



Compensatory growth of the kelp *Macrocystis integrifolia* (Phaeophyceae, Laminariales) against grazing of *Peramphithoe femorata* (Amphipoda, Ampithoidae) in northern-central Chile

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ABSTRACT

Compensation of tissue loss has been considered an alternative strategy for seaweeds that have no or only minor chemical or structural defense against herbivory. Compensatory responses are facilitated by resource transfer among different tissues and have been suggested for large kelps. *Macrocystis integrifolia* (Bory) is a common kelp species from northern-central Chile, which is characterized by high growth rates and the absence of lipophilic chemical defenses against herbivore grazing. Herein, we used the giant kelp *M. integrifolia* to test for compensatory growth in response to grazing by the nest-dwelling amphipod *Peramphithoe femorata* (Krøyer). Amphipods were allowed to graze inside nests on subapical blades of *M. integrifolia* sporophytes for 14 days. We measured growth and chemical composition (C, N, laminaran and mannitol) of apical and subapical blades of grazed and ungrazed (control) sporophytes. Our results revealed the capability of *M. integrifolia* to maintain elongation rates in grazed subapical blades, which were similar to those of subapical blades from ungrazed sporophytes. Apical blades grew slower in grazed than in ungrazed sporophytes indicating a trade-off between apical and subapical blades when herbivores were present. Thus, compensation occurs in blades directly attacked by grazers and is probably mediated by vertical resource allocation within sporophytes to subapical blades, a suggestion supported by the fact that stipe internodes in these regions grew more on grazed sporophytes. In general, our study indicates that *M. integrifolia* exhibits compensatory growth against the herbivore amphipod *P. femorata*, and we suggest that this could be an important strategy of large kelp species to tolerate moderate grazing intensities.

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1. Introduction

Compensation is defined as a strategy of plants to tolerate deleterious effects of herbivory (Strauss and Agrawal, 1999). The ecological and evolutionary consequences of herbivory have been primarily related to the fitness (i.e. reproductive success over time) of damaged individuals (Stowe et al., 2000). Many plants, however, are subjected to grazing before reproduction and if grazing damage is exhaustive some individuals might never become reproductive. Therefore, compensatory growth responses of terrestrial and aquatic plants have been a common research topic (reviewed in Lodge, 1991; Strauss and Agrawal, 1999).

Compensation seems to be a common tolerance strategy of terrestrial plants against herbivore damage (reviewed in McNaughton, 1983; Strauss and Agrawal, 1999; Stowe et al., 2000). This response is achieved through the modular structure of higher plants

(Silvertown and Gordon, 1989), which is efficiently integrated through specialized tissues for long-distance internal transport (i.e. translocation) of stored and photo-assimilated compounds (Trumble et al., 1993; Taiz and Zeiger, 2002). Hence, plants can quickly re-allocate resources to damaged parts when required. The most demonstrative evidence for compensation comes from mammalian herbivory on the scarlet gilia *Ipomopsis aggregata* (Pursh) V. Grant, in which grazing promotes a highly branched regrowth, resulting in a significantly higher biomass, and a higher number of flowers and fruits per plant in grazed than in ungrazed individuals (Paige and Whitham, 1987).

Experimental studies on compensation in seaweeds or aquatic plants are rare, and so far they have produced contradictory results. For example, the seagrass *Posidonia oceanica* (Linnaeus) Delile can compensate only at low and moderate but not at high levels of simulated herbivory (Vergés et al., 2008). Some coralline algae show compensation against limpets and chitons at non-lethal grazing pressures, i.e. when grazing activity does not destroy or remove growing meristems located deep inside the crust (Steneck, 1982; Littler et al., 1995); the compensatory response to grazing was

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explained by improved nutrient uptake due to epiphyte removal or the activation of new meristems. The rockweed *Fucus vesiculosus* Linnaeus (Phaeophyceae, Fucales) has been explicitly tested for compensatory growth, but did not show this strategy when herbivory was simulated by artificial clipping (Honkanen and Jormalainen, 2002). The absence of compensation by *F. vesiculosus* has been explained in terms of the high dependency of the apical growth meristems on basal assimilatory parts of the thallus. Small scale resource translocation occurs in *F. vesiculosus*, but the absence of well developed sieve tube elements (Schmitz, 1981) may be considered a structural and functional obstacle to compensatory responses.

