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Stressed, but not defenceless: no obvious influence of irradiation levels on antifeeding and antifouling defences of tropical macroalgae

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Abstract The production of defence metabolites is assumed to be costly in metabolic terms. If this holds true, low-light stress should reduce the ability of seaweeds to defend themselves chemically against herbivory and fouling. We investigated the effect of energy limitation on the defensive status of seaweeds by assessing their attractiveness to mesograzers and their activity against a bivalve macrofouler in comparison with non-stressed conspecifics. The macroalgae *Codium decorticatum* (Woodw.) M. Howe, *Osmundaria obtusiloba* (C. Agardh) R. E. Norris, *Pterocladiella capillacea* (S. G. Gmel.) Santel. and Hommer., *Sargassum vulgare* C. Agardh and *Stypopodium zonale* (Lamour.) Papenf. collected at the southeastern Brazilian coast were exposed to six levels of irradiation (between 1 and 180 μmol photons $m^{-2} s^{-1}$) for 10–14 days. After this period, algae from all treatment levels were: (a) processed as artificial food and offered to an amphipod community dominated by *Elasmopus brasiliensis* Dana and (b) extracted to test for differences in settlement rates of the fouling mussel *Perna perna* L. on filter paper loaded with the crude extracts. Generally, photosynthesis rates and growth were reduced under low light conditions. Attractiveness to herbivores and macrofoulers, however, was insensitive to energy limitation. We discuss possible expla-nations for the observed absence of a relationship between light availability and algal defence including the change in nutritional value of the algal tissue, the allocation of resources towards defence instead of growth and the absence of costs for defence.

Introduction

The progressive, man-induced increase in eutrophication that occurs in many coastal regions worldwide (e.g. Khan and Ansari 2005) and the regionally expected increase in precipitation (e.g. Fowler and Henessy 1995) may decrease the amount of light available for benthic macroalgae. Moreover, an increase in the abundance of suspended particles due to an enhanced storm frequency (e.g. Webster et al. 2005) or large-scale dredging activities (e.g. Erftemeijer and Lewis 2006) contribute to a change in light regimes in shallow water habitats. But even without anthropogenic stressors, algae are often light limited at the deep boundaries of their distribution. Low light stress should limit resource acquisition of seaweeds and reduce their total energy budget (Thomas et al. 1987; Dethier et al. 2005; Kavanaugh et al. 2009). As a consequence, the algae's capacity to defend themselves against herbivory should be reduced, since most hypotheses about defences in plants assume the build-up of resistance traits, such as chemical compounds, to be costly in metabolic terms (reviewed by Cronin 2001; Stamp 2003). These costs are thought to result from the synthesis, turnover, transport and storage of secondary metabolites (Hay and Fenical 1988; Purrington 2000). Moreover, it has been hypothesized that energy has to be diverted away from other life processes, such as growth, reproduction, acquisition of resources and maintenance, to abide resistance (Cronin 2001; Pavia and Toth 2008). Therefore, the environmental

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stress hypothesis (EST) predicts that plants under stress do not have sufficient resources to sustain chemical defence (White 1984; Rhoades 1985).

Hitherto, a few studies investigated whether environmental stress due to desiccation, unfavourable temperatures, nutrients, or light-limitation diminishes the production of secondary metabolites in macroalgal tissue and/or its susceptibility to herbivore grazing (Renaud et al. 1990; Yates and Peckol 1993; Cronin and Hay 1996a; Pavia and Toth 2000; Jormalainen et al. 2003; Heaven and Scrosati 2004; Dethier et al. 2005; Scheibling et al. 2008; Pansch et al. 2009). These, however, produced ambiguous results and none of them were performed in tropical systems, where grazing pressure is higher than in temperate systems (e.g. Bolser and Hay 1996) and where environmental factors such as light and temperature are not subjected to seasonal variations.

As sessile organisms with extensive surface areas, macroalgae are also exposed to fouling (e.g. Wahl 1989). This process, similar to grazing, can have severe direct and indirect effects (including light reduction) on their growth and spatial distribution (e.g. Duffy and Hay 2000; Wahl 2008). Consequently, many seaweeds are chemically defended against micro- and macrofoulers (reviewed by Clare 1996; Paul and Ritson-Williams 2008). According to the EST, environmental stress should also affect algal defence against epibionts.

