 2008).

For example, meristematic and reproductive blade portions should be proportionally more defended than non-meristematic vegetative blades, which are less important for the plant fitness (Steinberg 1984; Tugwell and Branch 1989; Tuomi et al. 1989; Van Alstyne et al. 1999; Toth and Pavia 2002b). However, these predictions are highly dependent on specific fitness values that are previously assigned for the tested tissues (see discussion in Pavia et al. 2002). This pattern might also vary between taxonomic groups (Tuomi et al. 1989; Van Alstyne et al. 1999; Pavia et al. 2002) or between particular algae that show distinct reproductive morphologies. For example, in kelps (Laminariales) higher chemical defenses (e.g., phlorotannin concentrations) were found in reproductive than in vegetative blades (Steinberg 1984; Paul and Fenical 1986; Tugwell and Branch 1989; Van Alstyne et al. 1999), while in the rockweeds (Fucales) the defense allocation showed the opposite pattern (Tuomi et al. 1989; Van Alstyne et al. 1999; Pavia et al. 2002).

The goal of the present study was to examine whether two kelp species from the northern-central coast of Chile, *Lessonia nigrescens* Bory 1826 and *Macrocystis integrifolia* Bory 1826 (Laminariales), display different defense strategies in response to herbivore (amphipod) attacks on blades. It is well known that stipes and holdfasts of perennial algae are more defended than non-meristematic blade portions (Tugwell and Branch 1989; Macaya et al. 2005; see also discussion in Pavia et al. 2002). However, blades of these large kelps fulfill different functions and defenses might vary depending on the function of a blade. In particular, both kelp species show important differences in their reproductive phenology; blades of *L. nigrescens* change during ontogeny from a vegetative to a reproductive stage that is characterized by the maturation of sori (Santelices et al. 1980; Edding et al. 1994; Hoffmann and Santelices 1997), while in *M. integrifolia* the sori are developed on specialized reproductive blades, called sporophylls (Neushul 1963). Based on this important difference of individual blade structures, we expected different defense strategies in these two algal species. Specifically, we hypothesized that in *L. nigrescens*, the vegetative blades, which are mainly photosynthetic structures, are more (or equally) protected than reproductive tissue parts, because, ontogenetically, they will develop sporangia and contribute to the fitness of the alga. Thus, vegetative blades of *L. nigrescens* not only participate in photosynthesis but also contribute to the future production of reproductive tissues later. However, after having fulfilled their vegetative function and once the sporulation took place, the need in defending the reproductive structures should diminish. On the other hand, *M. integrifolia* is expected to protect the reproductive blades

more than the vegetative ones because the alga could do without (part of) the latter, while the loss of reproductive blades means a significant loss for the plant with regard to its fitness. Herein we measured the consumption rates of mesograzers in three distinct feeding assays (fresh material, algal pellet, and extract pellet) to gain insights into structural, chemical (phlorotannin content), and nutritional (N-content) defense mechanisms of the two kelp species in the context of the ODT.

Materials and methods

Study site and organisms

The study was conducted at the end of the austral summer (March) 2007 in the Laboratorio de Botanica Marina at the Universidad Católica del Norte, Coquimbo, Chile. Vegetative and reproductive (bearing sori) blades of *Lessonia nigrescens* were collected in the exposed rocky intertidal zone at La Pampilla, Coquimbo (29°57′S, 71°20′W). In the case of *Macrocystis integrifolia*, blades and sporophylls were collected in the subtidal zone off Punta de Choros (29°14′S, 71°28′W). The amphipod *Parhyalella penai* Pérez-Schultheiss and Crespo 2008, which is a generalist mesograzer that feeds on a variety of different macroalgae including *L. nigrescens* and *M. integrifolia* (Macaya et al. 2005; Rothäusler et al. 2005), was used for testing algal palatability in the different feeding assays. This littoral amphipod species was called *P. ruffoi* Lazo-Wasem and Gable 2001 in earlier publications, but careful examination revealed that it is a new species, which led to the recent species description under a new name (Pérez-Schultheiss and Crespo 2008). This grazer can be found in accumulations of drift algae (diverse species) in the shallow subtidal zone of sheltered beaches from northern-central Chile. Amphipods for this study were collected from Playa Chica of Bahía La Herradura, Coquimbo by collecting accumulations of drift algae. The amphipods were separated from the algae by gently shaking them over a large tray filled with seawater.

Design of feeding assays

For each kelp species, both vegetative and reproductive blades were sampled from ten sporophytes and consequently the reproductive and vegetative tissues were dependent on each other. For both species and blade types, non-meristematic sections of ~15 cm length from middle parts of the blades were cut for the assays (ensuring that sori-bearing tissues from the reproductive blades were obtained). The large number of assays and analyses required subdivision of the materials, but we had sufficient materials for at least seven replicates in all feeding assays or tissue analyses with exception of the assays with algal pellets of *Macrocystis integrifolia*. Problems in preparation of the pellets caused additional loss of replicates, but we were able to recover at least three replicates in each of these two assays (choice and no-choice assays).