Giant kelps (Phaeophyceae, Laminariales) are the largest seaweeds of cold temperate coasts worldwide (Estes and Steinberg, 1988; Schiel and Foster, 2006). They form extensive subtidal forests, which support a wide variety of associated fauna (Pérez-Matus et al., 2007; Villegas et al., 2008). Many of the animals inhabiting kelp forests are mesograzers, which can have a significant impact on the maintenance and persistence of kelp populations (e.g. Tegner and Dayton, 1987; Graham, 2002). It has been proposed that some kelp species counteract negative effects of herbivory by rapid growth (i.e. compensatory growth; Toth and Pavia, 2002; Pansch et al., 2008). A growth response might be facilitated by resource translocation, as most kelps have specialized tissues for internal long-distance transport similar to that of higher plants, i.e. sieve tube elements (Schmitz, 1981; Raven, 2003). We are aware of two previous studies (Pfister, 1991; Steinberg, 1995) that tested compensatory responses in kelps with different approaches and contrasting results. Pfister (1991) demonstrated that removal by clipping of reproductive blades (sporophylls) of the intertidal kelp *Alaria nana* Schrader promoted the regeneration of reproductive tissues on the remaining vegetative frond, with a compensated surface area equivalent to that of control seaweeds. Steinberg (1995) investigated the growth response of the kelp *Ecklonia radiata* (C. Agardh) J. Agardh to grazing by the sea-urchin *Holopneustes purpureus* Agassiz, but no compensatory growth response was found for this species.

Compensatory growth in one part of the sporophyte might cause trade-offs in other parts. Trade-offs in resource allocation for growth can be inferred from studies on compound translocation within kelp fronds (e.g. Lobban, 1978; Gómez et al., 2007). Under ideal conditions (i.e. not damaged by grazing) young, actively growing apical blades receive energy required to sustain high growth rates from subapical assimilatory blades (Lobban, 1978; Schmitz, 1981). This pattern might, however, change in the presence of grazers. Energy channelled to or retained in attacked subapical blades might lack in other parts of the sporophyte, such as e.g. sporophylls or adjacent non-attacked blades. The energy status of kelps can be assessed from the proportions of chemical compounds within a frond (e.g. carbon, nitrogen; Gerard, 1982). Therefore, we expect that the translocation of particular compounds in response to localized herbivore attacks will produce differences in the chemical composition of apical and subapical blades from grazed and ungrazed sporophytes. The energy channelled to blades is moved through the stipe internodes. These structures have been suggested as the primary tissues in order to sustain the formation of apical blades at the apex of kelp sporophytes, especially those internodes in the medial zones of the frond (Clendenning, 1971). Since most energy required in attacked blades is probably channelled through the medial internodes we expect that these might grow in response to grazer attacks.

The objective of this study was to investigate whether sporophytes of *Macrocystis integrifolia* respond to herbivore attacks by compensatory growth. Considering the trade-offs that herbivory and compensatory growth might provoke within a sporophyte, we expect (i) that those portions under direct grazing attack will acquire more energy to compensate tissue losses, and (ii) that parts adjacent to the attacked sporophytes will show diminished growth or even losses due to the allocation of energy to attacked tissues. Herein, we tested these hypotheses by comparing the growth responses of grazed and ungrazed sporophytes.

2. Materials and methods

2.1. Study site and organisms

Giant kelp *M. integrifolia* and semi-sessile herbivorous amphipods *Peramphithoe femorata* were collected in a subtidal kelp forest at Los Vilos (31°54'S, 71°31'W) in northern-central Chile and immediately transported to the seawater laboratory at Universidad Católica del Norte in Coquimbo (29°57'S, 71°20'W). We selected kelp sporophytes with no or only few amphipod nests in order to avoid effects of previous grazing history on the experimental individuals. All grazers were carefully removed before preparing the algae for the experiment.

M. integrifolia is a perennial kelp, up to 10 m long. Sporophytes are anchored to the substratum by holdfasts. The frond arises from the holdfast with one or more stipes carrying numerous lateral blades, which develop from longitudinal divisions of the terminal blade advancing toward the apex. Before the first division reaches the apex, a series of new longitudinal divisions are produced with each future blade (Hoffmann and Santelices, 1997). The growth of *Macrocystis*, as in many Laminariales, is generated by three types of structures: the apical meristem at the apex of the frond, the intercalary meristem at the base of each blade and the intercalary epidermal meristem or meristoderm (Hoek, van den et al., 1995).

Amphipod amphipods such as *P. femorata* are seaweed grazers characterized as “kelp curlers” that build nest-like domiciles by curling one or several kelp blades using a cementing web-like amphipod silk (Appadoo and Myers, 2003). They usually graze on portions of the blade inside and outside the nest. *Peramphithoe* species are specialized on algae from the genera *Sargassum* and *Macrocystis* as host (Poore et al., 2008). Since *P. femorata* is a very common mesograzer on *Macrocystis* spp. in Chile, we consider this species as an appropriate model to test for compensatory growth of giant kelps against specialist marine herbivores.

