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Effects of limitation stress and of disruptive stress on induced antigrazing defense in the bladder wrack *Fucus vesiculosus*

Florian Weinberger[1,*], Sven Rohde[1,2], Yvonne Oschmann[1], Laila Shahnaz[1,3], Sergey Dobretsov[1,4], Martin Wahl[1]

[1]Leibniz-Institut für Meereswissenschaften (IFM-GEOMAR), Düsternbrooker Weg 20, 24105 Kiel, Germany
[2]ICBM, Carl-von-Ossietzky Universität Oldenburg, Schleusenstrasse 1, 26382 Wilhelmshaven, Germany
[3]Sindh Institute of Urology and Transplantation, Civil Hospital, Karachi 74200, Pakistan
[4]Department of Marine Science and Fisheries, College of Agricultural and Marine Sciences, Sultan Qaboos University, Al Khoud 123, PO Box 34, Muscat, Sultanate of Oman

ABSTRACT: We assessed the effects of light limitation and temperature shift on palatability and induced antiherbivore defense in the brown alga *Fucus vesiculosus* L. Incubation for 2 wk at light intensities above the compensation point of photosynthesis and in the absence of grazers increased the palatability of *F. vesiculosus* and its subsequent consumption by the omnivorous isopod *Idotea baltica* Pallas. This effect correlated with an increased C:N ratio and mannitol content in the algal tissue, presumably due to increased photosynthetic carbon fixation. Mannitol, the primary product of photosynthesis in *F. vesiculosus*, proved to be a feeding cue for *I. baltica*, and depletion of the mannitol pool may therefore account for the reduced palatability during light limitation. At light intensities above the compensation point of photosynthesis, *F. vesiculosus* responded with decreasing palatability when it was exposed to *I. baltica* grazing. Irrespective of the preceding light regime, such defense induction was prevented during incubation under light limitation. Thus, under low light, defense induction is not only inhibited, but also less necessary due to the relative absence of feeding cues. Upward or downward shifts in water temperature by approximately 10°C also inhibited inducible defense in *F. vesiculosus*. However, such shifts did not affect algal growth and were therefore the consequence of an impairment of specific defense-related components rather than of resource limitation, unless compensatory growth was given priority over defense.

KEY WORDS: Alga–herbivore interaction · Defense induction · *Idotea* · Seaweed–herbivore interaction · Stress effects

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INTRODUCTION

Herbivory is an important biotic factor affecting algal biomass, abundance, and distribution (Lubchenco & Gaines 1981, Van Alstyne et al. 2001). The effects of mesograzer consumption on algal individuals may last over relatively long periods because grazed individuals are rarely killed (Dethier et al. 2005). Consequently, the capacity to limit grazing to a tolerable level should be of substantial selective advantage. Such resistance may be either constitutive (stable in intensity) or regulated, i.e. deployed in the presence of grazers and reduced when grazing stops. Resistance in general is the sum effect of various different traits reducing edibility, such as tissue toughness or presence of chemical deterrents. The known examples of regulated algal resistance against herbivores are almost exclusively based upon biochemical mechanisms (Jormalainen & Honkanen 2008), which affect palatability and may be either activated or induced. In the case of activation,

*Email: fweinberger@ifm-geomar.de

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tissue disruption by herbivores provokes a transformation of pre-formed metabolites into active defense compounds (Paul & Van Alstyne 1992, Cetrulo & Hay 2000, Van Alstyne et al. 2001). Such activated defenses need to be stimulated and in this sense may be considered regulated. However, they are basically constitutive, because the defensive precursors are produced before herbivory occurs and have to be stored and maintained independently of the presence of grazers (Paul & Van Alstyne 1992, Karban & Baldwin 1997). As activated defense, induced defense needs stimulation, but defense genes instead of defensive precursor compounds are activated. The occurrence of inducible chemical defenses following herbivory, or mechanical simulation of herbivory, is well documented for a large number of terrestrial plants (Karban & Baldwin 1997, Agrawal 2005). Recent research suggests that induced resistance is also common among macroalgae (Toth & Pavia 2007, Jormalainen & Honkanen 2008, Rohde & Wahl 2008), and in some cases molecular signals may indicate the presence or activity of potential enemies to the alga and induce a de novo synthesis of defensive compounds (Coleman et al. 2007).

Several theories postulate selective advantages of induced defense (Agrawal & Karban 1999), e.g. where consumption pressure is variable (Karban & Baldwin 1997). The gene-to-gene model predicts a reduced risk of enemy adaptation to induced defensive traits (Keen 1990), while the cost-allocation model postulates an energetic advantage when defenses are only produced when needed (Cronin 2001). Defense costs are difficult to detect (Strauss et al. 2002) and their existence has been questioned (Mole 1994). However, a meta-analysis of 88 studies investigating plant-herbivore interactions found evidence of direct costs of constitutive and induced defense in 57% and 52% of all cases, respectively (Strauss et al. 2002). While numerous studies demonstrated defense induction against herbivores in marine macroalgae in recent years (reviewed in Toth & Pavia 2007), so far none have rigorously demonstrate incurring costs (e.g. Pansch et al. 2009, Appelhans et al. 2010; but see Dworjanyn et al. 2006 for costs of constitutive defense against fouling).

Because regulated defenses are dynamic, they may be particularly prone to environmental stress. Numerous terrestrial studies have demonstrated interactive effects of environmental and biotic factors on plant performance, but corresponding marine work is scarce (Dethier et al. 2005). The abiotic environment often determines the resources that are available to an organism. A reduction in resource availability can result either from direct limitation or indirectly from the disruptive physiological effects of extreme environmental conditions (Dethier et al. 2005). Both disruptive stress and limitation stress could therefore affect the macroalgal capacity to induce defenses — if they are costly and if resource shortage is not buffered by energy stores or re-allocation of resources from other processes such as growth. In the present study, we investigated the effects of both limitation stress and disruptive stress on induced defense in the rockweed *Fucus vesiculosus*, and we hypothesized that stress inflicted by light limitation and disruptive temperature shifts weakens the algal defensive performance.