The palatability of the different blades to the mesograzer *Parhyalella penai* was tested in feeding assays with: (1) fresh material, (2) agar-based food from air-dried and powdered algae (algal pellets), and (3) agar-based food made with crude extract of the algae dropped onto powder of the palatable green alga *Ulva lactuca* L. 1753 (extract pellets). Following logistic restrictions (availability of tissue), for fresh-algal material (i.e., natural food) we only conducted no-choice assays with fresh-algal material, accounting for autogenic changes of the living algal tissues by growth controls (Cronin and Hay 1996; Taylor et al. 2002; Toth and Pavia 2002a). It had been discussed by Peterson and Renaud (1989) that results from no-choice assays can reflect differences in attractiveness or palatability of various potential foods. Supporting this assumption, several recent studies had shown no-choice assays producing a similar outcome as choice assays (Taylor et al. 2002; Macaya et al. 2005; Macaya and Thiel 2008), as was also verified in a recent meta-analysis by Toth and Pavia (2007). In the case of the agar-based pellets (i.e., artificial food), we conducted both no-choice and choice assays.

Furthermore, the concentration of phlorotannins was measured, since these secondary metabolites have been shown to serve as defense substances in brown algae (Amsler and Fairhead 2006). Additionally, the concentration of nitrogen is commonly used as a proxy for food quality traits in algal tissues (reviewed in Mattson 1980; see also Cruz-Rivera and Hay 2000, 2003). In the present study, the N-concentration in algal tissues was measured in order to compare them with consumption rates of *Parhyalella penai* on the different algae tissues.

Preparation and evaluation of feeding assays

In the no-choice assays, one piece of fresh-algal material or one pellet was offered to eight individuals of the mesograzer *Parhyalella penai* (adult specimens, body length ~4–6 mm) in one Petri dish (9 cm diameter, filled with ~80 ml seawater). In the choice assays, the reproductive and the vegetative materials from the same algal individual were offered simultaneously. All feeding assays were conducted in a constant temperature room (15 ± 1°C) with a 12 h light cycle at an irradiance of 40 ± 10 μmol m^{-2} s^{-1} (fluorescent lamp, 40 W, Phillips). A maximum consumption period of 72 h was used during which we exchanged the water and replaced dead amphipods daily (mortality rates were generally very low in all assay combinations, with an absolute

maximum of two dead amphipods per Petri dish in 1 day; but this only occurred in very few replicates). If necessary, assays were stopped earlier to avoid a total consumption of fresh material or pellets. The data from the feeding assays were converted to consumption rates as mg (fresh weight) or percent of the total of 200 squares consumed by one individual of the amphipod *P. penai* within 24 h.

Feeding assays with fresh-algal material

After blotting the algal pieces (~0.3 g) with absorbent tissue paper, these were weighed to the nearest mg using an analytical balance (±0.2 mg). Following exposure to the amphipods for a maximum period of 72 h, the pieces were re-weighed. Another algal piece was kept under the same conditions without grazers as a growth control. The total consumption by the herbivores was then calculated using the formula described in Cronin and Hay (1996) as $C_{\text{real}} = T_i \times (C_f/C_i) - T_f$, where T_i and T_f are the initial and final wet weight of the algal material that was subject to grazing and C_i and C_f the initial and final wet weight of the growth control.

Feeding assays with algal pellets

The algal material was dried at room temperature in a dark paper box to avoid photolysis of light-sensitive compounds and then ground in an ultra-centrifugal mill. The pellets were prepared with 0.5 g of the algal powder and then mixed with 4 ml of distilled water. A specific amount of agar (0.36 g) was added to 6 ml distilled water and boiled three times in a microwave until a clear solution was visible. Once the agar cooled down to at least 40°C, the algal powder was added and mixed. This mixture was immediately poured onto pieces of a gauze mesh (mesh size 1 mm^2) consisting of 200 squares and pressed between two glass plates. After hardening, the pieces were offered to the grazer as agar-based food (pellet) in choice and no-choice feeding assays. Consumption rates of agar-based food were determined by counting the total mesh squares (1 mm^2 surface area) consumed after the feeding period.

Feeding assays with extract pellets

To examine whether differences in algal palatability are caused by chemical compounds, extracts from fresh-algal material were prepared, mixed with *Ulva* powder, incorporated into an agar-matrix, and offered to grazers as agar-based food. For the extraction, fresh material of the alga were shortly dried with tissue paper and cut into small pieces to facilitate the extraction procedure (Rothäusler et al. 2005; Fairhead et al. 2005a, b; Medeiros et al. 2007). Pieces of 3 g wet weight were weighed with an analytical balance and added to glass flasks (100 ml), which were then filled with 50 ml of a 1:1 hexane–methanol mixture (to extract most secondary metabolites; from polar to non-polar). The extraction lasted 48 h and the mixture of solvent and algal material was then filtered (coffee filters) into a small vial to separate the algae pieces from the extract. After evaporation, ~0.5 g dry *Ulva* powder was mixed with the crude extract obtained from 3 g wet weight (~0.5 g dry weight, dw). To achieve natural concentrations of extracted compounds in the agar-based food we used the following relationship for the pellet preparation: 0.5 g dw of the extracted algal material ≈0.5 g dw of the *Ulva* powder for the pellet. Subsequently, these extract pellets were prepared as described above.