2.2. Experimental design

Twentyfour sporophytes of *M. integrifolia* were placed individually in large outdoor tanks (90 L) with constant air supply and running unfiltered seawater. Twelve tanks were randomly assigned as grazed treatment and the other 12 as ungrazed control. We choose individual sporophytes of equal length (grazed 147.1 ± 17.4 cm, ungrazed 152.0 ± 24.1 cm; t -test $df = 22$, $p = 0.57$) and biomass (grazed 213.8 ± 16.3 g, ungrazed 217.5 ± 25.9 g; t -test $df = 22$, $p = 0.68$). For each sporophyte we measured the elongation rate of 3 apical and 5 subapical blades at 2-day intervals throughout the 14 days of the experiment. To achieve a fully orthogonal design we analyzed only the first 3 subapical blades (Fig. 1), although on each sporophyte we let *P. femorata* graze on 5 of these blades in order to obtain a representative effect of amphipod grazing on *M. integrifolia* (at high amphipod densities in the field, many sporophytes have ≥ 5 amphipod nests; L. Gutow et al., unpublished data). The subapical blades were curled up manually and held as a tube with a rubber band to mimic amphipod nests. One *P. femorata* female each was added to the 5 subapical blades of sporophytes in the assigned grazing treatments ($n = 12$ sporophytes). Preliminary experiments had shown that these amphipods readily adopt artificial nests made from rolled *Macrocystis* blades. We used only ovigerous females because we expected these to have the longest residence times in the nests as a consequence of maternal care activities. To ensure the persistence of the amphipods in the nest we revised the position of the grazers daily. Grazers that abandoned their nests were recovered from the tanks and immediately reintroduced to the nests during daily inspections. Amphipods that repeatedly abandoned their nest were exchanged with a new one of equal size. We also made artificial nests in the 5 subapical blades of the ungrazed treatment but left them without amphipods (control sporophytes; $n = 12$).

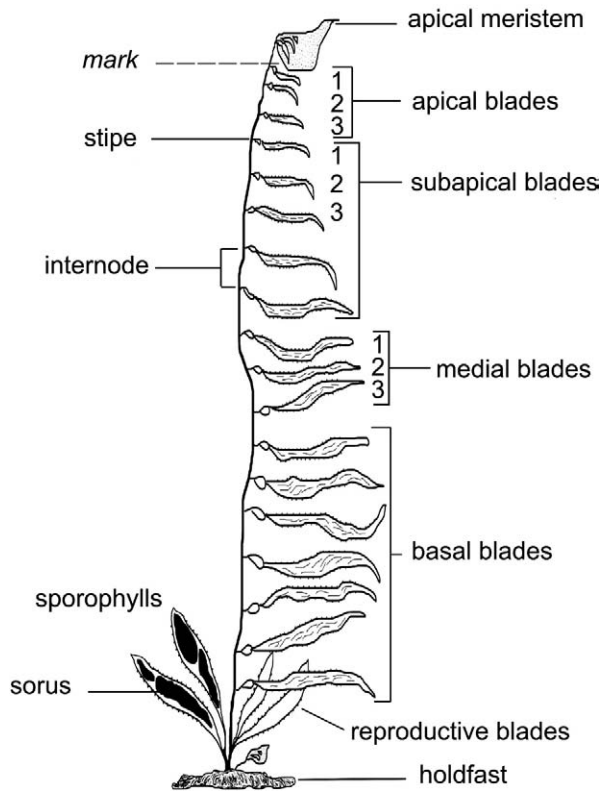


Fig. 1. Schematic representation of the different *Macrocystis integrifolia* parts quantified in the present study in order to examine compensatory growth responses against the amphipod grazer *Peramphithoe femorata*. At the beginning of the experiment, we marked the base of the apical meristem with a thread to avoid confusion while measuring its final length. We choose five subapical blades to shelter amphipods, though growth was only measured on the first three subapical blades.

2.3. Determination of growth in *M. integrifolia*

Growth rates of apical and subapical blades of *M. integrifolia* were determined by the hole-punch method (see e.g. Rothäusler et al., 2009) as elongation per unit time. A hole (diameter: 3 mm) was punched above the growing meristem of the studied blades with a cork borer. The hole was placed at 9 cm from the junction of the pneumatocyst and the blade. If an apical blade was not sufficiently long, the hole was placed in the centre of the total blade length in order to avoid its loss as distal parts of kelp blades are constantly sloughed off. The growth rate was estimated as the total daily displacement of the perforation away from the basal meristem (mm day^{-1}). At the end of the experiment, we also measured the final wet biomass (g) of the apical meristem, apical blades, subapical blades, medial + basal blades, reproductive blades, sporophylls, stipe and holdfast from grazed and ungrazed sporophytes, in order to identify grazer effects on those variables.