If environmental stress does affect the defensive capacity of seaweeds, the ensuing shift of plant–herbivore and plant–fouler interactions could substantially amplify the direct impact of stress on macroalgae (M. Wahl et al. Defense ecology of the bladder wrack (*Fucus vesiculosus*): 1. patterns of microfouling and antimicrobial protection, submitted; F. Weinberger et al. Defense ecology of the bladder wrack (*Fucus vesiculosus*): 2. Effects of temperature and light on antigrazing defense, submitted). The abundance as well as the vertical and horizontal distribution of habitat-forming macroalgae is at least partly controlled by grazers (Scheibling et al. 1999) or foulers (Rohde et al. 2008). Tropical algae, which are adapted to constant light intensities throughout the year, should be especially vulnerable to short-term fluctuations in irradiation. We tested these predictions with different macroalgal species from the southeastern Brazilian coast.

Materials and methods

Sampling sites and organisms

We conducted this study at the laboratories of the department of Marine Biology of the Universidade Federal Fluminense (UFF) in Niterói (Rio de Janeiro, Brazil) between January and May 2007. The five species of seaweeds tested were sampled at different sites on the SE coast of Brazil.

The green alga *Codium decorticatum* has a very stiff, water-filled thallus and occurs in tropical, temperate and even Antarctic waters. For our study, the seaweed was collected from 1 to 2 m water depth at Boa Viagem, Niterói, Rio de Janeiro (22°54′37″S, 43°7′47″W) in the polluted and eutrophic Guanabara Bay (Kjerfve et al. 1997). Here, a high organic load limits penetration of light and Secchi depths usually do not exceed 3 m (Tauiol and Yoneshigue-Valentin 2002).

Two species of red algae, *Osmundaria obtusiloba* and *Pterocladiella capillacea*, were sampled in Praia Rasa, Armação dos Búzios, Rio de Janeiro (22°44′05″S, 41°57′31″W). Here, light penetration is even lower due to constantly high amounts of suspended sediment in the water. Secchi depth was 60 cm on a sunny day. *Osmundaria obtusiloba* has a course, tough and bushy thallus and is sub-tropical to tropical, while *Pterocladiella capillacea* is more delicate and occurs in temperate and tropical regions. We collected the latter in the intertidal zone at Praia Rasa.

The two brown algae, *Sargassum vulgare* and *Stypopodium zonale,* were collected in Enseada do Forno, Armação dos Búzios, Rio de Janeiro (22°45′39″S, 41°52′30″W) from a water depth of 2–3 m. This embayment exhibited a Secchi depth of about 5 m. The thallus of *S. vulgare* is erect, leathery and branched, while that of *S. zonale* is flat and tough with almost dichotomous branching. Both species are widespread in temperate and tropical regions.

All algal individuals collected showed less than 10% cover by epibionts. They were transported to the laboratory in cooled containers and weighed within 1 h after sampling.

Algal palatability after the irradiation treatment was tested using an amphipod community dominated by *Elasmopus brasiliensis*, which, in previous studies, has been identified suitable for feeding assays with the chosen seaweeds (Weidner et al. 2004; da Gama et al. 2008). The grazers were collected in Boa Viagem, Niterói, where they occur in association with the common green alga *Ulva fasciata* Delile. They were kept in aerated buckets at 15°C and were fed with *U. fasciata*. Amphipods were not starved previous to the feeding assays, since starvation can alter the feeding preference of herbivores (Cronin and Hay 1996b).

We tested for antifouling activities of algal extracts by an attachment assay using the bivalve *Perna perna* (da Gama et al. 2003). Juvenile specimens between 1 and 2 cm were collected in Itaipu, Niterói, Rio de Janeiro (22°58′10″S, 43°02′47″W) a few hours prior to the bioassays and kept in aerated buckets.

Experimental setup

The setup consisted of a closed seawater system with nine water reservoirs (ca. 3,000 L in total) connected by pipes. Seawater constantly circulated in the system, passing a

filter of bivalve shell fragments, fibreglass wool, coral skeletons and protein skimmers. The experimental units, plastic aquaria ($12 \times 18 \times 11$ cm, 2 L), were spray-painted (silver) outside to exclude diffuse light. There was a constant flow of seawater at 30 L h^{-1} through the aquaria. Each of the six shelves containing experimental units was irradiated by four daylight fluorescent lamps (180 W) in a 12:12 h light:darkness cycle. The light of the 24 lamps was reflected by aluminium panels surrounding the shelves. Laboratory light intensity was measured by a quantameter (QSL-2101, Digital Output 2100 Series, Biospherical Instruments Inc.) and was determined to be in the range of typical irradiance levels measured at the sampling sites (ca 450 μmol photons m^{-2} s^{-1} just below the water surface).