Chemical composition of algal tissues

The phlorotannin content of the different tissue parts was measured at the Universidad Austral de Chile in Valdivia. Algal material was air-dried in darkness and room temperature and stored in silica gel. To determine the concentration of soluble phlorotannins, we used the Folin-Ciocalteu assay (Van Alstyne 1995) and compared the values with a phloroglucinol standard from a calibration curve. Algal samples of ~0.1 g dw were incubated in 10 ml of 70% acetone for 12 h at 4°C in total darkness following the extraction-method described in detail by Koivikko et al. (2005). Following multiple extractions, 1 ml of the Folin-Ciocalteu reagent was added to the phlorotannin extract, which was kept for 5 min before adding 2 ml of a sodium carbonate solution (0.2 g ml^{-1}). After 1 h, the absorbance at 730 nm was read on a SUV-2120 spectrophotometer (SCINCO, Korea).

Tissues were analyzed for their N-concentrations at the University of Rostock, Germany. Air-dried material was ground using a mortar and samples of 1–3 mg were loaded into small tin boats (6 × 6 × 12 mm) and packaged. These packages were burned (900°C) and total concentrations of nitrogen were measured automatically using acetanilide as an internal standard (Elementar Vario EL III, Germany).

Statistical analyses

All data were tested for normality with the Shapiro-Wilk's *W* test and if non-normal, square-root transformed before being used in parametric statistical tests. An arcsine transformation was used for percentage data. To analyze the differences between means of the treatments a *t* test for dependent samples was used since reproductive and vegetative tissues came from the same individual plant. This was done for all assays, and only in the case of feeding assays with algal pellets of *Macrocystis integrifolia*, a *t* test for independent samples was applied (because there were

insufficient dependent replicates). In case the data were not normally distributed after transformation, a non-parametric Wilcoxon matched pairs test was used to analyze differences between means of dependent data and a non-parametric Mann–Whitney *U* test was used for analyses of non-normal data from independent samples. Homogeneity of variances was checked using the Levene's test. All statistical tests were performed using the software STATISTICA 6.0 (StatSoft, Inc., USA).

Results

Palatability of vegetative and reproductive algal tissues

In the feeding assay with fresh-algal material of *Lessonia nigrescens* the amphipods consumed significantly more reproductive than vegetative material (Table 1; Fig. 1). When food was offered to amphipods in form of agar-based food pellets made from air-dried algal material of *L. nigrescens*, no significant differences were found in consumption rates, even though a trend was observed that the amphipods preferred the vegetative material in the choice feeding assay (Table 1; Fig. 2; algal pellets). Some problems appeared in no-choice assays with algal pellets, where amphipods consumed the total amount of squares in few pellets over night before the assay could be stopped. In the case of agar-based food made from *Ulva* powder containing algal crude extract, the amphipods consumed significantly more from the vegetative than from the reproductive material, both in choice and no-choice feeding assays (Table 1; Fig. 2; extract pellets).

When fresh-algal material of *Macrocystis integrifolia* was offered, the amphipods consumed significantly more vegetative than reproductive material (Table 1; Fig. 1). Although problems appeared in the preparation of algal pellets (slime production of the powdered algae when getting in contact with water, leading to the loss of several replicates), consumption rates differed significantly; a significant preference for reproductive material was found in both choice and no-choice assays (Table 1; Fig. 2; algal pellets). However, as mentioned for *Lessonia nigrescens*, in some

Table 1 Results from statistical analysis of the consumption rates on algal material [containing mechanical (m), nutritional (n) or chemical (c) traits], of the phlorotannin contents and the N-concentrations between reproductive (R) and vegetative (V) blade parts of *Lessonia nigrescens* and *Macrocystis integrifolia* in different assays using *t* tests for dependent samples

		Lessonia nigrescens				*Macrocystis integrifolia*			
		df	*t*	*P*		*df*	*t*	*P*	
Fresh material (mg)	m, n, c	8	3.714	0.006	V < R	6	−4.557	0.004	V > R
Algal pellets, no-choice (% squares)	n, c			0.735[a]	V = R	7[b]	2.390[b]	0.048[b]	V < R
Algal pellets, choice (% squares)	n, c	8	−1.564	0.156	V = R			0.034[c]	V < R
Extract pellets, no-choice (% squares)	c	8	−2.359	0.046	V > R	8	1.855	0.101	V = R
Extract pellets, choice (% squares)	c	8	0.019	0.006	V > R	9	3.616	0.006	V < R
Content of soluble phlorotannins (% dw)		6	3.078	0.022	V < R	6	8.970	<0.001	V < R
Nitrogen content (% dw)		6	4.823	0.003	V < R	7	3.355	0.012	V < R