We also quantified the length increment ($\text{mm } 14 \text{ day}^{-1}$) of stipe internodes in order to compare whether these structures respond in the same way as the respective blades. We measured the internode lengths between 3 apical, 3 subapical and 3 medial blades from grazed and ungrazed sporophytes at the beginning (L_i) and at the end (L_f) of the experiment. The growth rate was then calculated as G ($\text{mm } 14 \text{ day}^{-1}$) = $L_f - L_i$.

2.4. Determination of carbon, nitrogen, mannitol and laminaran content

We quantified carbon, nitrogen, mannitol and laminaran (reserve compound in brown algae) content of the first apical and subapical blades from grazed and ungrazed *M. integrifolia* sporophytes. Middle

parts of each blade were cut and dried at 38°C for 2 days, and then stored in plastic bags with silica gel for later analysis.

The C and N contents were analyzed at the University of Rostock, Germany. Dried algal material was ground to powder using a mortar and samples of 1–3 mg from the first apical and subapical blades were loaded and packaged into small tin cartridges ($1 \times 6 \times 12 \text{ mm}$). The packages were combusted at 900°C and the absolute contents of C and N were automatically quantified in an elemental analyzer (Elementar Vario EL III, Germany) using acetanilide as standard. The C and N contents were expressed as percentage of mg dry weight (% DW).

Laminaran was extracted from 10–20 mg dried, powdered material from apical and subapical blades, and quantified based on the enzymatic assay described by Gómez and Wiencke (1998). Mannitol content was obtained after extraction from 10–20 mg powdered material from apical and subapical blades, following the HPLC method described by Karsten et al. (1991).

2.5. Statistical analysis

All statistical analyses were carried out using SPSS 11.5. Normality and homogeneity of variance of data and residuals were tested using Shapiro–Wilks and Levene tests, respectively. Additional graphical evaluations were conducted for outliers and severe deviations from normality. If necessary, \log_{10} transformations were applied to meet the assumptions for parametric statistics (Zar, 1999). Elongation rates of apical and subapical blades from grazed and ungrazed sporophytes were compared by a mixed linear model with nested repeated measures with the fixed factors grazing status (G), time (T), blade zone (B) and blade number (P). The random factor included in the analysis was individual algae. In order to select the best structure of the repeated measures covariance matrix, we used the lower Akaike Information Criterion values, which allowed us to select the least information loss within our data (Akaike, 1981). Finally, the model included the effects of the fixed factors and their interaction [grazing (G), time (T) and ($G \times T$)], and the nested factors and their interactions [$B(G)$, $P(B(G))$, $T \times P(B(G))$, $T \times B(G)$]. Changes in blade elongation rates among treatments were tested using F statistics, which were calculated from iterative restricted (residual) maximum likelihood procedures.

To test for significant differences in elongation rates of selected stipe internodes between blades from grazed and ungrazed sporophytes, we used a fully orthogonal nested mixed linear model. The length increment from 3 apical, 3 subapical and 3 medial internodes between day 0 and day 14 was used for the analysis. We included fixed (grazing G , internode zone I , and internode number P) and random (algae) factors in the model. Hence, the sources of variation of the model were G , $I(G)$ and $P(G)$.

We compared the effect of amphipod grazers on the final biomass (g) variables as a one-factor MANOVA. We included the final biomass of the apical meristem, apical blades, subapical blades, medial + basal blades, reproductive blades, sporophylls, stipe and holdfast. If a significant

Table 1

Final total biomass (mean \pm standard error) of apical, subapical, reproductive and basal blades, sporophylls, apical meristems, stipes and holdfasts of grazed and ungrazed *M. integrifolia* were used as dependent variables in MANOVA ($n = 12$ per treatment, data were log transformed).

Source of variation	df	F	p	Grazed (g)	Ungrazed (g)
Apical meristem (g)	1	0.280	0.602	12 ± 2.1	13.6 ± 2.2
Apical blades (g)	1	0.821	0.375	17.6 ± 1.3	19.4 ± 1.4
Subapical blades (g)	1	7.567	0.012	24.6 ± 2.7	36.0 ± 3.1
Medial + basal blades (g)	1	0.241	0.628	71.8 ± 5.0	75.3 ± 5.0
Reproductive blades (g)	1	0.029	0.865	5.6 ± 2.7	6.1 ± 1.8
Sporophylls (g)	1	0.293	0.594	29.4 ± 6.6	34.2 ± 6.0
Stipe (g)	1	0.771	0.389	38.8 ± 1.6	36.9 ± 1.4
Holdfast (g)	1	0.038	0.847	39.5 ± 4.2	38.5 ± 2.5

Results are based on the inter-subject effects of grazing; bold probability value indicates significant differences between grazed and ungrazed sporophytes.

