

Effect of detachment on the palatability of two kelp species

Eva Rothäusler^{1,2} & Martin Thiel^{2,3,*}

¹Institut für Aquatische Ökologie, Albert-Einstein-Str.3, 18057 Rostock, Germany; e-mail: evarot@gmx.de;

²Facultad Ciencias del Mar, Universidad Católica del Norte, Larrondo 1281, Coquimbo, Chile; ³Centro de Estudios Avanzados en Zonas Áridas (CEAZA), Coquimbo, Chile

*Author for correspondence: e-mail: thiel@ucn.cl; fax: +56 51209812

Key words: defence, dislodgement, detachment, floating, macroalgae, palatability

Abstract

Many species of macroalgae survive after becoming dislodged from their primary substratum, but little is known about their capacity to express anti-herbivore defences after detachment. We examined the effect of detachment on the relative palatability of the two kelp species *Lessonia nigrescens* and *Macrocystis integrifolia* to mesograzers. Laboratory and field experiments were conducted on the northern-central coast of Chile to investigate whether (i) time after detachment and (ii) grazing on detached and attached algae could trigger internal defence mechanisms in the algae, which may have acted as deterrents to grazing. In order to examine palatability, feeding assays were run after each experiment using fresh algal pieces and artificial food. Time after detachment had a significant influence on palatability of *L. nigrescens* but not of *M. integrifolia*. During the first 12 days of detachment, detached *L. nigrescens* held in grazer-free laboratory tanks were not significantly more palatable than attached conspecifics from the field but thereafter detached individuals became more palatable. Floating individuals of *M. integrifolia* showed no effect of detachment, indicating that this alga maintains its defence after detachment. An experiment conducted in the field confirmed these results for *M. integrifolia*. An additional laboratory experiment confirmed that attachment status plays an important role on algal defence reaction for *L. nigrescens* when exposed to grazers. Detached and previously grazed individuals of this species were less palatable than grazer-free control algae, but grazing had no effect on palatability of attached algae. Our results indicate that kelps have varying capacities for development of anti-grazing responses once they become detached, possibly depending on their capacity to float and survive after detachment.

Introduction

Defence mechanisms against herbivorous grazers have been reported for numerous species of marine macroalgae (e.g. Van Alstyne, 1988; Paul & Van Alstyne, 1992; Sotka et al., 2002). These mechanisms include (i) morphological defences such as calcification of structures, increase in tissue toughness and/or modification of growth form (Littler & Littler, 1980; Hay et al., 1988; Hay, 1991) and (ii) chemical defences including the production of noxious or unpalatable chemical compounds, which are termed secondary metabolites, because usually they are not associated with the primary metabolism of the plant (Bazzaz

et al., 1987; Duffy & Hay, 2001). Among chemical defences three mechanisms have been recognized: (1) constitutive defence, where secondary metabolites are produced continuously, independent of attack or presence of grazers (e.g. Pavia & Toth, 2000), (2) inducible defence, where algae enhance production of secondary metabolites when under attack by herbivores (e.g. Sotka et al., 2002), and (3) activated defence triggered by injury and acting extremely rapidly (seconds to minutes) by converting a less potent stored secondary metabolite to a more potent one (e.g. Paul & Van Alstyne, 1992; Cetrulo & Hay, 2000). The three mechanisms function as herbivore deterrents, and they have been reported from a wide diversity of different

macroalgae, including brown, green, and red algae (e.g. Steinberg, 1984; Paul & Fenical, 1986; Van Alstyne, 1988; Peckol et al., 1996; Cetrulo & Hay, 2000; Pavia & Toth, 2000; Van Alstyne et al., 2001; Sotka et al., 2002; Taylor et al., 2002).

Two classical approaches have been employed to test for the presence of chemical defences in marine macroalgae. The first is based on an empirical method where algae that were exposed to either: (i) naturally occurring herbivory, (ii) experimental grazing levels or (iii) artificial injury *in situ*, are collected from the field and evaluated for the presence of chemical or morphological defences, or (iv) they were injured after collection (e.g. Van Alstyne, 1988, 1989; Paul & Van Alstyne, 1992; Peckol et al., 1996; Cronin & Hay, 1996a,b; Pavia et al., 1997; Hammerstrom et al., 1998; Cetrulo & Hay, 2000). In the second type of studies, algae are usually maintained detached in experimental outdoor flow-through systems, where they are grown in tanks of variable sizes and are treated with different grazing regimes and after exposure tested for the presence of defensive mechanisms (e.g. Toth & Pavia, 2001; Sotka et al., 2002; Taylor et al., 2002). Although this allows all algae in a tank to be exposed to the same light and nutrient regimes over time, it does not necessarily reflect the natural condition where algae are growing attached to a firm substratum.

Benthic algae, particularly in shallow areas, are exposed to varying degrees of wave and water currents, which may dislodge them from the substratum. This detachment can cause physical stress for the plants, possibly altering seaweed palatability, as is known in cases of desiccation (Renaud et al., 1990). Stressed algae often show a limited potential for chemical defence (Renaud et al., 1990; Cronin & Hay, 1996b), and it is expected that this is also true for detached algae. However, little is known about the effect of detachment on the presence of defence mechanisms in marine macroalgae even though this appears important in understanding the mechanisms of chemical defence in a natural situation. Understanding the relationship between detachment and defence appears important since assemblages of unattached seaweeds are commonplace in nature (e.g. Benz et al., 1979; Ólafsson et al., 2001; Hirata et al., 2001; Thiel & Gutow, 2004). Storms frequently cause detachment of benthic macroalgae (Norton & Mathieson, 1983), which then contribute to floating or drifting populations. Some macroalgae possess gas bladders (e.g. the giant kelp *Macrocystis pyrifera*) or a plant body that temporarily acts as a balloon (e.g. the entire thallus of *Colpomenia perigrina*)

(Norton & Mathieson, 1983), allowing them to float at the sea-surface. Other species of algae have no floating potential and after detachment sink to the sea-floor where they might contribute to a species-rich assemblage of drifting macroalgae in shallow waters (Benz et al., 1979; Norton & Mathieson, 1983; Norkko & Bonsdorff, 1996).

After detachment, macroalgae may be exposed to intense herbivory (biological stress). For example, floating macroalgae harbour many animals, including herbivores, that have originally been living on them and thus can quickly consume them (Ingólfsson, 1995, 1998). Also drift-algae in shallow waters are commonly inhabited by a wide diversity of mesograzers, in particular amphipods and isopods (Inglis, 1989; Geertz-Hansen et al., 1993; Ingólfsson, 2000; Brooks & Bell, 2001). Floating algae may survive for extended periods at the sea surface (Hobday, 2000) despite high abundances of grazers, but drifting algae often face high grazing pressure from a wide diversity of benthic grazers and may have little chance to survive for long (see e.g. Rodriguez, 2003). Thus, it can be hypothesized that kelp species that float after detachment may be capable of maintaining their defence after detachment, while kelp species that sink after detachment may lose their defence capacity shortly after detachment.