[a] Non-parametric Wilcoxon matched pairs test for analyses of non-normal data from dependent samples

[b] *t* test for independent samples, that we used because of insufficient dependent replication

[c] Non-parametric Mann–Whitney *U* test for analyses of non-normal data from independent samples

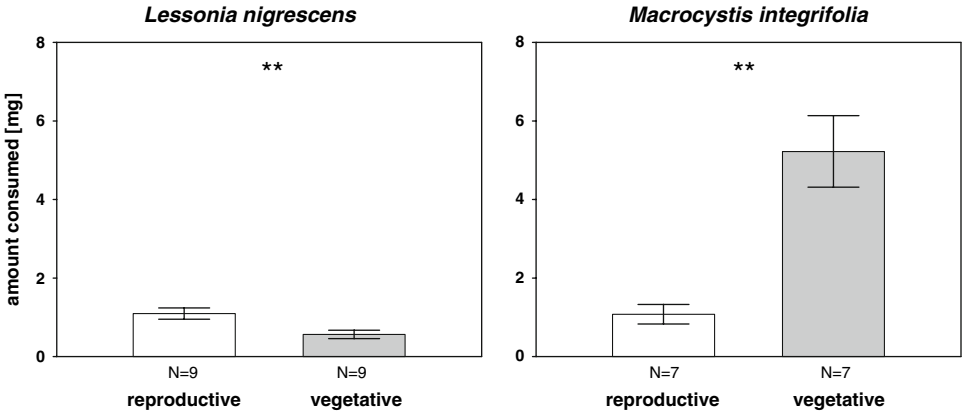

Fig. 1 Consumption of fresh material (mg) of *Lessonia nigrescens* and *Macrocystis integrifolia* by one individual of *Parhyalella penai* within 24 h. *White boxes* indicate reproductive and *grey boxes* vegetative material (each box: mean ± SE; * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$; *N* number of replicates). All assays lasted a maximum time period of 72 h

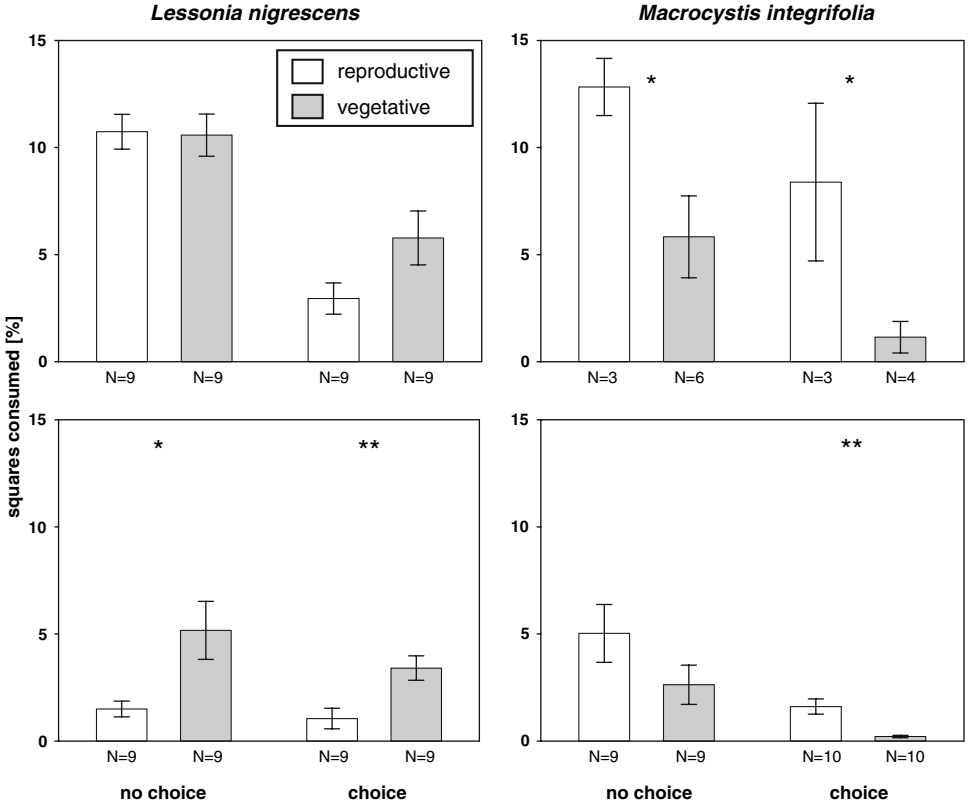

Fig. 2 Consumption of agar-based food (% of total squares) of *Lessonia nigrescens* and *Macrocystis integrifolia* by one individual of *Parhyalella penai* within 24 h on algal pellets (*above*) and extract pellets (*below*). *White boxes* indicate reproductive and *grey boxes* vegetative material (each box: mean ± SE; * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$; *N* number of replicates). All assays lasted a maximum time period of 72 h

replicates the amphipods consumed all available food in no-choice assays with algal pellets before the feeding assay was stopped. Similar as for the algal pellets, in the assays with extract pellets, amphipods preferred material of reproductive blades, albeit this was only significant in the choice assay (Table 1; Fig. 2; extract pellets).