Fucus vesiculosus occurs in boreal and cold temperate zones of the north Atlantic and is the main macroalgal constituent of the upper littoral zone in the western Baltic (Rönnbäck et al. 2007). Several abiotic stresses are common in this habitat, and some of them have been predicted to increase in the course of ongoing climate change. According to most climate-change scenarios, stress caused by high temperature, high nutrient load, fluctuating salinity, and low light will increase (IPCC Core Writing Team et al. 2007, BACC Author Team 2008). Presumably due to increased light limitation (resulting from a combined long-term effect of eutrophication favoring plankton blooms and epibiosis), *F. vesiculosus* has already retreated from the deeper parts of its distribution range in the western Baltic, which 35 yr ago extended to a depth of 10 m and nowadays goes only to depths shallower than 2 to 3 m (Vogt & Schramm 1991). However, in the western Baltic, *F. vesiculosus* shows positive growth to a depth of 5 to 6 m in summer at least (Rohde et al. 2008, Wahl et al. 2010) and is capable of tolerating periods of severe light limitation of weeks to months due to its capacity to store assimilated carbon in the form of mannitol and polysaccharides such as laminaran or alginate (Bidwell et al. 1972, Lehvo et al. 2001, Obluchinskaya et al. 2002). Prolonged winter periods of light limitation occur not only north of the Arctic circle (Voskoboinikov et al. 2006), but also in the western Baltic, where mean light intensities at the lower distribution limit of *F. vesiculosus* fall below the light compensation point of photosynthesis from the end of October to mid-March (Wahl et al. 2010). During such conditions, the tissue concentration of mannitol and alginate in *F. vesiculosus* drops by 60 to 70%, while net growth may continue under a net energy deficit for up to 2 mo (Lehvo et al. 2001, Obluchinskaya et al. 2002). Thus, the disappearance of *F. vesiculosus* from the depth range between 3 and 5 m requires additional explanations other than the direct light-reduction effect.

Besides light limitation, other potential abiotic stressors for *Fucus vesiculosus* are the ongoing and predicted shifts in temperature and its fluctuations. While 20°C is the highest water temperature that we find for periods longer than weeks within the natural

distribution range of *F. vesiculosus* (Lüning 1990), water temperature is already close to this limit in the upper 5 m of the western Baltic from June through August (M. Wahl unpubl.) and is predicted to rise by 3 to 5°C in the course of this century (BACC Author Team 2008). Moreover, *F. vesiculosus* may already experience extremely warm (30 to 40°C) and fluctuating conditions during periods of air exposure in summer (Pearson et al. 2009 for Portugal and Cornwall, Wahl et al. 2010 for the western Baltic). Thermal stress may either impact *F. vesiculosus* directly or affect its biotic interactions (Wahl et al. 2010), e.g. by provoking changes in its palatability or antifeeding protection.

Various aspects of the defensive mechanisms in *Fucus vesiculosus* have been studied since the report of antifeeding properties by Geiselman & McConnell (1981). *F. vesiculosus* induces defenses in response to clipping (Van Alstyne 1988, Yates & Peckol 1993, Hemmi et al. 2004), as well as in response to natural herbivory by its main consumer in the western Baltic, the isopod *Idotea baltica* (Rohde et al. 2004). *I. baltica* grazing results in reduced palatability of *F. vesiculosus* to this omnivore, and this effect is due to a changed chemical composition, demonstrated by palatability tests of *F. vesiculosus* using freeze-dried and pelleted material (Rohde et al. 2004), which is devoid of all thallus structure.

The effect of nutrient limitation (e.g. Yates & Peckol 1993, Pavia & Brock 2000), as well as of disruptive stresses such as UV radiation (Cronin & Hay 1996, Pavia et al. 1997) or desiccation (Dethier et al. 2005), on macroalgal defense has been investigated in a few studies. The results are somewhat controversial, both finding and refuting stress effects on defense. It must be noted that in most of these studies, tissue concentrations of phlorotannins were used as a proxy of induced defense. However, *Idotea baltica*, like many other marine consumers of algae (Targett & Arnold 1998), is not deterred by phlorotannin (Jormalainen et al. 2001) and grows well when fed with *Fucus vesiculosus* rich in phlorotannin (Hemmi & Jormalainen 2004). As with most other alga-herbivore interactions, the relevant defensive compounds in the interaction of *F. vesiculosus* with *I. baltica* are still unknown. Moreover, a reduced palatability may not only result from increased concentrations of defensive compounds, but also from lower concentrations of feeding cues, which again are unknown in most instances. Consequently, a direct measurement of palatability is necessary when assessing the trade-off between defensive costs and stress.

We therefore analyzed the effect of *Idotea baltica* grazing at different naturally occurring stress levels, from weak to severe, on the subsequent palatability of *Fucus vesiculosus* to *I. baltica*. To distinguish between stress effects on overall palatability and on chemical determinants of palatability, we ran feeding assays quantifying the preference of *I. baltica* for either live *F. vesiculosus* (characterized by mechanical and chemical properties) or for pelleted *F. vesiculosus* (characterized by chemical properties only). We (1) compared the inducibility of changes in both types of palatability, and we will call such changes 'induced defense'. We assessed the effect of light limitation upon (2) photosynthesis and (3) food quality (mannitol content and C:N ratio) of *F. vesiculosus*. We also assessed the effects of (4) light limitation and (5) previous temperature shifts upon induced defense in *F. vesiculosus*. We present the findings from a series of experiments, and evaluate feeding assays based upon effect sizes, in order to facilitate comparisons among experiments.

MATERIALS AND METHODS

All *Fucus vesiculosus* individuals used were collected from a rocky shore in the Kiel Fjord, western Baltic (54° 26′ N, 10° 11′ E), where they form dense, almost monospecific stands in the first meter below the water surface. The algae were transferred in coolers from the upper subtidal zone (0.2 to 0.7 m) to the laboratory, freed from grazers, and maintained at 15°C in aerated seawater until required (<24 h). The isopod *Idotea baltica* was collected twice per year (in spring and autumn) from Kiel Fjord and maintained on a long-term basis in a tank with seawater flow-through at 15°C, and mainly fed with *F. vesiculosus* and other seaweeds. *I. baltica* dry weight (mg) was inferred from body length (mm), using the function (authors' unpubl. data):

$$\log(\text{dry weight}) = 2.583 \times \log(\text{body length}) - 1.859 \quad (1)$$

Defense induction. All defense-induction experiments were run in constant-temperature rooms with ambient seawater supply at the Leibniz Institute of Marine Sciences (IFM-GEOMAR) in Kiel and conducted in transparent plastic aquaria (2.9 l). Ambient water was obtained from the nearby Kiel Fjord, sand-filtered, and sterilized with UV light (flow-through UV water sterilizer 500; hw Wiegandt). The water was stored in tanks for temperature adjustment before being supplied to the aquaria with a constant flow rate regulated with roller clamps to 0.25 l h^{-1}. No additional nutrients were provided. Light was provided by fluorescent tubes (Osram Fluora L 36 W/77 25X1) mounted in parallel above the aquaria, and was quantified at the algal surface using a LI-1400 quantameter (LI-COR

Biosciences). For the highest light level, reflectors were installed above the fluorescent tubes. For lower light levels, either no reflectors were installed, the elevation of the lamps over the aquarium was increased, or a window screen was mounted between the lights and aquaria. For complete prevention of irradiation, aquaria were covered with black plastic sheet. The light:dark period was 12:12 h in experiments conducted in winter (October to March) and 16:8 h in experiments conducted in summer (April to September). The daily mean of photosynthetically active radiation (PAR) was calculated as:

$$\text{PAR} \times \text{Light exposure time (h)} / 24\ \text{h} \quad (2)$$

For an overview of the light and temperature combinations that were tested in the different defense-induction experiments, see Table 1.