Table 2

Effect of grazing on the elongation rate of apical and subapical blades of *Macrocystis integrifolia* sporophytes maintained for two weeks with and without the amphipod *Peramphithoe femorata*.

Source of variation	Numerator df	Denominator df	F	p
Grazing	1	128	0.690	0.408
Time	6	132	181.963	<0.001
Time × grazing	6	132	0.386	0.887
Blade zone (grazing)	4	128	16.990	<0.001
Blade number (blade zone (grazing))	6	128	41.289	<0.001
Time × blade zone (grazing)	24	132	2.816	<0.001
Time × blade number (blade zone (grazing))	36	132	11.454	<0.001

The results come from a mixed linear model with nested repeated measures design and correspond to Fig. 2. Bold numbers indicate significant differences among treatments.

overall effect of grazing was found, inter-subject effects were analyzed in order to determine which variables were significantly different between grazed and ungrazed sporophytes (Scheiner, 1993). The effect of grazing on carbon, nitrogen, mannitol and laminaran contents (% DW) was also analyzed using one-way MANOVA.

3. Results

Amphipod grazing had a significant effect on the final total biomass of the selected variables (MANOVA Pillai's $T=0.620$, $F=3.053$

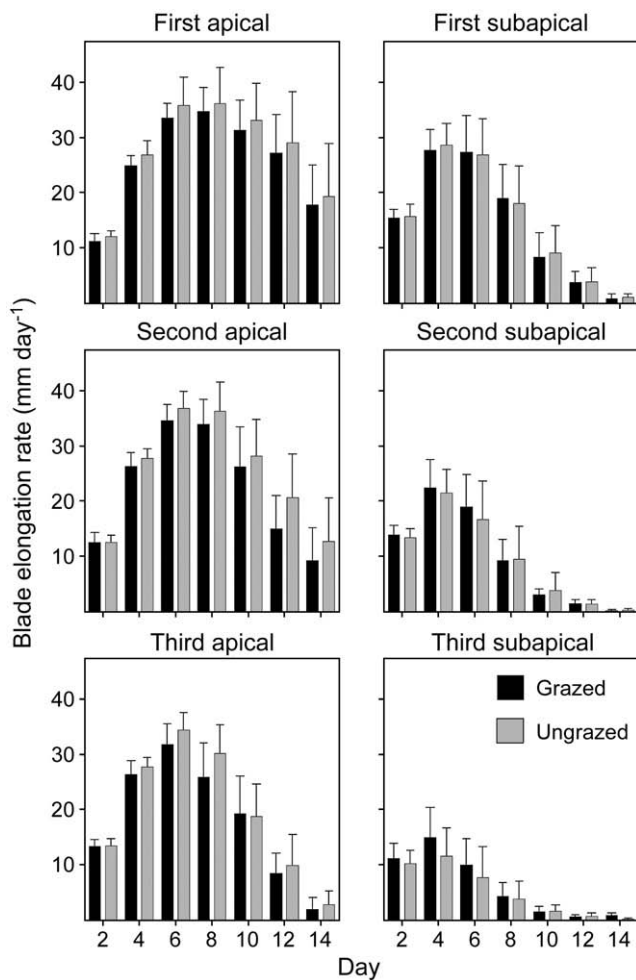


Fig. 2. Elongation rates (mm day^{-1}) of the three apical and subapical blades selected from grazed and ungrazed *Macrocystis integrifolia* sporophytes, which were measured over two weeks at two-day intervals ($n=12$ replicate sporophytes per treatment, mean \pm 95% confidence intervals).

Table 3

Effect of grazing by *Peramphithoe femorata* on the elongation rate of the first, second and third internodes between apical, subapical and medial blades nested within the grazing status of *M. integrifolia* sporophytes.

Source of variation	Numerator df	Denominator df	F	p
Grazing	1	198	6.711	0.01
Blade zone (grazing)	4	198	88.054	<0.001
Blade number (blade zone (grazing))	12	198	1.369	0.183

Values are based on a mixed linear model and correspond to Fig. 3. Bold numbers indicate significant differences between treatments.

$p=0.030$, power = 0.81). The inter-subject effects analysis revealed that the variation was mostly explained by differences in the final biomass of subapical blades (Table 1). At the end of the experiment (i.e. after 14 days), subapical blades from grazed sporophytes had a significantly lower biomass than subapical blades from sporophytes maintained without amphipods (Table 1). This result confirmed that grazing by *P. femorata* had a significant effect on biomass loss at the blade level.