Here we tested whether two kelp species from the SE-Pacific, that differ in their behaviour (floating or drifting) after detachment, maintain their defences, or whether they lose them due to physiological alterations caused by detachment. Plants of *Lessonia nigrescens* are negatively buoyant and sink to the seafloor after detachment where they may contribute to a large pool of drifting algae. In contrast, *Macrocystis integrifolia* floats after detachment and may travel for a long time with ocean currents (e.g. Helmuth et al., 1994). Field and laboratory experiments were conducted on the northern temperate coast of Chile in order to learn how detachment affects the palatability and thus the defence capacity of these two macroalgae.

Materials and methods

Both laboratory and field experiments were carried out to test the changes in palatability following extended detachment of the two kelp species. Laboratory experimentation was done with *Lessonia nigrescens* and *Macrocystis integrifolia* while field experimentation was carried out with *M. integrifolia* only. An additional laboratory experiment was conducted with *L.*

nigrescens to test whether defence can be induced in attached as well as in detached plants of this species.

Collection and culture conditions of macroalgae

Kelps used in the laboratory were collected by hand during low-tide in the vicinity of Coquimbo, Chile (Figure 1). After collection, algae were kept in a cooler at their ambient temperature and immediately transferred to seawater tanks at the Marine Seawater-Laboratory of Universidad Católica del Norte, Coquimbo. Grazers and epiphytes were carefully removed by hand from test algae prior to experiments. Laboratory experiments were either conducted in a large flow-through seawater tank, containing ≈ 1800 L of seawater, or in plastic aquaria measuring $10 \times 19 \times 13$ cm and containing ≈ 1.5 L of seawater. The tank was supplied with an air pipe and with flowing seawater. The large tank was used to test for the effect of extended detachment in *L. nigrescens* and *M. integrifolia* whereas the small aquaria were used for the induction experiment with *L. nigrescens*.

The small aquaria received filtered ($10 \mu\text{m}$ cotton cartridge) seawater that was continuously pumped from the shallow subtidal waters of Bahía La Herradura into 4 plastic reservoirs (70 L) from where it was

then redistributed. Flow regulators were used to supply each aquarium with an individually-controlled flow rate ($\approx 0.1 \text{ L h}^{-1}$). Aquaria were additionally maintained with continuous aeration. All laboratory experiments were conducted in outdoor tanks and aquaria with algae were shaded with a black plastic cloth in order to protect them from direct sunlight.

The field experiment with *M. integrifolia* was carried out at Isla Damas (Figure 1) under natural conditions. With this field experiment we also tested the effect of extended detachment.

Mesograzers used in experiments and feeding assays

The consumers used to determine algal palatability after the laboratory and field experiments were either the amphipods *Parhyalella ruffoi* and *Hyale hirtipalma* or the isopod *Isocladus bahamondei*. The amphipod *P. ruffoi* was also used in the grazing treatment in the induction experiment. All mesograzers were previously observed living and feeding on a variety of different macroalgae (e.g. Thiel, 2002), indicating that they are generalist grazers not specialized to a particular algal species. Amphipods and isopods were collected from mixed assemblages of drift algae at Playa Guayacán and from intertidal algae in La Pampilla (Figure 1).

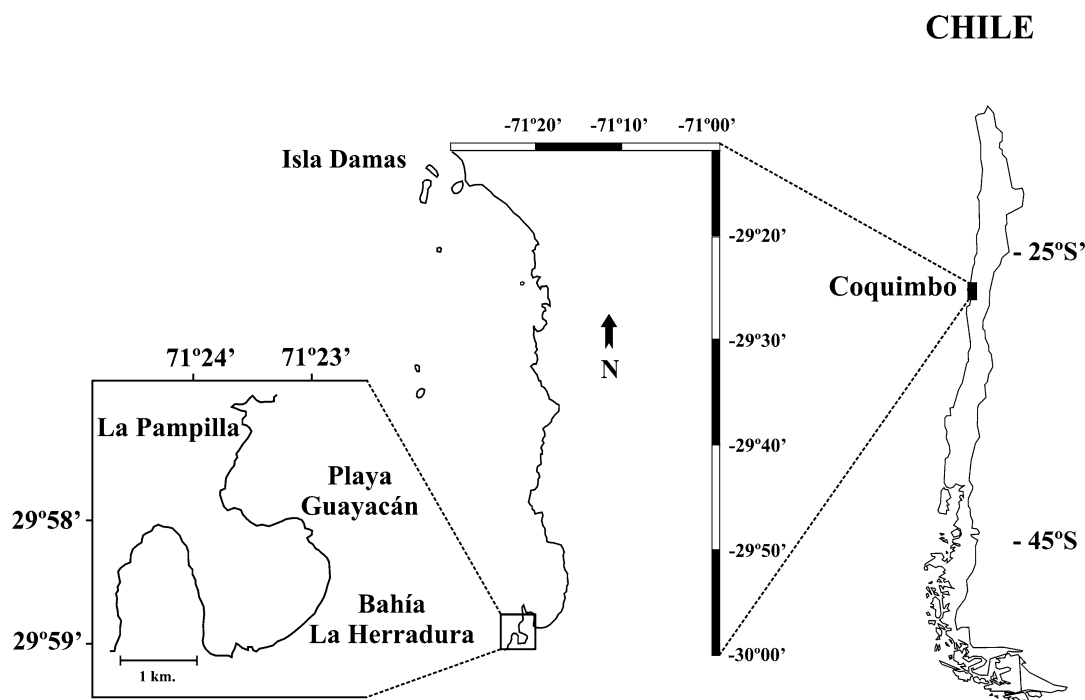


Figure 1. Map of Chile with the locations of the sampling sites in the vicinity of Coquimbo.

Freshly collected individuals of all grazers (*P. ruffoi*, *H. hirtipalma* and *I. bahamondei*) were used in feeding assays after each experiment in order to examine the palatability and thereby the defensive mechanisms of the selected macroalgae.

General design of the feeding assays to examine algal palatability

All feeding assays were conducted in a culture room (12 h L: 12 h D; 15 ± 1 °C; light intensity $40 \pm 10 \mu\text{m photons m}^{-2} \text{s}^{-1}$). Choice and no-choice feeding assays were done mainly with fresh algal pieces but also with artificial agar-based food (see below). In choice feeding assays the grazers were offered two algal pieces simultaneously, such that they had the possibility to express a dietary choice. In the no-choice feeding assays grazers could only feed on the offered alga or they would starve. Feeding assays were conducted in petri-dishes (diameter ≈ 8.8 cm; volume ≈ 30 mL). For feeding assays with fresh algal pieces, we determined the amount consumed in mg, and for assays with artificial pieces we counted the number of squares consumed (see below for details). All feeding assays were carried out with five replicates ($n = 5$), unless noted otherwise.