At the beginning of each defense-induction experiment, 6 to 12 *Fucus vesiculosus* individuals (for the exact number of replicates, see Table 1) of equal size and without severe grazing damage or epibionts were collected (1 algal individual was comprised of the tissue growing from a single holdfast). They were divided into a sufficient amount of comparable apical pieces of 4 to 5 g that each individual could be incubated both in the presence and absence of *Idotea baltica* under each light and temperature condition of a given experiment.

Defense induction was inferred from the feeding preference of *Idotea baltica* among *Fucus vesiculosus* individuals that were previously exposed or unexposed to *I. baltica*. To assess this, pieces from each *F. vesiculosus* individual were randomly distributed to the pairs of aquaria in all light and temperature conditions and allowed to acclimatize for 3 d under the given conditions. After acclimatization, induction was initiated. To this end, one aquarium of a replicate pair of aquaria under each treatment combination received a defined number of *I. baltica* individuals (5 to 8 in different experiments, depending on the size of the animals and corresponding to approximately 35 mg dry weight in all experiments), while the second aquarium was left grazer-free. Grazers and abiotic stressors or stress combinations were allowed to act for the following 14 d.

Table 1. Defense-induction experiments. Sea surface temperature (SST) is the mean water temperature (recorded in intervals of 8 min) during 2 wk prior to the collection of *Fucus vesiculosus*. Correspondingly, ambient photosynthetically active radiation (PAR) is the mean PAR reaching the sea surface during these 2 wk (also recorded in intervals of 8 min during day and night). Temperature and mean PAR are the conditions during defense induction. n: number of replicates (individual *F. vesiculosus* specimens that were treated)

Month	SST (°C)	Ambient PAR (µE m^{-2} s^{-1})	Treatment no.	Temp. (°C)	Mean PAR (µE m^{-2} s^{-1})	Feeding assay	n
Mar 2006	0.8	95.5	1	15	37.5	Live	12
			2	15	15	Live	12
			3	20	37.5	Live	12
			4	20	15	Live	12
Jan 2007	6.5	22.9	5	15	40	Live	8
			6	15	25	Live	8
			7	15	10	Live	8
Apr 2007	9.2	223.8	8	15	40	Live	8
			9	15	25	Live	8
			10	15	5	Live	8
May 2007	10.5	470.2	11	15	37.5	Pellet	8
			12	15	5	Pellet	8
May 2008	13.7	594.7	13	8	46.7	Live + pellet	8
			14	12	46.7	Live + pellet	8
			15	16	46.7	Live + pellet	8
			16	21	46.7	Live + pellet	8
			17	23	46.7	Live + pellet	8
Aug 2008	18.7	333.5	18	8	46.7	Live + pellet	8
			19	11	46.7	Live + pellet	8
			20	15	46.7	Live + pellet	8
			21	20	46.7	Live + pellet	8
			22	22	46.7	Live + pellet	8
Feb–Mar 2009	2.2	92.7	23	15	0	Live + pellet	6
			24	15	15	Live + pellet	6
			25	15	32.5	Live + pellet	6
			26	15	62	Live + pellet	6

Net biomass change during the treatment phase was quantified as wet-weight change. For this purpose, algal pieces from aquaria with and without grazers were carefully blotted dry and weighed to the nearest 0.001 g at both the beginning and end of the trial.

For the analysis of C:N ratio and mannitol content, *Fucus vesiculosus* pieces from aquaria with and without grazers were frozen (–20°C), freeze-dried, ground in a cooled mill (IKA), and stored in a freezer (–20°C). The C:N ratio was analyzed according to Sharp (1974) using a Carlo Erba NA-1500-CNS-analyzer. Mannitol was extracted and analyzed as described in Vas'kovskii & Isai (1972), with the difference that periodate oxidation was stopped after 10 s.

Feeding assays. After the treatment phase, the palatability of *Fucus vesiculosus* exposed versus unexposed to grazing was compared in 2-way choice feeding assays, using naive *Idotea baltica* as a grazer. Two different

assays were used in this test phase. In one of these assays, *F. vesiculosus* was tested alive ('live assay'), while in the second assay *F. vesiculosus* was offered as food pellets ('pellet assay').

The live assay was conducted as follows. Grazed and ungrazed *Fucus vesiculosus* pieces (one piece of each) from the same individual alga and light/temperature condition were carefully blotted dry, weighed, and transferred to a 2.9 l aquarium containing seawater, to which a defined number of *Idotea baltica* were added (3 to 5 in different experiments, depending on the size of the animals and corresponding to approximately 20 mg of dry weight in all experiments). Additional grazed and ungrazed algal pieces (one of each) were weighed and transferred to another aquarium to serve as controls for autogenic changes in mass during the bioassay (Peterson & Renaud 1989). The algal pieces were reweighed after 3 d and the biomass consumed was calculated as:

$$\text{Biomass consumed} = T_0 \times (C_f/C_0) - T_f \quad (3)$$

where T_0 and T_f were wet weights of the algae before and after the feeding trials, and C_0 and C_f were the weights of the growth controls; i.e. C_f/C_o represents autogenic changes in mass (Sotka et al. 2002).

For the pellet assay, grazed and ungrazed algal pieces from the same individual and light/temperature condition were frozen, lyophilized, and ground for 2 min in a cooled mill (IKA). The algal powder was stored in airtight plastic tubes in a freezer (–20°C) until food pellets were prepared as follows: 180 mg of agar (Carl Roth) were mixed with 2.5 ml of deionized water and heated to boiling in a microwave oven. The diluted agar was then poured into a mixture of 0.5 g *Fucus vesiculosus* powder and 2 ml of deionized water, and after short stirring with a spatula, the mixture was poured onto wax paper that was covered by mosquito gauze (mesh size: 1 × 1 mm). A second piece of wax paper was used as cover and the mixture was compressed with a panel until it gelled. After removal of the wax paper, the mixture of agar and *F. vesiculosus* powder had attached to the net, and squares of defined size (7 × 7 or 15 × 15 cells in different experiments) were cut out. Two pellets containing powder from grazed and ungrazed parts of the same individual and the same light/temperature condition were placed into a Petri dish (Greiner; 9 cm diameter) after one of them had been marked by removal of one corner (1 to 3 squares). We shifted the marking among treatments to randomize its potential effect on grazer behavior. For each *F. vesiculosus* individual and light/temperature condition, 3 Petri dishes were prepared in this way as pseudo-replicates. Averaging the grazer preferences across these 3 dishes helped to reduce the variability among grazer individuals. One *Idotea baltica* individual and 30 ml seawater were added to each Petri dish before they were incubated in darkness at 15°C. The incubation of the Petri dishes was stopped individually when 25 to 75% of at least 1 of the 2 pellets had been eaten. Petri dishes where food detached from the mosquito net or no consumption took place within 48 h were not evaluated. In all other Petri dishes, the consumed and remaining amounts of both pellets were quantified by counting the number of squares that had and had not been consumed by *I. baltica*, respectively.