We found a significant interaction among the elongation rates of the different blades from the apical and subapical sections of grazed and ungrazed *M. integrifolia* sporophytes and the duration of the experiment (Table 2). Elongation rates decreased from the first to the third subapical blade, while elongation of all apical blades was nearly equivalent over time (Fig. 2). Over the two weeks, the observed variability was stronger among apical than among subapical blades. In most cases elongation rates of apical blades tended to be lower in grazed than in ungrazed sporophytes, while elongation rates of subapical blades were similar in grazed and ungrazed sporophytes (Fig. 2).

Internode elongation rates were significantly different between grazed and ungrazed sporophytes and also among apical, subapical and medial internodes (Table 3). A large amount of the variation was due to treatment effects on internode elongation in subapical and medial sections of the experimental seaweeds, while internode elongation of apical blades did not differ between the two treatments (Fig. 3). Total sporophyte length of grazed and ungrazed seaweeds was not statistically different at the end of the experiment (t -test, $df=22$, $p=0.37$). However, we found that mean elongation rate (as the difference between final and initial length) of grazed sporophytes was significantly higher than elongation rate of ungrazed seaweeds (t -test, $df=22$, $p=0.005$).

Carbon, nitrogen, laminaran and mannitol content did not differ significantly between apical and subapical blades in grazed and ungrazed sporophytes, respectively (Table 4). Mean carbon and nitrogen contents of apical and subapical blades exhibited a tendency of higher

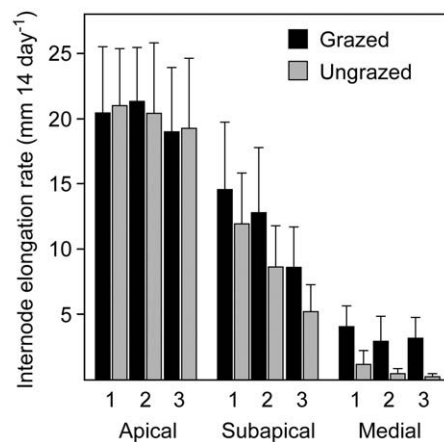


Fig. 3. Elongation rates (mm 14 day^{-1}) of three internodes situated between apical, subapical and medial blades of grazed and ungrazed *Macrocystis integrifolia* sporophytes ($n=12$ replicate sporophytes per treatment, mean \pm 95% confidence intervals).

Table 4

Overall MANOVA results on carbon, nitrogen, laminaran and mannitol contents (% DW) of apical and subapical blades of *Macrocystis integrifolia* maintained for 14 days with and without the herbivorous amphipod *Peramphithoe femorata* ($n = 12$ per treatment, except for mannitol where $n = 9$).

Source of variation	Pillai's <i>T</i>	<i>F</i>	<i>df</i> (hypothesis, error)	<i>p</i>	Power
Grazing	0.095	0.787	4, 30	0.543	0.22
Blade zone	0.145	1.271	4, 30	0.303	0.35
Grazing*blade zone	0.044	0.345	4, 30	0.845	0.18

Values correspond to Fig. 4.

values in grazed than ungrazed treatments (Fig. 4). Laminaran contents, in contrast to our expectations, showed higher values in grazed than ungrazed subapical blades. On the other hand, apical blades revealed a tendency of higher values in the ungrazed treatment (Fig. 4). The mannitol contents of both apical and subapical blades revealed a trend (albeit non-significant) of lower values in grazed than in ungrazed sporophytes (Fig. 4).

4. Discussion

4.1. Response of *Macrocystis* to grazers

Grazing of the herbivorous amphipod *P. femorata* caused a substantial biomass loss in subapical blades of the kelp *M. integrifolia*. Despite significant damage, grazed blades upheld high elongation rates throughout the entire experiment, comparable and sometimes even higher than growth of subapical blades from control sporophytes. We consider this a strong indication for compensation in attacked subapical sections of *M. integrifolia* sporophytes. This compensatory growth in subapical blades apparently produced a trade-off in apical blades, which grew less in the grazed treatments.

The potential for compensation in *M. integrifolia* has been tested in the present study by simulating moderate grazing pressures. Under controlled experimental conditions, Rothäusler et al. (2009) imposed up to ten times the grazing pressure of the present study on *Macrocystis* spp. sporophytes. Similar to the present study, they observed a significant reduction in frond biomass and decreased elongation rates of apical blades from grazed sporophytes. At very high grazing pressure, sporophytes may not be able to compensate losses due to herbivory. In *M. pyrifera* (Linnaeus) C. Agardh populations from California, an over-infestation by the kelp-curling amphipod *Amphithoe humeralis* Stimpson provoked serious damage to kelp forests during late-spring and early-summer (Graham, 2002). In natural populations these extreme events might be rather sporadic (e.g. under "El Niño" conditions). Under normal conditions the number of amphipods per sporophyte is moderately low (L. Gutow et al., unpublished data), permitting compensatory responses.