Preparation of artificial food

After completing the respective algal treatments, a lipophilic crude extract was obtained from one piece of each alga. The extraction was done for 48 h with dichloromethane according to a 1:2 ratio (1 g algae wet weight: 2 mL dichloromethane). Dichloromethane extracts only the lipophilic compounds, and thus any non-lipophilic compounds, which may also contain feeding deterrents, are disregarded by this procedure. The lipophilic extract was dropped on freeze-dried and finely powdered *Ulva lactuca*. The dichloromethane then evaporated leaving behind the compounds extracted from the algal tissues. To determine the amount of *Ulva* powder for every sample, algae were weighed before adding the dichloromethane. A ratio 3:1 (g alga wet weight: g dried *Ulva* powder) was used in order to obtain approximately similar proportions between the dried powder and the wet mass of the algae. After evaporation of the lipophilic extract, 8 mL distilled water was added to the *Ulva* powder. A specific amount of agar (0.36 g) was mixed with 10 ml distilled water and heated to boiling point in a microwave. After the agar cooled down to 40 °C the *Ulva* powder with the lipophilic crude extract was added. The agar/*Ulva* mix

was poured into a mold lying over a fly mesh (mesh size 1 mm²) and 200 squares were cut out after hardening. The artificial food went directly into feeding assays.

The experiments

1. *Effect of extended detachment on the palatability of two kelps in an experimental tank:* This laboratory experiment was run in the large outdoor tank between March and May 2003. The aim was to examine whether kelps detached from their primary substratum become more palatable to mesograzers after 3, 6, 12, 24 and 42 days of detachment compared to attached conspecifics taken directly from their natural substratum in the field. Detached algae were held in a grazer-free environment in the laboratory tank, while attached conspecifics in the field were exposed to the natural grazing regime.

Apical parts of *M. integrifolia* were collected from Isla Damas and those of *L. nigrescens* from La Pampilla. Algae were maintained together with two other detached algal species (*Ulva* sp. and *Cryptomenia obovata*) in the large tank since detached algae also accumulate in multi-species assemblages in the field. The algae in the tank (laboratory treatment) were stirred briefly, twice a day, to provide some water movement. Palatability was tested after 3, 6, 12, 24 and 42 d in choice feeding assays with detached individuals from the tank (laboratory treatment) and attached individuals from the field (field treatment) that were collected on the same day. In each petri-dish ($n = 5$) we placed three amphipods (*P. ruffoi*) and allowed them to feed for three days.

2. *Effect of detachment on the palatability of *Macrocystis integrifolia* in the field:* In order to test the detachment effect on the palatability of apical parts of *Macrocystis integrifolia*, a field experiment was carried out from January to May 2003 at Isla Damas. Both floating and natural attached plants were exposed to natural grazer conditions in the field. Algae were detached from the primary substratum in January 2003, placed in wide-mesh bags (mesh size: 65 mm), and tethered at the sea surface for the duration of the experiment. Palatability was tested after 6, 24, 42, 67 and 97 d in choice feeding assays with apical blades from tethered floating and from natural attached plants. In each petri-dish ($n = 5$) we placed five amphipods (*H. hirtipalma*) and allowed them to feed for three days. Additionally, we conducted identical feeding assays in which we used six isopods (*I. bahamondei*) instead of the amphipods.

3. *Induction experiment with attached versus detached *Lessonia nigrescens**: An outdoor laboratory experiment was conducted during austral fall (May) 2003 to examine whether attached or detached *L. nigrescens* plants are more susceptible to amphipod grazing. A total of 35 complete juvenile plants (≈ 18 g) of the brown alga *Lessonia nigrescens* were sampled randomly during low tide in the intertidal zone of La Pampilla (see Figure 1). During collection, all juvenile plants were carefully removed with their complete holdfast from the substrate using a scalpel. The holdfasts of these juvenile plants had a diameter of approximately 8 cm and a blade length (5–8 blades per plant) of 10–15 cm. Five plants were frozen at -40°C immediately after sampling in order to represent the natural level of defence (natural attached algae). The remaining 30 plants were distributed individually over 30 aquaria, each with a volume of 1.5 L and continuously flowing seawater. To each of the aquaria we added one complete juvenile plant of *L. nigrescens* but in one half of the aquaria ($n = 15$) the algae were maintained as detached individuals whereas in the other half ($n = 15$) the juvenile plants were carefully sewn with their holdfasts onto the aquaria wall, representing attached plants. All algae continued to grow during the experiment, and the “attached” plants firmly grew onto the plastic wall of the aquarium.

The experiment was separated into an acclimation and a treatment phase, each lasting 10 d. The acclimation phase was included to adjust the defence level after an unknown consumption history in the field. After the acclimation phase, small apical blades were taken from 5 attached and from 5 detached plants and stored in the freezer for 2 d at -40°C . All frozen algae pieces were later compared in artificial food feeding assays (see above).

In the following treatment phase, the factor direct grazing with *P. ruffoi* was tested to examine whether grazing attacks by amphipods might induce defences in detached as well as in attached algae. The algae were exposed to two different grazing levels (direct grazing and grazer-free control): 10 amphipods *P. ruffoi* each were added to one half of the detached *L. nigrescens* ($n = 5$) and to one half of the attached *L. nigrescens* ($n = 5$), while the other half of the detached ($n = 5$) and attached *L. nigrescens* ($n = 5$) were left without grazers as control treatments. After the treatment phase, small apical blades were cut off from all algae in order to carry out feeding assays with artificial food as well as with fresh algae pieces.

We conducted choice-feeding assays with 4 individuals and no-choice feeding assays with 2 individuals of *P. ruffoi*. Choice-feeding assays with artificial food (made from the frozen apical blades) were carried out after the acclimation phase to examine for differences between (A1) the natural attached and the control detached algae pieces, and between (A2) natural attached and control attached pieces. After the treatment phase, choice-feeding assays were carried out (T1) between grazed detached and control detached pieces, (T2) between grazed attached and control attached algae pieces, (T3) between control attached and control detached, and (T4) between grazed attached and grazed detached. Additionally no-choice feeding assays were done (A3; T5). All feeding assays with artificial food lasted for 2 d. The fresh algae choice and no-choice feeding assays were conducted with the same treatment combinations as described for the artificial food assays, with the exception that the assays were terminated after 3 d.

Statistical analysis

The same statistical procedure was used for the laboratory and the field experiment, testing for the effect of extended detachment and grazing on algal palatability. Choice feeding assays, testing for differences in palatability (consumption) between attached and detached algae were analyzed with a t-test for dependent samples for each sampling date, rather than with a 2-way ANOVA, because some data sets contained negative consumption values caused by algal growth during the assays. Prior to analysis the data were inspected for normality, using Cochran’s test. When data were normal a t-test was used, or alternatively a non-parametric Wilcoxon matched pairs test was conducted.

The consumption data from the induction experiment with *L. nigrescens* were examined for normality using the Cochran’s test and $\ln(x + 1)$ transformed if necessary. Choice feeding assays were analyzed with a t-test for dependent samples. No-choice feeding assays were analyzed with a 2-way ANOVA, with the fixed factors grazing and attachment status, or with the corresponding non-parametric Kruskal-Wallis test. When the ANOVA revealed significant differences, a post-hoc Tukey HSD was applied.