The pellet assay was also used in order to quantify the feeding preference of *Idotea baltica* for pellets containing of not containing mannitol (Roth). Pure agar is not consumed by *I. baltica*, and a sample of dried *Ulva* sp., a green macroalga devoid of mannitol (Kylin 1915), was therefore used as main ingredient of the pellets instead of *Fucus vesiculosus* powder, and either mixed with mannitol or not.

Net photosynthesis. Net photosynthesis under the different light treatments was estimated by measuring O_2 production with a Clark-type GOX20 oxygen electrode (Greisinger Electronic). The electrode was calibrated in seawater from the Kiel Fjord that was extensively bubbled with air or N_2, in order to obtain fully oxygenated or anoxic water, respectively. Aquaria containing 2.9 l of seawater, an oxygen electrode, and *Fucus vesiculosus* at a density of 10 g l^{-1} were sealed air-tight with adhesion film (Melitta Toppits) and exposed at 15°C to the light intensities used in the treatment phase. The water in the aquaria was mixed using a magnetic stirrer. O_2 concentrations in the water were recorded for 30 min, and rates of net production or consumption were calculated.

Data analysis. Standardized effect sizes were calculated in order to allow for comparisons among different defense-induction experiments. For the live assay, which records absolute amounts of consumed biomass on a continuous scale, the effect size g was calculated according to Hedges (1981) as:

$$g = (T - C)/s^{\star} \quad (4)$$

where T and C represent the average biomass consumed of previously grazed and ungrazed *Fucus vesiculosus*, respectively, and $s^{\star}$ is the pooled standard deviation of both groups. The value g was bias-corrected and 95% confidence intervals (CI) of g were constructed (Hedges & Oilkin 1985).

For the pellet assay, which recorded numbers of gauze squares consumed by *Idotea baltica* in the pellets on a non-continuous scale, the effect size was w, which is the approximate relative risk of consumption of a given pellet type. To calculate this size, the gauze squares counted in different pseudo-replicates of the

same individual and test condition were summed (Fisher & van Belle 1993). The value w was then calculated as an odds ratio

$$w = [(0.5 + t^+)/(0.5 + t^-)]/[(0.5 + c^+)/(0.5 + c^-)] \quad \text{(Fisher \& van Belle 1993)} \quad (5)$$

where t represents numbers of squares of pellets containing grazed *Fucus* or mannitol; c represents the same for pellets containing ungrazed *Fucus* or no mannitol; + and – indicate squares that were eaten or not eaten, respectively. Binominal proportion 95% CI of w were constructed according to Fisher & van Belle (1993). CI of w that exclude 1 indicate a significant association of eaten and not-eaten mesh among both pellets and thus a significant preference of *I. baltica* for one of these (χ^2 test, p = 0.05; Fisher & van Belle 1993). Both g and log(w) indicate a preference of *I. baltica* for grazed *F. vesiculosus* when they are >0, while effect sizes <0 indicate an inverse preference.

Fucus vesiculosus growth rates were calculated as a percentage change in biomass per day.

Unless mentioned otherwise, statistical tests were conducted using the software package Statistica 6.0 (StatSoft). Differences in C:N ratios, algal growth, and consumption in the feeding assays among pairs of grazed treatments and ungrazed controls were analyzed by a pairwise Wilcoxon test. Effect sizes, C:N ratios, and algal growth obtained in the different treatments of single experiments were compared by repeated-measures ANOVA (observations made with different parts of the same algal individual were regarded as repeated measures). For comparisons among different experiments, factorial ANOVA was used. In several cases, data had to be Box-Cox-transformed to obtain normality (Kolmogorov-Smirnov test, p = 0.05) and homogeneity of variance (Levine's test, p = 0.05) using the Minitab 13 software package. Tukey tests were performed as post hoc tests (p = 0.05).

The light-compensation point of net photosynthesis was computed by linear regression of O_2 production rates and PAR, using the Prism 5.0 software package. Non-linear regression of effect sizes and PAR was conducted with the same software, using the logistic dose-response function:

$$\text{Effect size} = [A + (B - A)] \times (1 + 10^{[\log(EC50) - \log(PAR)]})^{-1} \quad (6)$$

in which A and B represent minimal and maximal effect sizes, respectively, and EC50 represents the necessary PAR for a half-maximal effect size.

RESULTS

Comparison of bioassays

Overall, the type of bioassay used (live algae versus pellets) did not affect the outcome of the preference tests, and effect sizes obtained for identical samples did not differ between the 2 assay types (Table 2). However, assays conducted with living *Fucus vesiculosus* were more variable: the 95% CI obtained for Hedges' g ranged from 0.98 to 1.24, while they were between 0.08 and 0.18 for log(w).

Light effects

The light-compensation point of photosynthetic oxygen production in *Fucus vesiculosus* at 15°C was 16.8 µE m^{-2} s^{-1} (95% CI: 10.3 to 22.2 µE m^{-2} s^{-1}) (Fig. S1 in the supplement at www.int-res.com/articles/suppl/m427p083_supp.pdf). Specimens incubated for 1 wk at a daily PAR close to this light intensity (15 µE m^{-2} s^{-1}) did not have significantly lower mannitol content than specimens incubated at 62 µE m^{-2} s^{-1} (repeated-measures 2-way ANOVA and Tukey test, p = 0.05) (Table S1 in the supplement; Fig. 1). However, incubation in darkness for the same time period resulted in a clear reduction of mannitol by approximately 40% (Table S1 in the supplement; Fig. 1). Similarly, a reduced C:N ratio was observed in *F. vesiculosus* after 2 wk of mean daily exposure to 5 µE m^{-2} s^{-1}, as compared to 2 light intensities above the light-compensation point (repeated-measures 2-way ANOVA and Tukey test, p = 0.05) (Table S2 in the supplement; Fig. 2A). The mannitol content was significantly reduced in the presence of *Idotea baltica* (Table S1 in the supplement; Fig. 1), while the C:N ratio was not significantly affected by these grazers (Table S2 in the supplement; Fig. 2A).