4.2. Compensatory growth in large kelps

Compensatory responses to herbivores have been repeatedly inferred for large brown algae from the orders Fucales and Laminariales (Steinberg, 1995; Honkanen and Jormalainen, 2002; Toth and Pavia, 2002; Pansch et al., 2008). Besides their large sizes, Fucales and Laminariales differ from other seaweeds in that they have a high degree of differentiation and specialized cells involved in translocation processes, i.e. equivalent to the sieve-tube elements of vascular plants (Raven, 2003 and references therein), which is one of the most important morphological adaptations involved in compensatory growth of vascular plants (reviewed by McNaughton, 1983; Stowe et al., 2000). The lack of compensation in *F. vesiculosus* was inferred to be due to limited tissue differentiation within the thallus of this rockweed (Honkanen and Jormalainen, 2002). *M. integrifolia*, in contrast, has a substantially higher degree of morphological differ-

entiation than most *Fucus* species, which suggests an increased potential for compensatory growth against herbivore grazing.

Giant kelp (*Macrocystis* spp.) features ontogenetic changes in the photosynthetic capacity of blades in different sections of the sporophyte. Photosynthetic capacity per unit blade area increases with distance from the apical meristem (Clendenning, 1971). The resource allocation pattern within the thallus of giant kelp *Macrocystis* spp. is therefore characterized by high-velocity transport of photo-assimilates from basal and medial to apical blades (37–78 cm/h; Parker, 1965; Schmitz and Srivastava, 1979). In the present study, however, compensatory growth of subapical blades indicates intensified transport of assimilates to those subapical blades instead of increased transport to newly formed apical blades in response to grazing by *P. femorata*, which is the normal pattern of translocation in this large kelp. Intensified translocation from lower positioned blades to subapical blades might require a stronger internode structure in order to generate more cellular components involved in this transport. Although we only measured internode length, the production of more specialized cells involved in translocation (e.g. medullary filaments; Parker, 1965) might also lead to an increase in internode width.

The chemical composition of blades was similar to those reported for *M. integrifolia* populations from the north-eastern Pacific coast (e.g. Rosell and Srivastava, 1984; 1985). Although no significant differences in carbon, nitrogen, laminaran and mannitol content between apical and subapical blades were observed along with missing effects of grazing on these compounds, the data indicate a tendency of higher carbon and nitrogen values in grazed compared to ungrazed treatments. Using ^{14}C -labelling techniques, Hellebust and Haug (1972) reported that in the blade of *Laminaria digitata* (Hudson) J.V. Lamouroux the amino acid alanine played a quantitatively more important role than mannitol in the internal transport of photo-assimilates. In addition, glutamic and aspartic acid are commonly translocated through the transporting sieve elements (Schmitz, 1981). Based on these considerations, it is reasonable to assume that particular amino acids might be involved in the growth response of grazed *M. integrifolia* sporophytes by increased transport rates to attacked sections of the plant. Future studies should examine whether

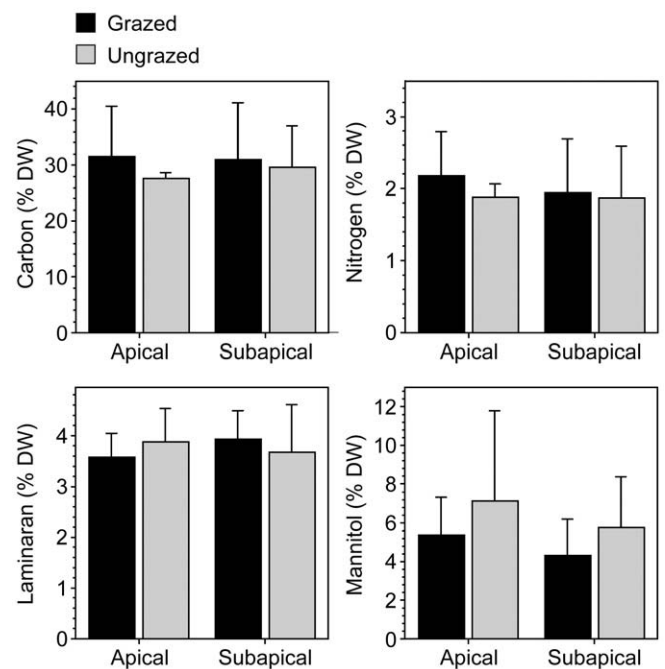


Fig. 4. Carbon, nitrogen, laminaran and mannitol contents (% DW) of the first apical and subapical blades from grazed and ungrazed *Macrocystis integrifolia* sporophytes (mean \pm SD, $n = 12$ replicates, except for mannitol where $n = 9$).

there exists a quantitative trade-off in these translocated products between adjacent blades (grazed versus ungrazed) of the same individual.