Mean differences in consumption rates between fresh reproductive and vegetative material were much stronger in *Macrocystis integrifolia* (mean difference 33.16 mg) than in *Lessonia nigrescens* (mean difference 4.33 mg); (*t* test $P < 0.001$).

Chemical composition of vegetative and reproductive tissues

The content of soluble phlorotannins differed significantly between reproductive and vegetative material in both algal species (Table 1; Fig. 3). Phlorotannin concentrations in reproductive blade parts of *Lessonia nigrescens* were close to 4.9% dw, while vegetative blades contained less than 3.5% dw. In *Macrocystis integrifolia*, the pattern was similar, showing phlorotannin concentrations close to 4.6% dw in reproductive and about 2.1% dw in vegetative tissue. In both *L. nigrescens* and *M. integrifolia* the nitrogen content varied between 1.3 and 2.3% dw, with values significantly higher in reproductive than in vegetative tissues (Table 1; Fig. 3).

Discussion

The results of this study show that the interaction between amphipod grazers and algae proceeded as previously hypothesized when fresh-algal material was offered. The amphipods preferred the reproductive tissues of *Lessonia nigrescens*, which suggests that these parts are less defended or simply tastier for the amphipods. In *Macrocystis integrifolia*, vegetative blades were strongly preferred over reproductive blades, indicating a low defense level in vegetative tissues. Effect sizes (i.e., differences in consumption rates between vegetative and reproductive tissues) have in this case been much higher in assays using fresh material of *M. integrifolia* (Fig. 1). However, in both kelp species, grazers that were offered fresh material consumed more of the tissues with lower assigned fitness values, which provides support for the ODT (valuable tissues are more defended). Surprisingly, feeding assays with pellets based on air-dried algal material or algal extracts (i.e., after destroying structural characteristics of the algae) showed the opposite pattern in grazer preferences for both algal species. This suggests that structural or mechanical traits of fresh-algal tissues seem to be more efficient in deterring amphipod grazers than chemical compounds present in agar-based pellets.

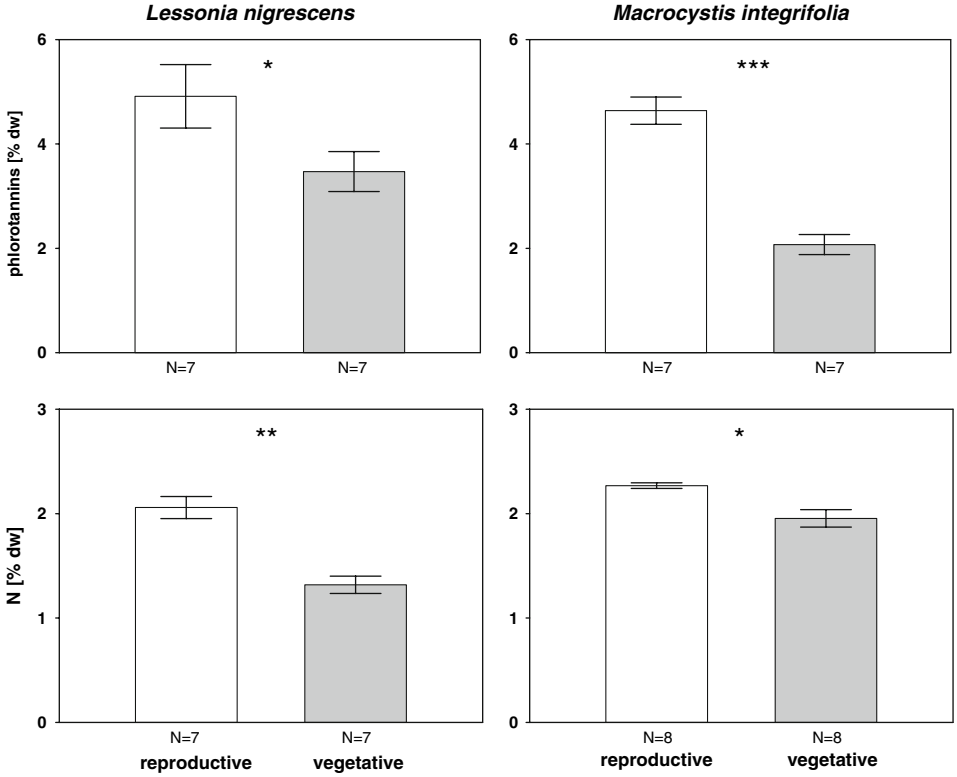

Fig. 3 Content of soluble phlorotannins and nutrients (% dry weight) in reproductive (*white boxes*) and vegetative (*grey boxes*) tissues of *Lessonia nigrescens* and *Macrocystis integrifolia* (each box: mean ± SE; * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$; *N* number of replicates)