Results

1. *Effect of extended detachment on the palatability of two kelps in an experimental tank*: In the assays designed to measure the effect of detachment

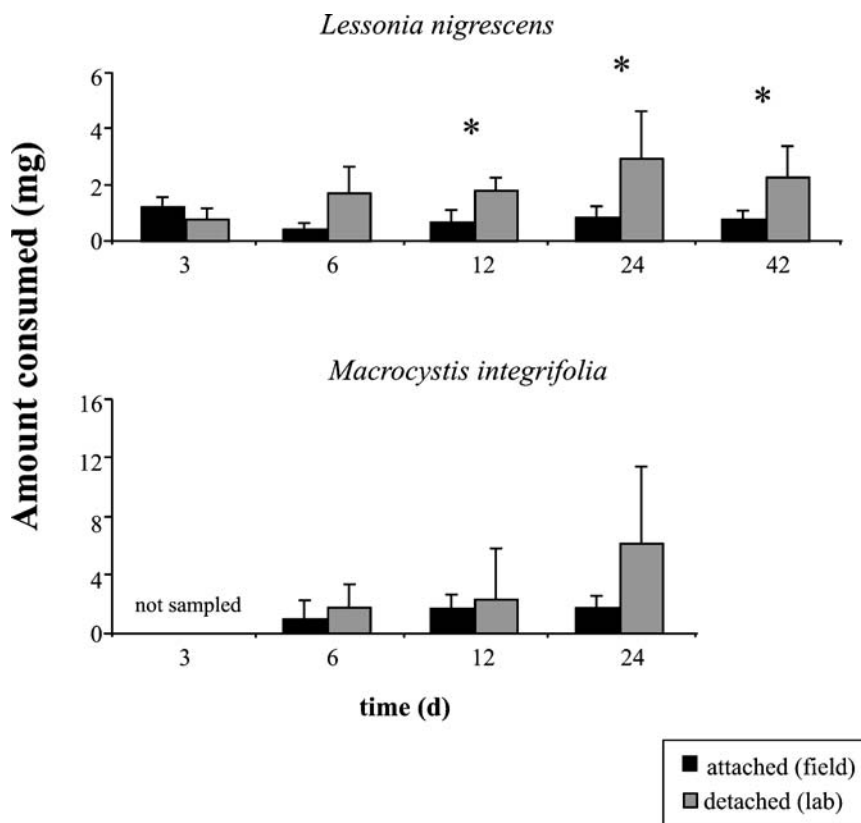


Figure 2. Mean consumption (mg) by the amphipod *Parhyalella ruffoi* of *Lessonia nigrescens* and *Macrocystis integrifolia* after different days of detachment. At each sampling date, grazers were offered a choice between plants from the field (attached) and plants from the experimental tank (detached); error bars represent + 1 SD ($n = 5$). (* $p < 0.05$).

on palatability of the two kelps *Lessonia nigrescens* and *Macrocystis integrifolia*, a clear tendency was evident for *L. nigrescens* (Figure 2). The palatability of detached *L. nigrescens* held in the grazer-free laboratory tank increased over time. At days 12, 24 and 42 after detachment, the grazer *P. ruffoi* consumed significantly more from the detached pieces (tank) than from the attached pieces (t -test for dependent samples, $t = -7.097$, $df = 4$, $p = 0.002$, Wilcoxon matched pairs test $p = 0.043$ and Wilcoxon matched pairs test $p = 0.043$, respectively, $n = 5$) (Figure 2). Consequently, for *L. nigrescens* the time after detachment appeared to play an important role in the expression of defensive mechanisms. In contrast, the giant kelp *M. integrifolia* did not reveal any significant differences during any of the sampling dates (t -test for dependent samples, $p > 0.05$).

2. *Effect of detachment on the palatability of Macrocystis integrifolia in the field*: The time after detachment (6, 12, 24, 42, 67 and 97 days) had no apparent

effect on the palatability of *M. integrifolia* blades from detached compared to attached plants (Figure 3). No statistical differences were detected between the algae tips neither for the feeding assays using the amphipod *H. hirtipalma* nor for those with the isopod *I. bahamondei* (Table 1).

Table 1. Result of t -test for dependent samples for the mean consumption (mg) of *Macrocystis integrifolia* by the two grazers after different time intervals. wx = Value from Wilcoxon matched pairs test

Time intervals (d)	d.f.	<i>Hyale hirtipalma</i>		<i>Isocladus bahamondei</i>	
		t	p	t	p
6	4	0.007	0.995	2.347	0.079
12	4	1.173	0.306	1.273	0.272
24	4	-0.074	0.945	0.227	0.831
42	4	0.038	0.971	0.524	0.627
67	4	1.632	1.178	1.608	0.183
97	4	0.865	0.436	wx	0.08

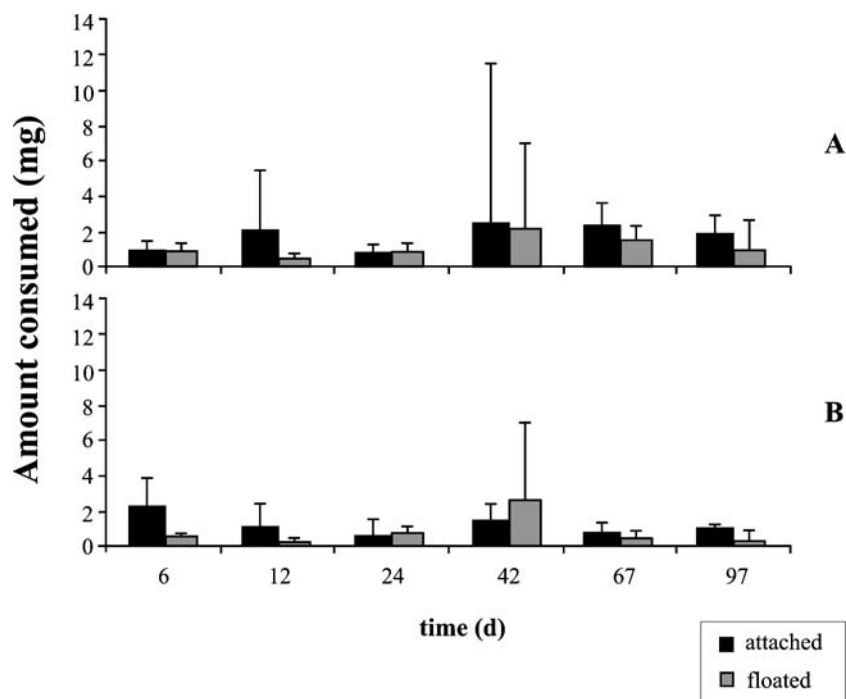


Figure 3. Mean consumption (mg) of *Macrocyctis integrifolia* by the grazers: (A) *Hyale hirtipalma* and (B) *Isocladus bahamondei* after different sampling dates (d). Grazers were offered simultaneously an artificially floated alga piece and an alga piece from plants growing on substratum (attached); error bars represent +1 SD ($n = 5$).

3. *Induction experiment with attached versus detached Lessonia nigrescens*: After the acclimation phase, when offering the amphipod *P. ruffoi* a choice between artificial food made from attached and detached algae (A1), and between those made from control or natural algal pieces (A2), no statistical differences were detected (Figure 4A, *t*-test for dependent samples, $t = 0.507$, $df = 4$, $p = 0.639$ and Figure 4B, *t*-test for dependent samples, $t = 0.040$, $df = 4$, $p = 0.97$). The same result was found for the no-choice feeding assays with artificial food (A3) (Figure 4C, Kruskal-Wallis test, $p = 0.685$).