Pilot study: identification of light compensation points

To identify light intensities that constitute a stress, we assessed the light intensities at which oxygen production and consumption were balanced, i.e. the light compensation point, for each algal species. Per species, twelve aquaria were equipped with equal amounts of algal material, which was weighed after spinning it 30 times with a salad spinner. We chose the amounts of algal biomass per aquarium in a way that self-shading (determined by visual judgment) was avoided. Total amounts thus differed between species: *C. decorticatum*: 160 ± 10 g, *O. obtusiloba*: 20 ± 1 g, *P. capillacea*: 20 ± 1 g, *S. vulgare*: 20 ± 1 g, *S. zonale*: 20 ± 1 g. We generated six different light levels by placing 0, 1, 2, 4, 6 and 8 layers of black gauze with a mesh size of 1×1.5 mm on top of the aquaria. All light levels were replicated twice.

The algae were acclimated to laboratory conditions for 1 week to allow them to adapt to lab light intensities, e.g. by an increase in pigmentation. After acclimation, all experimental units were decoupled from the water cycle, completely filled up with seawater and sealed off with transparent cling foil. We accounted for the light reduction by cling foil when we established the light regimes. Oxygen content in the aquaria was then measured three times a day with an oxymeter (GOX 20, Geisinger electronic GmbH) over a time period of 72 h. Aquaria in which no increase in oxygen tension occurred during light periods should have experienced light intensities at which algae were at or close to their light compensation point.

Main experiment: light irradiation treatment

Algae were exposed to light regimes above, at and below their compensation point for up to 2 weeks. Treatment levels were 1–2, 4–7, 15–20, 30–50, 70–90 and 130–180 μmol photons m^{-2} s^{-1} (light:dark = 12:12 h). Only in case of *P. capillacea* the treatment was stopped after 10 days, since

the algae showed signs of necrosis. Algal biomass per container was 160 ± 10 g for *C. decorticatum*, 30 ± 1 g for *O. obtusiloba*, 30 ± 1 g for *P. capillacea*, 5 ± 1 g for *S. vulgare*, 20 ± 1 g for *S. zonale*. Ten amphipods were added to each aquarium to ensure that defences were not reduced for lack of stimulus (e.g. Rohde et al. 2004). To hinder amphipods from escaping, we placed sheets of transparent PVC on top of the aquaria and covered tube apertures with a plastic mesh.

We established four replicates per irradiation level, while another three aquaria per level contained no grazers and served as a reference to measure autogenic change in algal biomass (Peterson and Renaud 1989). They were connected to the same circulatory system, allowing the exchange of water-borne cues between grazed and non-grazed units. This ensured equal conditions across all parallels with the exception of tissue loss due to grazing. Replicates were randomly distributed within the setup. We removed and replaced dead grazer individuals once a week.

Algal growth during irradiation treatment

Algal biomass in each replicate was determined before and after the irradiation treatment by measuring wet weight to the closest mg after spinning thallus fragments 30 times with a salad spinner.

Algal palatability after irradiation treatment

For the preparation of food pellets, we freeze-dried algal material, ground it and incorporated the powder into a agar mixture, which was then spread onto a mesh matrix (1×1.5 mm mesh size; Hay 1996; Rohde et al. 2004). We cut pellets of twelve by twelve mesh squares, which were stored in a refrigerator for a maximum time span of 1 day. Due to its stiffness, *P. capillacea* could not be ground and was therefore extracted (see below) and the residue incorporated in agar enriched with powder of *U. fasciata*.

We performed no-choice feeding assays for all four species of algae, since assays with more than two choices are difficult to evaluate (e.g. Manly 1993). We produced four algal pellets per replicate and averaged consumption across them. Each pellet was placed in a Petri dish filled with sea water to which four to six (equal amounts in same algal species experiments) amphipods were added. Dishes were left in a constant temperature room at 15°C with a 12:12 h light:dark cycle for maximum 2 days or until the first pellet was completely consumed. The water in the Petri dishes was exchanged once a day. We then determined amphipod consumption by counting the number of mesh squares cleared by more than 50%.

In feeding assays with *S. zonale,* we observed up to 100% mortality among the amphipods preceded by a

temporal paralysis of all individuals. We therefore terminated the assays with this seaweed species.

Activity against the macrofouler *P. perna* after irradiation treatment

Following da Gama et al. (2003), we prepared extracts from freeze-dried algal fragments with hexane and methanol for 24 h after which we filtered them and vaporized the solvent. This process was repeated three times and the residues were sealed off airtight and stored in a deep freezer at −20°C. Before testing, the hexane and methanol extracts of a given algal individual were united. The extraction from *S. zonale* was undertaken in the dark, since brown algal polyphenols are known to decompose when exposed to light (Amsler and Fairhead 2006).