Palatability of fresh tissue and nutritional qualities

Both algae had higher N-concentrations (% dw) in reproductive than in vegetative tissues (as also found for *Alaria marginata* by Steinberg 1984), which might result from active spore production within the reproductive tissues (Reed et al. 1996). Following general assumptions (Mattson 1980; Duffy and Paul 1992; Cruz-Rivera and Hay 2003), fresh reproductive material of these two algae should thus be more valuable for amphipods (and more consumed) because of its higher nutritional quality. However, this was not supported by our results, since preferences in food choice by amphipods were not always consistent with higher N-concentrations within the preferred food. Amphipods consumed significantly more reproductive than vegetative fresh tissue from *Lessonia nigrescens*, but preferred vegetative blades from *Macrocystis integrifolia*. The reverse pattern was found in feeding assays with agar-based food (algal and extract pellets) in both algae, which led us to assume that feeding preferences in fresh-algal tissue are based on structural or mechanical tissue characteristics rather than on nutritional or chemical traits. This had also been suggested by Steinberg (1985) for chemically weakly defended (or undefended) algae. Morphological characteristics of algal tissue had previously been emphasized to affect feeding preferences of grazers (Littler and Littler 1980; Steneck and Watling 1982). The pattern observed herein for small amphipods might have been different with other grazers that are less affected by tissue hardness (e.g. sea urchins or gastropods) (Rothäusler et al. 2005).

In *Macrocystis integrifolia*, we found vegetative blades to be much softer and thinner than reproductive blades (personal observation), which might explain the extremely high consumption rates of vegetative fresh material. Similar results were found by Steinberg (1984) for *Alaria marginata* (Laminariales), which also has distinctive reproductive and vegetative tissues; the herbivorous snail *Tegula funebralis* consumed much more fresh material from vegetative blade portions than from the reproductive portions. Steinberg (1984) also measured the tissue toughness in *A. marginata* with a "penetrometer", and he showed that reproductive tissues are tougher than vegetative tissues, supporting our suggestions for *M. integrifolia*. Furthermore, it is important to consider that *M. integrifolia* is producing large amounts of mucus, which might have been more intense in reproductive tissues, and could have affected amphipod preferences in fresh feeding assays. The production of mucus by the alga *Carpoglossum confluens* is thought to reduce the level of competition from other algae and to deter animals from being on or around the alga (Edgar 2000). Wotton (2004) discusses further roles of mucus (exopolymers) in aquatic systems. As an example, mucus might prevent damage by abrasion, forming a slippery layer on macroalgal fronds. It was also observed that some algal species (Fucales) release spores with large

amounts of viscous mucus that might reduce the dispersal of spores (Brawley and Johnson 1992; Brawley et al. 1999). Unfortunately, to date, the effect of mucus on algae grazer interactions has not been experimentally tested.

In *Lessonia nigrescens*, our findings suggest that feeding rates might also be influenced by structural or mechanical traits. Here the higher consumption of fresh reproductive material might be explained by constraints due to physiological changes that must occur when the tissue transforms from vegetative to reproductive. Furthermore, the reproductive structures are known to decay and be shed off after releasing spores (F. Tala, personal communication), which might expose the undefended inner parts (Tugwell and Branch 1989; Shibata et al. 2004) of the blade (medulla) to amphipod grazers. Possibly, the spores themselves might be easily consumed and digested by crustacean mesograzers, a situation observed in interactions between red algae and micro-grazers (Buschmann and Santelices 1987). Additionally, in *L. nigrescens* blades accumulate products of photosynthesis during maturation (e.g. polysaccharides), which might be particularly concentrated in older reproductive blades (Gómez et al. 2007). The high N-concentrations that we found in mature reproductive tissues of *L. nigrescens* might be a reflection of these processes, and might drive the feeding preferences of the amphipods.

Palatability of pellets and the role of phlorotannins

Phlorotannin concentrations (% dw) were higher in reproductive than in vegetative tissues in both algal species, as previously suggested by Van Alstyne et al. (1999) for algae belonging to the order Laminariales (see also Steinberg 1984; Tugwell and Branch 1989). Phlorotannins have also been shown to occur in very low concentrations of ~1% dw in vegetative blades of the congener *Macrocystis pyrifera* (Steinberg 1985; Winter and Estes 1992). Following general assumptions, one could expect that based on these differences in phlorotannin concentrations, reproductive tissues are more defended than vegetative tissues. Surprisingly, we found no consistent evidence for this assumption in feeding assays with fresh material (in *Lessonia nigrescens*) or in feeding assays with agar-based food (in *Macrocystis integrifolia*). This led us to assume that the grazer *Parhyalella penai* does not respond to extracted phlorotannins, at least not at the concentrations found in the two studied algae. There might be long-term effects of phlorotannin consumption, e.g., on reproductive or food-assimilation rates of the amphipod grazers (Cruz-Rivera and Hay 2000; Targett and Arnold 2001), but this was not examined herein. It also should be considered that the highly water-soluble phlorotannins might have leached out of the prepared food pellets during the assays (Jormalainen et al. 2005), possibly reducing the deterrence effect on *P. penai*.