In the assays designed to measure the palatability in response to direct grazing after the treatment phase, we found statistical differences for both choice feeding assays (T1) with artificial and fresh algal pieces. Amphipods *P. ruffoi* consumed in the two assays (Figures 5A and 6A, respectively) significantly more of the detached ungrazed control pieces than from the detached grazed pieces (artificial food: Figure 5A, *t*-test for dependent samples, $t = -3.249$, $df = 3$, $p = 0.047$, $n = 4$; fresh algal pieces: Figure 6A, *t*-test for dependent samples, $t = -3.766$, $df = 3$, $p = 0.032$, $n = 4$). Thus, if differences in palatability

were detected, these occurred in the treatments where detachment and grazing were combined. No further significant preferences were detected for the remaining artificial food and live algal choice feeding assays (T2–T4) (Figures 5B–D and 6B–D, respectively). The grazer *P. ruffoi* did not discriminate between attached or detached algal pieces treated by grazing, or controls. The two artificial food and fresh no-choice feeding assays (T5) (5E and 6E, respectively) did not display any significant differences in the palatability concerning the factors attachment status or grazing (Table 2).

Discussion

Our results indicate that extended detachment influenced the palatability of *Lessonia nigrescens* but not of *Macrocyctis integrifolia*. Grazing also seemed to have an effect on the palatability of detached *L. nigrescens*, which were less palatable when previously exposed to grazers compared to grazer-free controls. For attached plants of *L. nigrescens* no such differences in palatability were found, which suggests that time after detachment may have an influence on defensive responses

Table 2. Results of 2-way ANOVA after the treatment phase for the mean consumption (mg) of *Lessonia nigrescens* from no-choice feeding assays by the grazer *Parhyalella ruffoi*

No-choice feeding assays treatment phase	Artificial food				Fresh alga			
	df	MS	F	P	df	MS	F	P
Attachment status (A)	12	52.874	0.352	0.854	15	0.000053	0.077	0.786
Grazing (G)	12	715.534	0.476	0.503	15	0.000001	0.001	0.972
G * A	12	474.439	0.316	0.584	15	0.000042	0.061	0.809

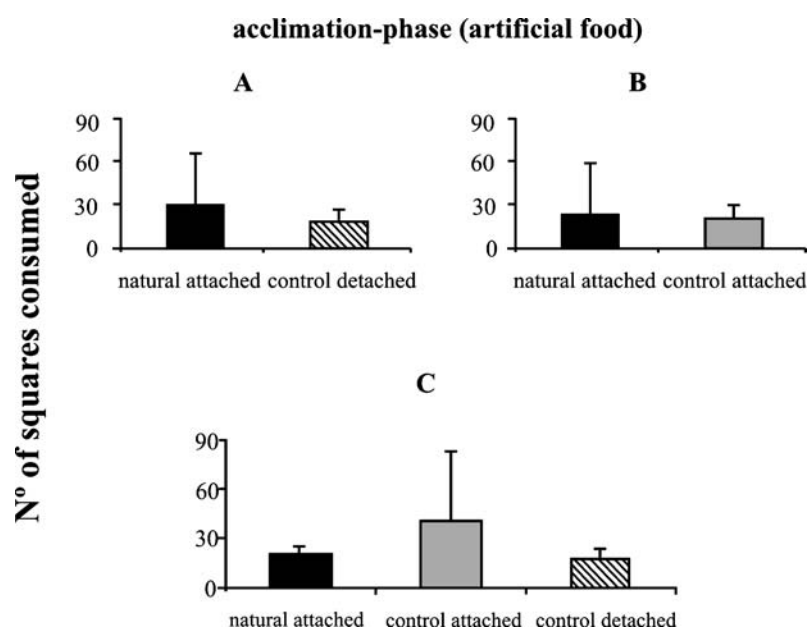


Figure 4. Mean (\pm SD) number of squares consumed of artificial *Lessonia nigrescens* by *Parhyalella ruffoi* after the acclimation phase. (A & B) choice feeding assays, and (C) no-choice feeding assays; error bars represent +1 SD ($n = 5$).

(and possibly other physiological processes) in marine macroalgae.

Detachment effect

Some species of macroalgae only exist as populations of detached individuals, such as for example the brown algae *Sargassum natans* and *S. fluitans*. Parr (1939) mentioned that both Fucales show no signs of attachment and lack reproductive organs. Their floating thalli represent an effective long-distance dispersal mechanism (Deysner & Norton, 1982) and may support survival of populations via asexual reproduction. Also, kelps that usually grow as attached individuals may persist after detachment. Detachment does not mean death for these plants, which may become entangled in kelp forests or float freely for extended periods (Hobday, 2000). During this floating time some

(e.g. the kelp *M. integrifolia*) may even be reproductive (Macaya et al., 2005). Other algae sink to the seafloor after detachment, where they may form dense accumulations of drift algae (Norton & Mathieson, 1983; Ingólfsson, 1995; Norkko & Bonsdorff, 1996). Following detachment, algae may start to deteriorate (e.g. Hobday, 2000), which could be due to loss of antiherbivore defence making detached algae more palatable compared to attached conspecifics.

We found that the detached kelp *L. nigrescens* became more palatable to mesograzers 12 d after detachment if held without grazers (Figure 2). One reason for this could be that algae invested more energy to growth, which resulted in fewer resources available to the production of deterrents (Herms & Mattson, 1992). Rapid growth might also be a mechanism to tolerate future grazer attack. Detached algal species are exposed to a variety of environmental conditions (e.g. light, nu-