Table 2. *Fucus vesiculosus*. Repeated-measures ANOVA of effect sizes obtained in 2 different feeding bioassays with specimens that originated from 14 different treatments (Treatments 13 to 26 in Table 1). The treatments were used as between-subject factor and the type of feeding assay was used as within-subject factor

Source	SS	df	MS	*F*	p
Between-subject effects					
Intercept	31.659	1	31.659	27.83	<0.0001
Treatment	53.931	13	4.149	3.65	<0.0001
Error	94.408	83	1.137		
Within-subject contrasts					
Type of assay	0.364	1	0.364	0.30	0.583
Treatment × Type of assay	15.492	13	1.192	1.00	0.463
Error (Sample)	99.383	83	1.197		

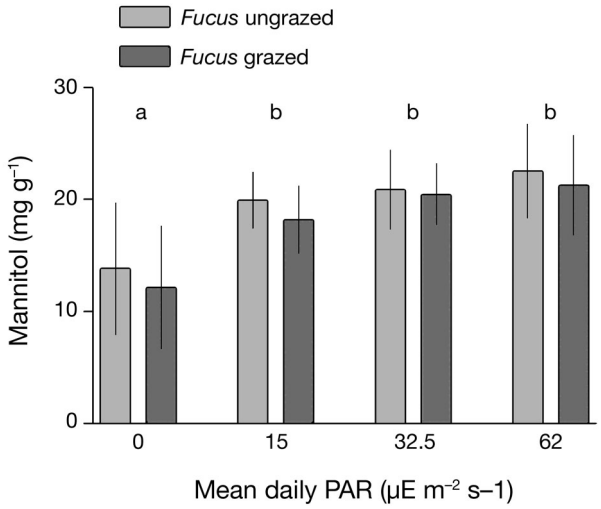

Fig. 1. *Fucus vesiculosus*. Concentration of mannitol in tissue after incubation at different light intensities and in the presence (grazed) and absence (ungrazed) of *Idotea baltica*. Treatments 23 to 26 in Table 1; data are mean ± 95% CI. Different letters indicate light intensities that resulted in significantly different mannitol concentrations (2-way ANOVA; Table S1 in the supplement)

Even in the absence of grazers, a significant weight loss of *Fucus vesiculosus* was observed at subcompensation irradiance (5 µE m^{-2} s^{-1}; Fig. 2B). In contrast, light intensities above the light-compensation point allowed for positive growth in ungrazed algae. Net growth in previously grazed algae at all light intensities tended to be lower than in previously ungrazed algae. This effect was significant at 25 and 40 µE m^{-2} s^{-1} (Fig. 2B; Table S3A in the supplement). In the presence of grazers, *F. vesiculosus* always tended to lose biomass except for previously grazed algae (i.e. with induced defenses, see below), which gained biomass under the highest irradiance (Fig. 2C). The difference in wet-weight increase between induced and uninduced *F. vesiculosus* at 40 µE m^{-2} s^{-1} was significant ($p < 0.05$) when the Wilcoxon test was used. However, 2-way ANOVA detected neither a significant overall effect of grazing during the treatment phase, nor a significant interactive effect of grazing and PAR (Table S3B in the supplement). Overall, preceding grazing (treatment phase) seemed to reduce *Idotea baltica* consumption during the test phase (Fig. 2D). Nearly no consumption was observed with previously

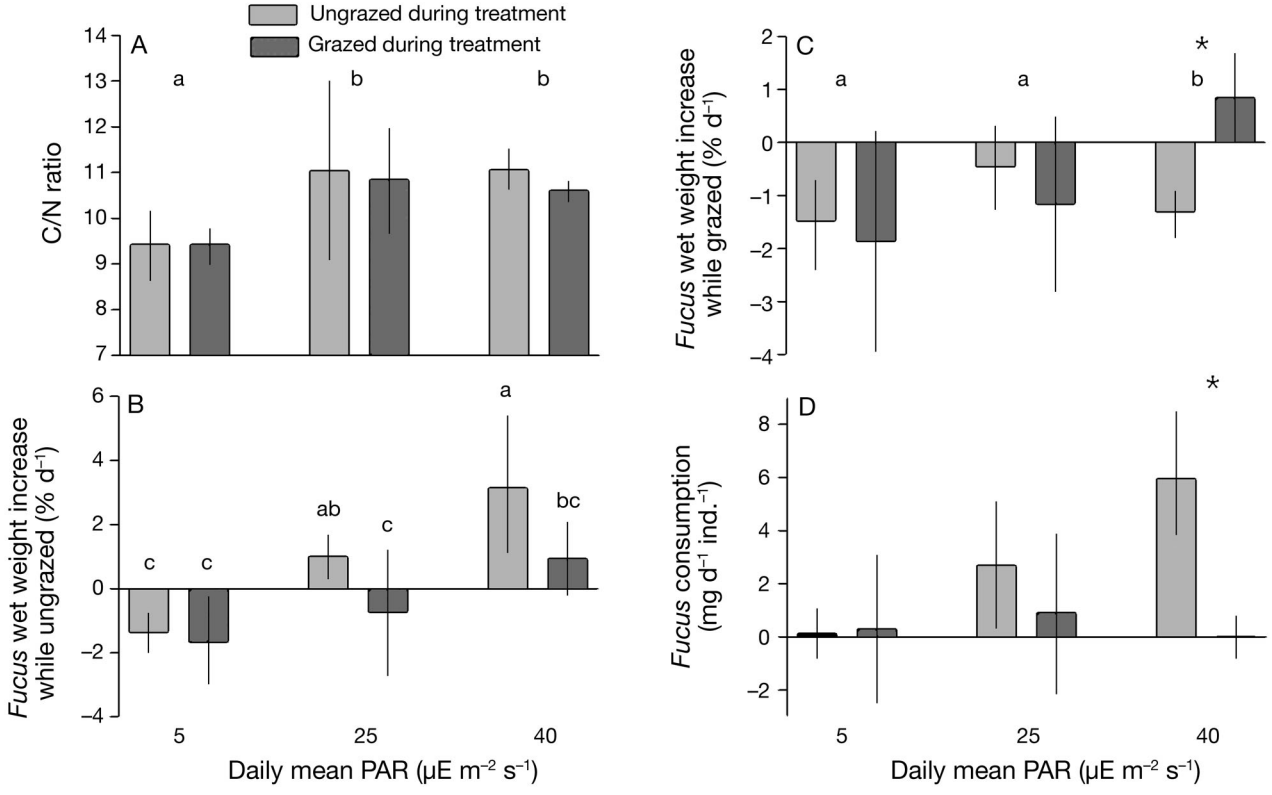

Fig. 2. *Fucus vesiculosus*. (A) C:N ratios, (B,C) growth, and (D) palatability after 14 d of treatment at 15°C and at 3 different light intensities with and without the presence of *Idotea baltica*. Treatments 8 to 10 in Table 1; data are mean ± 95% CI. (A) C:N ratios at the end of the treatment phase. (B,C) Growth rates in the (B) absence or (C) presence of *I. baltica* after previous presence or absence of the grazer. (D) Consumption by *I. baltica* in a 2-way choice assay. The grazers could choose between living thallus pieces that had and had not been exposed to *I. baltica* during the preincubation period. Different letters indicate light intensities (A,C) or treatments (B) that are significantly different (2-way ANOVA; Tables S3 & S4 in the supplement). *Significantly different data after treatment with and without grazers (Wilcoxon test, $p < 0.05$). PAR: photosynthetically active radiation

grazed *F. vesiculosus* irrespective of light conditions. In contrast, the consumption of previously ungrazed algae increased with irradiance. At 40 µE m^{-2} s^{-1} but not at lower irradiance, the preference for previously ungrazed *F. vesiculosus* relative to previously grazed *F. vesiculosus* was significant (Wilcoxon test).