Martinez (1996) demonstrated that individuals of *Lessonia nigrescens* with higher contents in phlorotannins (~5 mg g^{-1} dw) were less palatable to herbivorous snails and fish than individuals with lower phlorotannin contents (~1 mg g^{-1} dw). These values, however, seem to be extremely low (~0.1–0.5% dw) when compared to our results and concentrations cited for other brown algae. Nevertheless, there appear to be effects of phlorotannins on larger grazers (Martinez 1996), but no or only minor effects on small crustacean mesograzers (e.g., *Parhyalella penai*) as seen in our study. There might be other reasons why the phlorotannin content is higher in reproductive than in vegetative blades since phlorotannins are also involved in primary functions such as, e.g., structuring cell walls (Schoenwaelder and Clayton 1999; Arnold and Targett 2003). It must be emphasized that synthesis and allocation of phlorotannins in brown algae are complex and require further examination. Since the production of soluble phlorotannins is almost exclusively a function of cortex cells (Shibata et al. 2004) and thus, a large proportion of the blade tissues do not contain phlorotannins, some micro- and mesograzers might be able to distinguish the different tissue types, feeding mainly on the phlorotannin-free zones in, e.g., the reproductive tissues of *L. nigrescens*.

We observed a repelling effect in agar-based food from reproductive material of *Lessonia nigrescens* to the grazer *Parhyalella penai*. Although this pattern was weak in assays with algal pellets it was highly significant in assays with extract pellets, i.e., where we excluded the simultaneous effect of nutrients from *L. nigrescens*. However, just the opposite pattern was found in *Macrocystis integrifolia*. Considering that we used hexane and methanol to extract compounds from the algal tissue, we can expect a wide spectrum of polar as well as non-polar compounds being present in the crude extracts (Amsler and Fairhead 2006). Since methanol does not extract phlorotannins very efficiently (Koivikko et al. 2005), phlorotannins might even be under-represented in the extract pellets when compared to non-polar compounds. Consequently, our suggested explanation cannot only be based on phlorotannins but needs to include possible effects of a wide variety of additional extracted metabolites. Although a suite of non-phlorotannin secondary metabolites like galactolipids or hydrophilic non-phenolic compounds is known (Harper et al. 2001), relatively few studies have assayed their deterring roles (Amsler and Fairhead 2006; Maschek and Baker 2008). Since phlorotannin-rich tissues did not show consistent deterring effects on the grazer in this study, we might expect untested deterring secondary metabolites being responsible for differing consumption rates in assays with agar-based food. As a support for this assumption, Rothäusler and Thiel (2006) found slight chemically mediated defense in non-polar extracts (i.e., not containing polar

Table 2 Concentrations of phlorotannins and relationships between different tissues, in chemical deterrents and nitrogen contents as well as in grazer consumption of fresh tissues and artificial food for several species of macroalgae

	Phlorotannins ~[% dw][a]	Content of chemical deterrents	Nitrogen content	Consumption fresh tissue	Artificial food
Laminariales					
Lessonia nigrescens[b]	3.5–4.9	V < R	**V ≤ R**	**V ≤ R**	*V > R*
Macrocystis integrifolia[b]	2.1–4.6	V < R	V < R	*V > R*	V < R
Macrocystis angustifolia[c]		V < R, H, S			
Ecklonia maxima[c]		V < R, H, S < M			
Laminaria pallida[c]		V, R < H, S, M			
Laminaria hyperborea[d]	1.2–1.6	yV = oV	yV = oV	yV > oV	
Laminaria hyperborea[e]	1.8–3.2	yV, oV < yM < oM			
Alaria marginata[f]	1.0–5.0	V < R	V = R	*V > R*	
Alaria marginata[g]	1.0–2.1	V, M < R			
Alaria nana[g]	2.0–3.1	V < R, M			
Fucales					
Pelvetia compressa[g]	2.8–5.7	V, M > R			
Fucus gardneri[g]	3.2–4.9	M > V > R			
Fucus vesiculosus[h]	1.6–5.4	oV, yV, oR > yR	oR < yR		
Fucus vesiculosus[i]	9.0–10.5	aV < bV	**aV ≥ bV**	***aV*** **≥** ***bV***	
Ascophyllum nodosum[j]	3.5–7.5	S > V > R		*S < V < R*	
Ascophyllum nodosum[k]	3.8–6.3	aV < bV		*aV > bV*	
Sargassum filipendula[l]				aV, aS > bV > Bs	
Dictyota ciliolata[m]		yV < oV		*yV > oV*	*yV > oV*
Desmarestia anceps[n,o]	10.0–12.0	V > S			V, H > S
Desmarestia menziesii[n,o]	5.0–5.3	V = S			*V = H = S*