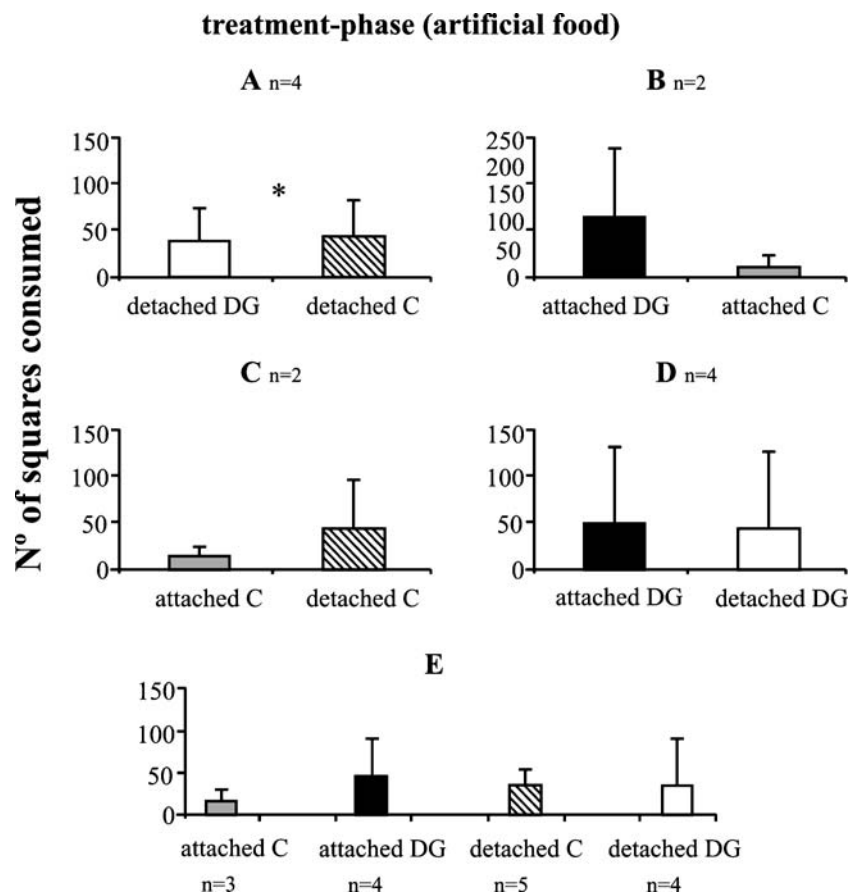


Figure 5. Mean (\pm SD) number of squares consumed of artificial *Lessonia nigrescens* by *Parhyalella ruffoi* after the treatment phase. Choice feeding assays are represented by the graphs A, B, C and D, while no-choice feeding assays are represented by the graph E. (DG = direct grazing treatment, C = control treatment). Error bars represent +1 SD. Significance values are from *t*-test for dependent samples.

trients, temperature, herbivory) differing from those affecting attached plants (Norton & Mathieson, 1983). Possibly the conditions encountered by detached individuals are sub-optimal and thus they may have few extra resources available for defence. *Lessonia nigrescens* does not possess floating structures and consequently will sink to the bottom after detachment. Similarly, as in the natural environment after detachment, in the deep tank used in the first experiment, the detached thalli of *L. nigrescens* faced subtidal conditions while normally they are growing in the intertidal zone (Hoffmann & Santelices, 1997) where they are exposed to the air during each low tide. Thus plants may have experienced physiological stress in the tank after 12 d because they lay at the bottom of the tank at a depth of about 1 m. As a result, the detached plants in the tank received less light than attached control plants in the natural environment. If these changes in palatability after 12 d of detachment indeed were stress-induced changes then

this process would be consistent with the result from Renaud et al. (1990) who found that desiccation of the unpalatable brown alga *Padina gymnospora* increased its palatability to sea urchins via loss of chemical defence. Physical features such as, for example, tissue toughness (Watson & Norton, 1985) may also affect herbivore choice. Possibly, over the extended detachment time in the experimental tank the detached individuals lost their toughness (resistance to penetration) and were therefore more susceptible to the grazer than freshly collected attached algae from the field. The results from Littler et al. (1983) indicated that herbivore preference is a function of the degree of seaweed toughness.

In contrast to these results, where an increase in palatability was observed beginning at day 12 after detachment, no detachment effect was observed for *L. nigrescens* after the 10 d of detachment during the acclimation phase (A1) in the small containers

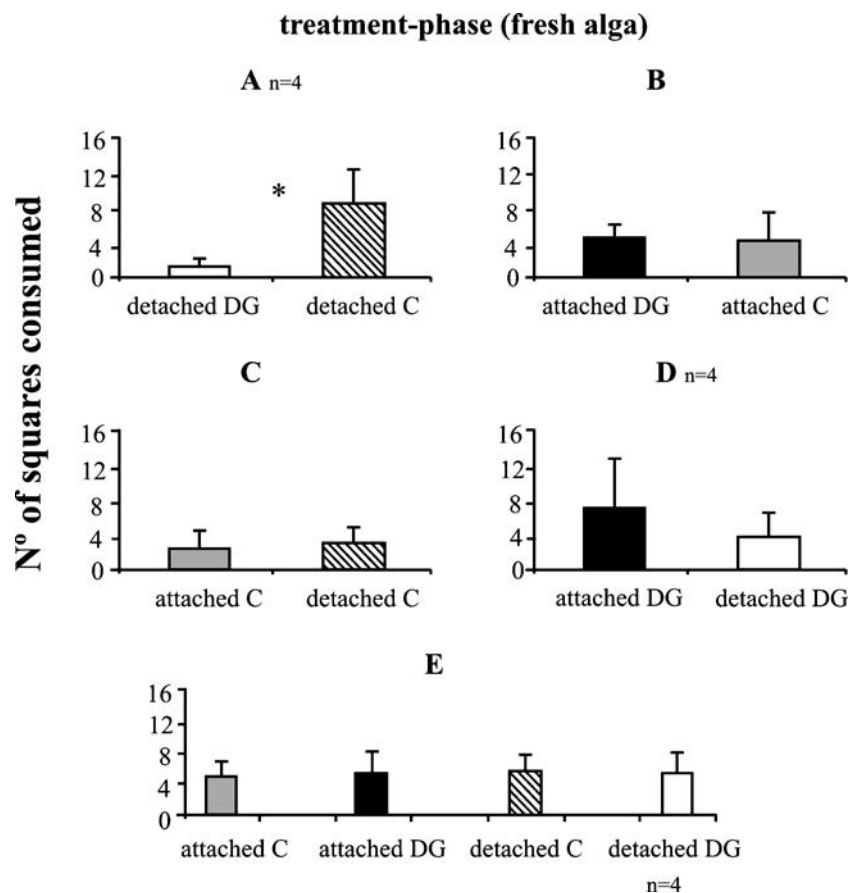


Figure 6. Mean consumption (mg) of fresh *Lessonia nigrescens* after the treatment phase by *Parhyalella ruffoi*. Choice feeding assays are represented by the graphs A, B, C and D, while no-choice feeding assays are represented by the graph E. (DG = direct grazing treatment, C = control treatment). Error bars represent +1 SD ($n = 5$). Significance values are from t -test for dependent samples. (*If the number of replicates are not noted above or below bars they are present with $n = 5$)

(Figures 4A). Since later a detachment effect (increase in palatability) occurred in this experiment, detachment effects apparently become important around 10–14 days after detachment.

No effect of extended detachment was observed for the palatability of the kelp *M. integrifolia* after 24 and 97 days of floating, neither in laboratory (Figure 2) nor in field experiments (Figure 3), suggesting that both detached and attached individuals were (i) either undefended or (ii) maintained their defence. We suggest that a permanently expressed defensive capacity exists in the apical tips of *M. integrifolia*. *Macrocystis pyrifera* plants that detach from their substratum float to the surface and may remain buoyant for a maximum of between 65 and 109 days before beaching or sinking (Hobday, 2000). Floating of this and other kelp plants is made possible by gas-filled pneumatocysts (Lobban & Harrison, 1994). Detached individuals con-

tinue to function physiologically for some time after detachment (Hobday, 2000). It has also been shown that individuals of *M. integrifolia* can reproduce after becoming detached (Macaya et al., 2005). Consequently, it might be advantageous for this macroalga to maintain its defensive capacity even after becoming detached. Zubia et al. (2003) reported that detached individuals of two floating kelp species, in comparison to attached conspecifics, had almost similar chemical composition (lipids, proteins, minerals, amino acids). This suggests that macroalgae, which have the capacity to float, may possess and maintain anti-herbivore defences after detachment.