Exposure of *Fucus vesiculosus* to relatively high light thus seemingly increases its attractiveness for *Idotea baltica.* An additional experiment was therefore conducted in order to test the hypothesis that *I. baltica* is attracted by mannitol, the main primary product of photosynthesis in *F. vesiculosus*. Food pellets containing as the basic reference ingredient ground *Ulva* sp. (naturally devoid of mannitol) and mannitol or no mannitol were offered to *I. baltica* in 2-way choice assays (Fig. 3). Increasing mannitol concentration resulted in increasing preference of *I. baltica.* This effect was significant (χ^2 test, $p < 0.05$) when mannitol was present at natural concentrations (–17.5 mg g^{-1}).

Further experiments conducted at 15 to 16°C confirmed that the defense inducibility is light-dependent (Fig. 4). A logistic dose-response function could be fitted to these data ($r^2 = 0.7316$, $p = 0.044$; Fig. 4A). According to this function, the necessary mean daily PAR for induction of a half-maximal reduction of palatability was 3.6 µE m^{-2} s^{-1} (95% CI: 0.6 to 20.5 µE m^{-2} s^{-1}). Because of the data variability, no significant linear or logistic regression between the effect size obtained in assays with living *Fucus vesiculosus* and the mean daily light intensity during the preincubation was detected (Fig. 4B; $p < 0.05$). However, as in the pellet assay, the 2 lowest light intensities tested (0 and 5 µE m^{-2} s^{-1}) resulted in the 2 highest (least negative) effect sizes.

Regression analysis further revealed that no significant correlation between the effect sizes obtained with both bioassays and the mean outdoor light intensity in Kiel Fjord (ranging from 22.9 to 594.7 µE m^{-2} s^{-1}; Table 1) during the 2 wk prior to *Fucus vesiculosus* collection existed (data not shown).

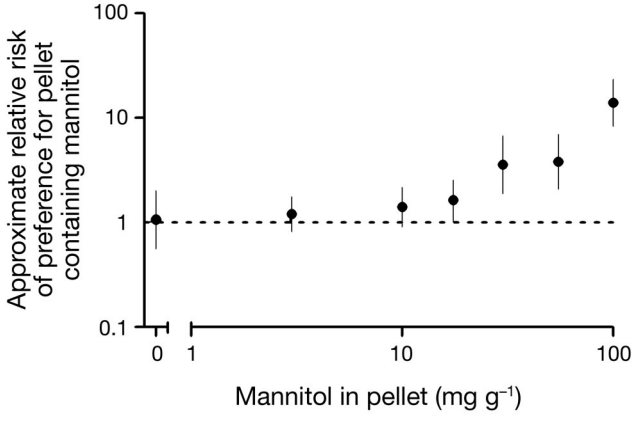

Fig. 3. *Idotea baltica*. Feeding preference for food pellets containing mannitol at different concentrations, relative to pellets devoid of mannitol. Data are mean ± 95% CI

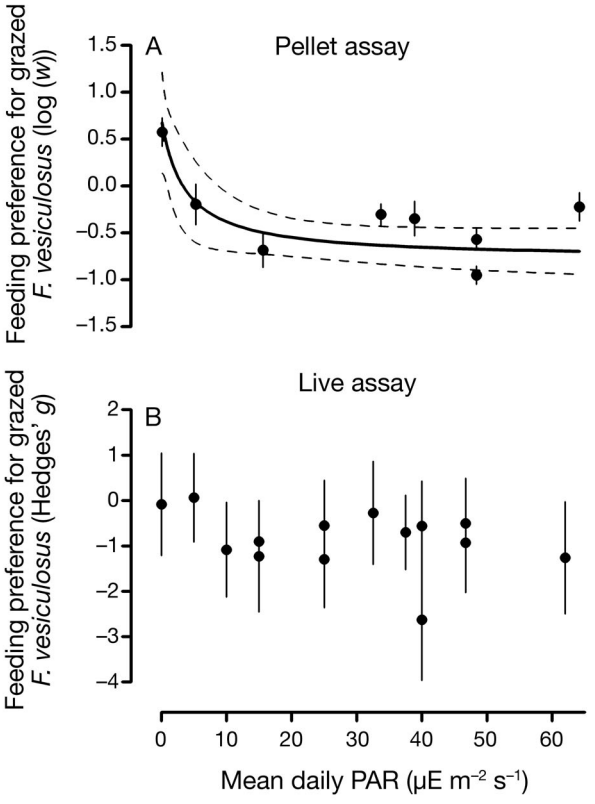

Fig. 4. *Fucus vesiculosus* and *Idotea baltica.* Feeding preference of *I. baltica* for induced *F. vesiculosus* that had been treated at 15°C at different light intensities. Effect sizes < 0 indicate reduced palatability after exposure to *I. baltica.* (A) *F. vesiculosus* was offered freeze-dried in an artificial diet. Treatments 11, 12, 15, 20, and 23 to 26 in Table 1. Solid line represents the best fitting logistic dose-response function (dashed lines are 95% CI; n = 8). (B) *F. vesiculosus* was offered alive. Mean ±95% CI. Treatments 1, 2, 5 to 10, 15, 20, and 23 to 26 in Table 1. PAR: photosynthetically active radiation

Temperature effects

In a first experiment, *Fucus vesiculosus* was collected in March, when the mean ambient water temperature was 0.8°C. The material was treated with and without *Idotea baltica*, at mean daily light intensities of 15 and 37.5 µE m^{-2} s^{-1}, and at each light condition at 15 and 20°C. ANOVA revealed that light affected growth significantly, while temperature had no overall effect, but interacted with light (Table S4A in the supplement; Fig. 5A). Irrespective of the light regime, *I. baltica* grazing during the treatment phase resulted in reduced palatability at 15°C, but not at 20°C