If food preferences reflect expectations based on chemical deterrents, they were italicized, if food preferences correspond with nitrogen contents, they were highlighted with bold letters

V vegetative blades, *R* reproductive tissue, *S* stipes, *H* holdfasts, *M* meristems, *y* young, *o* old, *b* basal, *a* apical

[a] Approximated mean phlorotannin values in the different thallus parts, corresponding to the comparisons of the "contents of chemical deterrents", min–max

[b] Present study, [c] Tugwell and Branch 1989, [d] Toth and Pavia 2002a, [e] Toth and Pavia 2002b, [f] Steinberg 1984, [g] Van Alstyne et al. 1999, [h] Tuomi et al. 1989, [i] Honkanen et al. 2002, [j] Pavia et al. 2002, [k] Toth et al. 2005, [l] Taylor et al. 2002, [m] Cronin & Hay 1996, [n] Fairhead et al. 2005a, [o] Fairhead et al. 2005b

phlorotannins) of *L. nigrescens*. On the other hand, there might be other substances in crude extracts (polar and non-polar) from, e.g., reproductive *M. integrifolia* that attract these amphipods (Pansch et al. unpublished data). Van Alstyne et al. (2001) and Van Alstyne and Houser (2003) showed simultaneously deterring and attracting (dependent on the metabolite concentrations) functions of activated secondary metabolites in macroalgae responding to sea urchins.

Finally, not all herbivores are deterred by phlorotannins (Jormalainen et al. 2003), indicating that some species have adapted to tolerate or utilize these algal compounds (Targett and Arnold 1998; Pavia et al. 1997; Jormalainen et al. 2003). Variations in effectiveness are even found at a biogeographic scale; the comparatively low polyphenol contents found in North American Phaeophycean have been found to deter a range of herbivores, but Australasian invertebrate herbivores are unaffected by high levels of phlorotannins (Steinberg and Van Altena 1992), which underlines the suggestion that phlorotannins have roles other than defense in these species. A similar situation occurs when induction of defensive responses of phlorotannins to grazing have been examined; in some cases, there is evidence of induction (Peckol et al. 1996; Lüder and Clayton 2004), whereas some authors have failed to demonstrate induced increases of phlorotannins in various species of Laminariales (Yates and Peckol 1993; Martinez 1996; Pavia et al. 1997; Toth and Pavia 2002b). Overall, the role of phlorotannins as herbivore deterrents remains ambiguous, mainly because the anti-herbivore effectiveness is also a function of herbivore-specific factors (e.g., characteristics of gut, enzymatic adaptations, etc.,).

Conclusions and outlook

The brown alga *Lessonia nigrescens* seems to follow a strategy in which fresh vegetative tissues are efficiently defended against amphipod grazing (Table 2). These photosynthetically active tissues have the potential to produce sori and to contribute to reproduction of the alga, thereby exhibiting an important role for future fitness. Reproductive structures seem to be little defended or somehow attractive to amphipod mesograzers. In fact, in some algal species, grazing on reproductive structures might improve spore release and enhance dispersal (Porter 1976; Buschmann and Santelices 1987; Santelices and Ugarte 1987), thus improving algal fitness. Algae with distinct reproductive blades such as the giant kelp *Macrocystis integrifolia* seem to follow a different strategy. Reproductive blades are presumably of higher value for these kelp species and thus much stronger defended than vegetative blades (Table 2). Particularly in *M. integrifolia*, this also coincides with the basal position of reproductive blades and their exposure to benthic grazers such as sea urchins and snails. On the other hand, vegetative blades seem to be highly susceptible to amphipod grazing. This alga had been shown to coincidentally express remarkable growth rates in vegetative plant portions (Stekoll and Else 1990) and might therefore compensate grazer-induced losses of vegetative blades by growth as suggested by Carpenter (1986).

Herein, it was shown that defense traits for both algae (*Lessonia nigrescens* and *Macrocystis integrifolia*) can be explained by the ODT. Although the fitness values assigned to reproductive and vegetative tissues are rather qualitative estimates, they seem to correspond with the defense strategies of the two studied algae. Results from former studies (Table 2) indicate that phlorotannin values differ between different tissues over small spatial scales (as also seen in the present study). However, in some cases the grazer preferences seem to vary independent from chemical deterrents and possibly responding to food quality of the studied tissues or other factors. Our results suggest a trade-off in amphipod preferences that depends mainly on (i) tissue toughness, but also on (ii) nutritional quality, and (iii) untested secondary metabolites of the food. How these different traits interact in driving the feeding preference of mesograzers needs to be examined in future studies.

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