Grazing effect

Grazing may have a strong effect on the community structure of macroalgae in coastal marine systems

(Lubchenco & Gaines, 1981). Algae frequently suffer attack by herbivores but many attached macroalgae can suppress palatability in order to deter herbivores. However, how do algae react against grazers after becoming detached from their primary substratum and while drifting on the sea floor or floating on the water surface? Detached algae are often colonized by dense assemblages of mesograzers, in particular amphipods and isopods (Geertz-Hansen et al., 1993; Ingólfsson, 2000; Brooks & Bell, 2001). It could thus be expected that these detached algae may be either (i) very susceptible to these grazers because of the physiological stress caused by detachment, or (ii) use defensive strategies in order to avoid high levels of herbivory.

In our first experiment, grazers were excluded during the entire duration of the extended detachment time, while in the attached control (plants from the field) grazers might have been present. The holdfasts of attached *L. nigrescens* for example, usually have many internal cavities used as a habitat by diverse invertebrate species (Hoffmann & Santelices, 1997; Thiel & Vásquez, 2000). This suggests that during the entire experiment the attached individuals of *L. nigrescens* (Figure 2), which were collected freshly from the field, may have been well defended against the tested grazer irrespective of the grazer pressure in the field (constitutive defence). However, detached plants may not have diverted extra-energy to production of deterrents when grazers were absent and thus lost their defensive capability. A similar pattern was detected for *L. nigrescens* (T1) in the induction experiment (Figures 5A and 6A). Detached plants, previously exposed for 10 days to grazers, were less palatable than grazer-free individuals. This supports our hypothesis that detached plants maintain their defensive capacity when grazers are present but lose it when grazers are absent.

Grazing appeared to have no influence on the palatability of apical parts from attached and detached kelp *Macrocystis integrifolia* since grazers exhibited no preferences in any of the tested choice-combinations (Figures 2 and 3). A comparatively homogeneous palatability was obtained in the laboratory experiment (Figure 2) between the grazer-free detached apical parts from the tank and the attached apical parts from the field where grazers usually are present. A similar pattern was observed in the field experiment (Figure 3) where both detached and attached algae were exposed to possible grazer attacks for 97 days. This suggests that detached and attached plants are equally defended, irrespective of the grazer intensity (constitutive defence).

Macrocystis kelp forests are inhabited by a variety of invertebrate and vertebrate herbivores, many of which use the kelp plants as food (North, 1994). The tested plant parts, namely the upper parts of the plants, are principally consumed by fishes. A constitutive defence would be advantageous against herbivores that are very mobile, such as fish, that move to more palatable food after a few bites of distasteful food (Paul & Van Alstyne, 1992; Hay, 1996).

Conclusions

We report evidence that detached kelps *L. nigrescens* and *M. integrifolia* are defended against herbivores. Detached plants of *L. nigrescens* only maintained defence when grazers were present. In the absence of grazers, detached plants of *L. nigrescens* might invest energy for survival or attempt to overcome physiological stress, while attached individuals may have sufficient energy to maintain constant defence. In contrast to *L. nigrescens*, where detached plants may lose their defensive capability, apical parts of *M. integrifolia* apparently maintain their defence even after being detached for 97 days. In summary, our results indicated that kelps, once detached from their natural substratum, can respond with defence against herbivorous enemies but they responded differently, possibly depending on their capacity to float (in the case of *M. integrifolia*) or the presence of grazers (in the case of *L. nigrescens*).

Acknowledgments

We are extremely grateful to AS Chapman for many constructive comments that helped to improve the manuscript substantially. Financial support was obtained via fellowships from the GAME-project to ER (project leaders Martin Wahl and Markus Molis at UKiel) and through project FONDECYT 1010356 to MT. We are grateful to the staff of the botany laboratory at UCN for their continuous support during this study. Also special thanks to all the biologists from the BEDIM laboratory at UCN.

References

- Bazzaz FA, Chiariello NR, Coley PD, Pitelka LF (1987) Allocating resources to reproduction and defense. *BioScience* 37: 58–67.