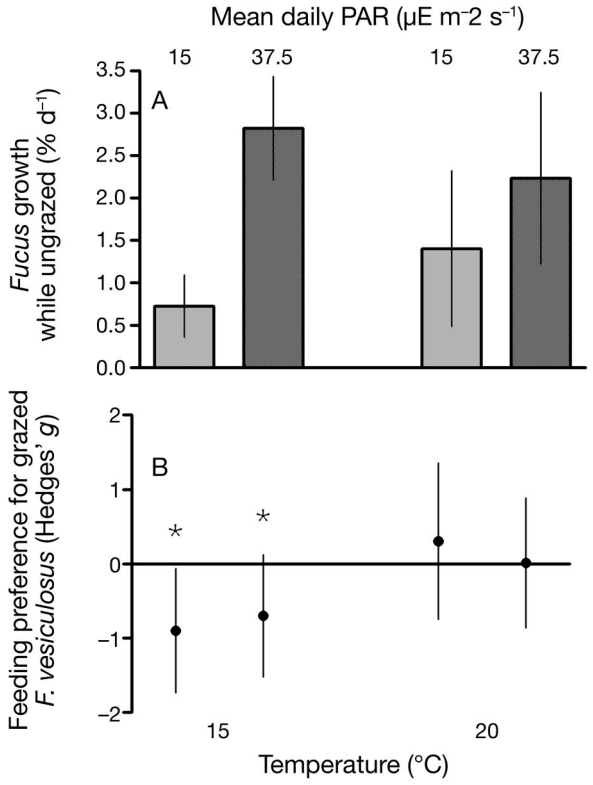

Fig. 5. *Fucus vesiculosus*. Growth rates during (A) and results of feeding assays after (B) treatment at different light and temperature combinations. Growth was measured as change in biomass (±95% CI) during the treatment phase. *Significant differences in the palatability of grazed and ungrazed specimens (Wilcoxon test, $p < 0.05$). Treatments 1 to 4 in Table 1. PAR: photosynthetically active radiation

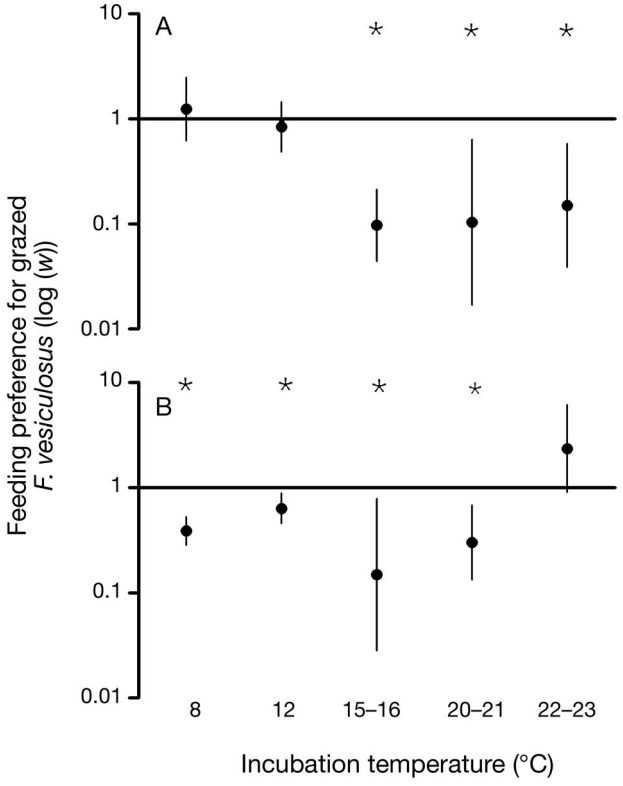

Fig. 6. *Fucus vesiculosus* and *Idotea baltica*. Relative feeding preference (effect size log(w) ± 95% CI) of *I. baltica* for food pellets containing *F. vesiculosus* that had been collected at (A) 18.7°C (Treatments 18 to 22 in Table 1) and (B) 13.7°C (Treatments 14 to 17 in Table 1) and exposed or unexposed to *I. baltica* at identical photosynthetically active radiation levels (46.5 µE m^{-2} s^{-1}), but different temperatures. Effect sizes < 0 indicate that previous exposure to *I. baltica* resulted in reduced palatability for *I. baltica*. *Significant differences in the palatability of grazed and ungrazed *F. vesiculosus* (Wilcoxon test, $p < 0.05$)

(Fig. 5B; Wilcoxon test, $p < 0.05$). ANOVA confirmed that temperature, but not light, affected the palatability reduction after grazing significantly, while light interacted with temperature (Table S4B in the supplement).

The experiment was repeated in May with *Fucus vesiculosus* that had been collected at 14°C. This time the algae were treated at only one light condition (mean daily PAR: 35 µE m^{-2} s^{-1}), but over a larger temperature range. In order to exclude possible temperature effects during the subsequent 2-way choice feeding assay, they were offered in pelleted form at a standard temperature of 15°C. In this second experiment, the algae were able to induce defenses between 8 and 21°C, but not at 23°C (Fig. 6A). The experiment was repeated in the same way in August with *F. vesiculosus* that had been collected at a mean ambient water temperature of 20°C. This time the algae induced defenses between 15 and 22°C, but not at 8 and 11°C (Fig. 6B).

Irrespective of the ambient temperature during collection (tested with material collected at 0.8, 6.5, 9.2, 10.5, 14, and 20°C), *Fucus vesiculosus* was always capable of a defense response when the treatment was conducted at a mean daily PAR of 35 to 40 µE m^{-2} s^{-1} and a temperature of 15°C (data not shown).

DISCUSSION

Both light limitation stress and disruptive stress resulting from temperature shifts may impair the capacity of *Fucus vesiculosus* for defense induction against grazing by *Idotea baltica*. We found comparable results with both bioassay types employed, both using live algae or reconstituted algae as feed. Each assay has certain advantages and disadvantages. The live assay allows for quantification of the overall palatability of *F. vesiculosus*, which may depend upon chemical as well as structural traits. However, the possibility that *F. vesiculosus* changes its palatability

while the assay is conducted cannot be fully excluded, which probably accounts for the relatively large variability in the data. More stringent test conditions are possible with the pellet assay, which results in less variability. However, this assay only quantifies chemical properties, since the algal structure is destroyed. The congruent results obtained with both assays suggest that variations in the palatability of *F. vesiculosus* after different treatments must be primarily due to variable chemical defense and/or attraction.

In *Fucus vesiculosus*, the light-compensation point of net photosynthesis varies with season. At the Danish Belt, a PAR of approximately 35 µE m^{-2} s^{-1} was required to compensate for respiration between April and September, compared to 15 µE m^{-2} s^{-1} between October and February (Middelboe et al. 2006). Corresponding with these data, respiration of *F. vesiculosus* from Kiel was in the present study compensated at 17 µE m^{-2} s^{-1} in early March, while positive growth in summer was impossible when the mean daily PAR was <35 µE m^{-2} s^{-1} (Rohde et al. 2008). We therefore exerted severe light limitation on *F. vesiculosus* when it was exposed to daily mean intensities of PAR <10 µE m^{-2} s^{-1}. This is confirmed by the reduced C:N ratio and growth rate of *F. vesiculosus* within 2 wk at 5 µE m^{-2} s^{-1}, as well as by the reduced content of mannitol after 2 wk of incubation in darkness. At light levels above the compensation point, the grazers reduced net growth directly by removing biomass, but part of this effect persisted even after the grazers had been removed, presumably because grazing had destroyed parts of the meristematic tissue or had caused other metabolic costs, such as defense production. The somewhat reduced mannitol content in grazed *F. vesiculosus* also hints at metabolic costs due to grazing.