The observed trade-off between apical and subapical blades might even be stronger in the natural environment than in our laboratory experiments. Herein we also made artificial nests on subapical blades of ungrazed sporophytes in order to ensure that effects are truly caused by the grazing activity of the amphipods. In a natural situation blade-rolling and grazing are intimately linked and ungrazed blades are usually not rolled up to a nest. Rolling up blades to artificial nests will reduce the area of photosynthetically active surfaces and thus weaken primary production of ungrazed subapical blades, as has been reported for leaf-rolling caterpillars on terrestrial plants (Sagers, 1992). These considerations support the suggestion that compensatory growth produces a trade-off within individual sporophytes, because the observed effects should have been even stronger if ungrazed blades would not have been rolled up.

Macrocystis is one of the most productive seaweeds in the world's oceans (Schiel and Foster, 2006; Graham et al., 2007). This high productivity, however, does not seem to be supported by chemical defense to counteract detrimental effects of herbivory. *M. integrifolia* does not exhibit inducible chemical defense by non-polar secondary metabolites against generalist amphipod mesograzers (Macaya et al., 2005; Macaya and Thiel, 2008). Polar anti-herbivore compounds such as phlorotannins (Amsler and Fairhead, 2006) have not yet been tested as chemical defense in *M. integrifolia*, which is known to contain relatively high quantities of reactive phlorotannins (see Pansch et al., 2008). Phlorotannins have secondary functions as defensive compounds and primary roles in cell-wall construction (Schoenwaelder and Clayton, 1999; Arnold and Targett, 2003). The compensatory growth of grazed subapical blades might be related to increased phlorotannin concentrations acting as both deterrent compounds against *P. femorata* or as cell-wall components to sustain high growth rates of attacked blades. However, the role of phlorotannins as grazer deterrents in *Macrocystis* is rather speculative and further studies are required to solve this question.

4.3. Compensation in aquatic environments

Compensatory growth against grazing does not seem to be uncommon in aquatic plants, as recently documented for the freshwater pondweed *Potamogeton pectinatus* L. (e.g. LaMontagne et al., 2003; Nolet, 2004). In this species, compensation is achieved by increased reallocation of resources from storage organs such as roots, tubers and rhizomes to leaves and meristems. The same physiological mechanism allowed for compensatory growth of the seagrass *P. oceanica* (Vergés et al., 2008). Therefore, compensation involves a redistribution of assimilated energy by aquatic plants facilitated by the capability to store compounds in special below-ground organs.

In seaweeds, the ability to compensate losses caused by grazers appears to be limited to species with specialized translocation structures and conditions of moderate grazing pressure. Our results indicate that compensatory growth by *M. integrifolia* might be achieved in response to tissue losses inflicted by the mesograzer *P. femorata*. Compensatory growth in the seagrass *P. oceanica* also occurred under low and moderate grazing pressures (Vergés et al., 2008). A similar response has been observed in crustose coralline red algae to non-lethal consumption of tissues by specialized grazers such as limpets (Steneck, 1982) and chitons (Littler et al., 1995), which are of relatively low mobility. In these crustose seaweeds, the meristematic tissues responsible for growth are located deep inside the crust, far from the superficial grazing impact of these herbivore molluscs. In contrast, elevated grazing pressures exerted by stronger herbivores, e.g. sea urchins, might drastically reduce the potential for compensatory growth of macroalgae or seagrasses. This could also explain the results of Steinberg (1995) who found no compensatory growth in the kelp *E. radiata* grazed by the sea urchin *H. purpurascens*. According to the author, in his experiments sea urchins

occasionally removed up to 50% of the biomass of individual algae, reflecting an extremely high grazing pressure.

5. Conclusion

Herein, we observed the potential of attacked blades of *M. integrifolia* sporophytes to compensate losses under moderate herbivore pressure. We consider this as a strategy to maintain the amphipods and their nests as far away as possible from the sensitive apical meristem, which is the primary site of kelp growth. This response most likely depends on the reallocation of specific internal resources other than laminaran and mannitol (e.g. amino acids) to damaged blades. Therefore, our results suggest that kelp responses to herbivores are integrative and plastic within kelp sporophytes, though working mainly at moderate grazing levels and limited or absent abiotic stress. Specific environmental conditions such as “El Niño” Southern Oscillation (ENSO) might constrain kelp compensation against grazers, since the co-occurring ecological changes may cause herbivore outbreaks. Low nutrient availability and high temperatures during El Niño phases may further suppress kelp physiological performance. Studies examining compensatory responses under nutrient limitation, high UV-radiation, elevated temperatures or intensified grazing pressures, and the interactions among these factors will help us to better understand kelp dynamics in fluctuating environments.

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