- Benz CB, Eiseman NJ, Gallaher EE (1979) Seasonal occurrence and variation in standing crop of a drift algal community in the Indian River, Florida. *Bot. Mar.* 22: 413–420.
- Brooks RA, Bell SS (2001) Mobile corridors in marine landscapes: Enhancement of faunal exchange at seagrass/sand ecotones. *J. Exp. Mar. Biol. Ecol.* 264: 67–84.
- Cetrulo GL, Hay ME (2000) Activated chemical defenses in tropical versus temperate seaweeds. *Mar. Ecol. Prog. Ser.* 207: 243–253.
- Cronin G, Hay ME (1996a) Induction of seaweed chemical defenses by amphipod grazing. *Ecology* 77: 2287–2301.
- Cronin G, Hay ME (1996b) Susceptibility to herbivores depends on recent history of both the plant and animal. *Ecology* 77: 1531–1543.
- Deyscher L, Norton TA (1982) Dispersal and colonization in *Sargassum muticum* (Yendo) Fensholt. *J. Exp. Mar. Biol. Ecol.* 56: 179–195.
- Duffy JE, Hay ME (2001) The ecology and evolution of marine consumer-prey interactions. In Bertness MD, Hay ME, Gaines SD (eds), *Marine Community Ecology*: Sinauer Associates, Sunderland, MA, USA, pp. 131–159.
- Geertz-Hansen O, Sand-Jensen K, Hansen DF, Christiansen A (1993) Growth and grazing control of abundance of the marine macroalga, *Ulva lactuca* L in a eutrophic Danish estuary. *Aquatic Bot.* 46: 101–109.
- Hammerstrom K, Dethier MN, Duggins DO (1998) Rapid phlorotannin induction and relaxation in five Washington kelps. *Mar. Ecol. Prog. Ser.* 165: 293–305.
- Hay ME, Paul VJ, Lewis SM, Gustafson K, Tucker J, Trindell R (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 ME (1991) Fish-seaweed interactions on coral reefs: Effects of herbivorous fishes and adaptations of their prey. In Sale PF (ed.), *The ecology of fishes on coral reefs*, Academic Press, California, pp. 371–413.
- Hay ME (1996) Marine chemical ecology: What's known and what's next?. *J. Exp. Mar. Biol. Ecol.* 200: 103–134.
- Helmuth BS, Veit RR, Holberton R (1994) Long-distance dispersal of a subantarctic brooding bivalve (*Gaimardia trapesina*) by kelp rafting. *Mar. Biol.* 120: 421–426.
- Herms DA, Mattson WJ (1992) The dilemma of plants to grow or defend. *Q. Rev. Biol.* 67: 283–335.
- Hirata T, Tanaka J, Iwami T, Ohmi T, Dazai A, Aoki M, Ueda H, Tsuchiya Y, Sato T, Yokohama Y (2001) Ecological studies on the community of drifting seaweeds in the south-eastern coastal waters of Izu Peninsula, central Japan. I. Seasonal changes of plants in species composition, appearance, number of species and size. *Phycol. Res.* 49: 215–229.
- Hobday AJ (2000) Age of drifting *Macrocystis pyrifera* (L.) C. Agardh rafts in the Southern California Bight. *J. Exp. Mar. Biol. Ecol.* 253: 97–114.
- Hoffmann A, Santelices B (1997) *Flora marina de Chile central*, Ediciones Universidad Católica de Chile, Santiago, Chile, 434 pp.
- Inglis G (1989) The colonisation and degradation of stranded *Macrocystis pyrifera* by the macrofauna of a New Zealand sandy beach. *J. Exp. Mar. Biol. Ecol.* 125: 203–217.
- Ingólfsson A (1995) Floating clumps of seaweed around Iceland: natural microcosms and means of dispersal for shore fauna. *Mar. Biol.* 122: 13–21.
- Ingólfsson A (1998) Dynamics of macrofaunal communities of floating seaweed clumps off western Iceland: A study of patches on the surface of the sea. *J. Exp. Mar. Biol. Ecol.* 231: 119–137.
- Ingólfsson A (2000) Colonization of floating seaweed by pelagic and benthic animals in southwestern Iceland. *Hydrobiologia* 440: 181–189.
- Littler MM, Littler DS (1980) The evolution of thallus form and survival strategies in benthic marine macroalgae: Field and laboratory tests of a functional form model. *Am. Nat.* 116: 25–44.
- Littler MM, Littler DS, Taylor PR (1983) Evolutionary strategies in a tropical barrier reef system: Functional-form groups of marine macroalgae. *J. Phycol.* 19: 229–237.
- Lobban CS, Harrison PJ (1994) *Seaweed ecology and physiology*, Cambridge University Press, Cambridge, 366 pp.
- Lubchenco J, Gaines SD (1981) A unified approach to marine plant-herbivore interactions. I. Populations and communities. *Ann. Rev. Ecol. Syst.* 12: 405–437.
- Macaya E, Boltaña S, Hinojosa I, Macchiavello J, Valdivia N, Vásquez N, Buschmann A, Vásquez J, Vega J, Thiel M (2005) Presence of sporophylls in floating kelp rafts of *Macrocystis* spp. (Phaeophyceae) along the Chilean Pacific Coast. *J. Phycol.* 41: 913–922.
- Norton TA, Mathieson AC (1983) The biology of unattached seaweeds. In Round FE, Chapman DJ (eds.), *Progress in Phycological Research*: Elsevier SP, Amsterdam, pp. 333–376.
- North WJ (1994) Review of *Macrocystis* biology. In Akatsuka I (ed.), *Biology of Economic Algae*. SPB Academic Publishing, The Hague, Netherlands, pp. 447–527.
- Norkko A, Bonsdorff E (1996) Population responses of coastal zoobenthos to stress induced by drifting algal mats. *Mar. Ecol. Prog. Ser.* 140: 141–151.
- Ólafsson E, Ingólfsson A, Steinarsdóttir MB (2001) Harpacticoid copepod communities of floating seaweed: Controlling factors and implications for dispersal. *Hydrobiologia* 453/454: 189–200.
- Parr AE (1939) Quantitative observations on the pelagic *Sargassum* vegetation of the western North Atlantic. *Bull. Bingham Oceanogr. Collection* 6: 1–94.
- Paul VJ, Fenical W (1986) Chemical defense in tropical green algae, order Caulerpaceles. *Mar. Ecol. Prog. Ser.* 34: 157–169.
- Paul VJ, Van Alstyne KL (1992) Activation of chemical defenses in the tropical green algae *Halimeda* spp. *J. Exp. Mar. Biol. Ecol.* 160: 191–203.
- Pavia H, Cervin G, Lindgren A, Åberg 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 GB (2000) Inducible chemical resistance to herbivory in the brown seaweed *Ascophyllum nodosum*. *Ecology* 81: 3212–3225.
- Peckol P, Krane JM, Yates JL (1996) Interactive effects of inducible defense and resource availability on phlorotannins in the North Atlantic brown alga *Fucus vesiculosus*. *Mar. Ecol. Prog. Ser.* 138: 209–217.
- Renaud PE, Hay ME, Schmitt TM (1990) Interactions of plant stress and herbivory: Intraspecific variation in the susceptibility of a palatable versus an unpalatable seaweed to sea urchin grazing. *Oecologia* 82: 217–226.
- Rodríguez SR (2003). Consumption of drift kelp by intertidal populations of the sea urchin *Tetrapygus niger* on the central Chilean coast: Possible consequences at different ecological levels. *Mar. Ecol. Prog. Ser.* 251: 141–151.

- Sotka EE, Taylor RB, Hay ME (2002) Tissue-specific induction of resistance to herbivores in a brown seaweed: The importance of direct grazing versus waterborne signals from grazed neighbors. *J. Exp. Mar. Biol. Ecol.* 277: 1–12.
- Steinberg PD (1984) Algal chemical defense against herbivores: Allocation of phenolic compounds in the kelp *Alaria marginata*. *Science* 223: 405–406.
- Taylor RB, Sotka E, Hay ME (2002) Tissue-specific induction of herbivore resistance: Seaweed response to amphipod grazing. *Oecologia* 132: 68–76.
- Thiel M (2002) The biogeography of littoral algal-associated peracarids along the Chilean coast. *J. Biogeogr.* 29: 999–1008.
- Thiel M, Vásquez JA (2000) Are kelp holdfasts islands on the ocean floor? Indication for temporarily closed aggregations of peracarid crustaceans. *Hydrobiologia* 440: 45–54.
- Thiel M, Gutow L (2004) The ecology of rafting in the marine environment. I. The floating substrata. *Oceanogr. Mar. Biol. Ann. Rev.* 42: 181–264.
- Toth GB, Pavia H (2001) Lack of phlorotannin induction in the kelp *Laminaria hyperborea* in response to grazing by two gastropod herbivores. *Mar. Biol.* 140: 403–409.
- Van Alstyne KL (1988) Herbivore grazing increases polyphenolic defenses in the intertidal brown alga *Fucus distichus*. *Ecology* 69: 655–663.
- Van Alstyne KL (1989) Adventitious branching as a herbivore-induced defense in the intertidal brown alga *Fucus distichus*. *Mar. Ecol. Prog. Ser.* 56: 169–176.
- Van Alstyne KL, Wolfe GV, Freidenburg TL, Neill A, Hicken C (2001) Activated defense systems in marine macroalgae: Evidence for an ecological role for DMSP cleavage. *Mar. Ecol. Prog. Ser.* 213: 53–65.
- Watson D, Norton TA (1985) The physical characteristics of seaweed thalli as deterrents to littorine grazers. *Bot. Mar.* 28: 383–387.
- Zubia M, Payri CE, Deslandes E, Guezennec J (2003) Chemical composition of attached and drift specimens of *Sargassum mangarevense* and *Turbinaria ornata* (Phaeophyta: Fucales) from Tahiti, French Polynesia. *Bot. Mar.* 46: 562–571.