The palatability of *Fucus vesiculosus* increased with irradiance in naive algae (that had not previously been exposed to grazing) but not in algae that had induced defenses in response to previous grazing. As previously suggested by us (Rohde et al. 2004) and others (Toth & Pavia 2007), a mechanism of induced defense against *Idotea baltica* obviously exists in *F. vesiculosus*. Our results suggest that this capacity is dependent on sufficient irradiation.

The necessary mean daily PAR for induction of a half-maximal defense response against *Idotea baltica* was approximately 4 to 8 µE m^{-2} s^{-1} when *Fucus vesiculosus* was maintained at its temperature optimum (Lüning 1990) of 15 to 16°C. This light intensity is substantially lower than the light-compensation point of photosynthesis. During periods of darkness, *F. vesiculosus* uses storage compounds in order to maintain its energy metabolism and other functions that are essential for survival (Lehvo et al. 2001, Obluchinskaya et al. 2002). Potentially, these resources may also be used to a certain extent for induced antigrazing defense. Their gradual depletion at more severe conditions of light limitation might then explain the absence of induced defense at these conditions. However, the time periods of light limitation applied in our experiments did not exceed 2 wk. They were therefore considerably shorter than those of several months that are usually experienced and tolerated by *F. vesiculosus* during winter. Moreover, algae collected in early summer — when carbon storage in *F. vesiculosus* reaches its maximum (Lehvo et al. 2001, Obluchinskaya et al. 2002) — proved to be similarly incapable of defense induction during light limitation like those specimens collected in late winter, when carbon depletion is maximal. These considerations suggest that not all carbon-storage compounds can be mobilized by *F. vesiculosus* for defense induction. Or, lack of resources is possibly not the main reason for the absence of induced defense in *F. vesiculosus* during light limitation.

White (1984) suggested that low-light stress may increase the susceptibility of vascular plants to insect herbivores by increasing the concentrations of nitrogenous compounds that herbivores use as feeding cues. However, in the present study, previously ungrazed tissue of *Fucus vesiculosus* had a higher palatability for *Idotea baltica* when it was exposed to relatively high PAR and actively growing than when it was light-limited. Obviously the reduced C:N ratio in light-limited *F. vesiculosus* was not correlated with increased attraction of *I. baltica*, suggesting that nitrogenous compounds in *F. vesiculosus* are not a relevant feeding cue for this isopod, which intensely preys on smaller crustaceans and uses them as a source of nitrogen (Franke & Janke 1998). Instead, compounds that are produced in *F. vesiculosus* during light exposure seem to increase its attractiveness. The modifying effect of presence or absence of light upon the attractiveness of *F. vesiculosus* was observed after relatively short incubation periods of <2 wk, which suggests that a primary rather than a long-term CO_2-storage compound acted as feeding cue. Mannitol is the main primary CO_2-storage compound of *F. vesiculosus* after photosynthesis (Bidwell et al. 1972). In the present study, a diet containing mannitol clearly attracted *I. baltica*, which confirms that omnivorous crustaceans often use carbohydrates as feeding cues (for an overview, see Corotto & O'Brien 2002) and which corresponds with earlier reports of increased fitness in *I. baltica* fed with *F. vesiculosus* rich in mannitol (Hemmi & Jormalainen 2004). The tissue concentration of mannitol in *F. vesiculosus* from Finland ranges from 20 mg g^{-1} wet weight in winter to 70 mg g^{-1} wet weight in summer (Lehvo et al. 2001). Concentrations at the lower end of this range were also detected by us in February and concentrations in the same range clearly

attracted *I. baltica*. Mannitol accumulation during light exposure may therefore increase the attractiveness of *F. vesiculosus*. Such increased attraction should result in increased feeding pressure and in an increased requirement of additional (induced) defense.

The proposed scenario corresponds with the fact that *Idotea baltica* is virtually absent from *Fucus vesiculosus* in the German Baltic Sea and in winter, but not from other algae in the close vicinity of *F. vesiculosus* (Weinberger et al. 2008). Since the presence of *I. baltica* alone is not sufficient to induce antifeeding defense, consumption is required (Rohde et al. 2004) and an absence of consumption due to low palatability at low light conditions should result in no triggering of defense. The question therefore arises whether antifeeding defenses are not induced under light limitation because the resources are lacking or because the necessity is not created. A definite answer will perhaps only be possible once the induced compounds have been identified and the resources and signaling pathways required for their production are known.

In addition to low-light stress, abrupt changes in water temperature in either direction by 7 to 8°C impaired the ability of *Fucus vesiculosus* to induce defense. Absolute temperatures were not important. Given that approximately 20°C is the highest long-term water temperature within the natural distribution range of *F. vesiculosus* (Lüning 1990), we expected that upward shifts of temperature to 20°C or more should exert stress, while downward shifts from 19°C should release stress. However, both shifts impaired the capacity for induced defense. At the same time, these shifts did not prevent algal growth, which indicates that a possible resource depletion due to disruptive stress was not as severe as under light limitation. Possibly, abrupt temperature shifts caused the (temporary) dysfunction of specific enzymes or other cellular components relevant for defense induction. Apparently, short-term oscillations in temperature during air exposure do not definitely disrupt defense induction in *F. vesiculosus*, because this capacity is present in field-collected material even from the most shallow depths, where temperature fluctuations can be extreme.

In conclusion, the present study indicates that the induced defense of *Fucus vesiculosus* against its main consumer in the western Baltic Sea requires exceeding a minimum threshold of light and stable temperature conditions. Induced defense against *Idotea baltica* is mainly employed during phases of strong photosynthesis, when the tissue is rich in mannitol and attractive for the herbivore. During phases of light limitation, induced defense is largely inhibited. However, this is compensated by reduced attraction of *I. baltica*, due to a reduced content of mannitol. *F. vesiculosus* thus appears capable of coping with its main consumer in the Baltic Sea even during periods of relatively severe light-limitation stress. Different from light limitation, abrupt shifts of water temperature by 7°C or more rarely (if ever) occur in *Fucus vesiculosis* habitats. For this reason, *F. vesiculosus* may not have evolved the capacity to compensate with alternative mechanisms for the disruption of induced defense by temperature stress.

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