Per algal replicate three filter papers, 9 cm in diameter, were weighed and soaked with a re-suspended extract–solvent mix. To ensure natural test concentrations, the quantity of extract per filter paper was identical to the quantity extracted from an equal mass of dried algal tissue. We used four subreplicates per replicate (ultimately averaged), whereas another sixteen filter papers treated with solvent only served as controls.

For the no-choice assays, we followed the procedure described by da Gama et al. (2003). Mussels were exposed to the extract-loaded filter papers, and the total amount of byssus threads attached was counted after staining them with bengal rose protein stain. Due to the low biomass available, no assays were performed with extracts from *S. vulgare*.

Statistical analysis

All data were tested for normality using the Shapiro-Wilks-W test before further statistical analysis. If the data were non-normally distributed, the Box-Cox procedure identified the simplest transformation to achieve normality. Percentage data were arcsine transformed. Data were tested for homogeneity of variances using Levene's test. If normality and homoscedasticity could not be achieved, we used parametric tests but lowered the α-level to 0.01. In linear and polynomial regression analyses, light reduction was used as a predictor for algal biomass variation, amphipod consumption and byssus thread production. Additionally, a one-factorial ANOVA followed by Tukey's HSD post-hoc tests tested for significant differences between controls and treatments in the mussel tests.

Results

Algal oxygen production generally increased with increasing light availability (Fig. 1). We found maximum oxygen production at lower than at the highest light level in three cases (Fig. 1). Light compensation points of the different species were: *Codium decorticatum* between 5 and 9 μmol photons $m^{-2} s^{-1}$, *Osmundaria obtusiloba* between 2 and 5 μmol photons $m^{-2} s^{-1}$, *Pterocladiella capillacea* between 1 and 5 μmol photons $m^{-2} s^{-1}$, *Sargassum vulgare* between 1 and 5 μmol photons $m^{-2} s^{-1}$ and *Stypopodium zonale* between 5 and 11 μmol photons $m^{-2} s^{-1}$.

In all species, except for *P. capillacea*, there were significant relationships between biomass change and light intensity (Fig. 2). These are best described by a 2nd degree polynomial for *C. decorticatum*, with the maximum growth occurring at medium light intensities, and by a positive linear model in all other cases (*C. decorticatium*: $r^2 = 0.91$, $P < 0.01$, $y = 1.34 + 0.61 \times x - 0.003 \times x^2$; *O. obtusiloba*: $r^2 = 0.36$, $P = 0.01$, $y = 10.60 \times 0.09 \times x$; *P. capillacea*: $r^2 = 0.20$, $P = 0.18$; *S. vulgare*: $r^2 = 0.95$, $P < 0.01$, $y = -21.70 + 0.33 \times x$; *S. zonale*: $r^2 = 0.50$, $P < 0.01$, $y = -3.06 + 0.08 \times x$).

The light regimes did not affect the palatability of any of the investigated algal species (Fig. 3; *C. decorticatium*: $r^2 = 0.11$, $P = 0.29$; *O. obtusiloba*: $r^2 < 0.01$, $P = 0.90$; *P. capillacea*: $r^2 = 0.11$, $P = 0.31$; *S. vulgare*: $r^2 = 0.13$, $P = 0.08$; *S. zonale*: $r^2 = 0.03$, $P = 0.44$). Interestingly, overall mortality of amphipods in contact with pellets produced from *S. zonale* was almost double to that of all other species.

None of the investigated seaweed species showed a significant relationship between irradiation and susceptibility to fouling assessed as the amount of byssus threads attached to extract-loaded filter papers (Fig. 4; *C. decorticatium*: $r^2 = 0.01$, $P = 0.87$; *O. obtusiloba*: $r^2 = 0.07$, $P = 0.22$; *P. capillacea*: $r^2 = 0.08$, $P = 0.41$; *S. zonale*: $r^2 = 0.15$, $P = 0.06$). However, in the cases of *C. decorticatum* (ANOVA: $df = 6$, $F = 20.21$, $P < 0.01$), *P. capillacea* (ANOVA: $df = 6$, $F = 6.18$, $P < 0.01$) and *S. zonale* (ANOVA: $df = 6$, $F = 15.68$, $P < 0.01$) mean byssus thread attachment rates were significantly lower when *Perna perna* was in contact with extracts, irrespective of the treatment level, than in the control assays. This is indicative of a repulsive effect of all extracts. For *O. obtusiloba*, significant differences to the controls could only be found in assays with algae kept at 4–7, 30–50 and 70–90 μmol photon^{-2} s^{-1} (ANOVA: $df = 6$, $F = 3.64$; $P = 0.01$). Mussel mortality rates differed between treatment and control dishes for *P. capillacea*. Here, mortality was up to 100% higher in assays with extracts than in the controls.

Discussion

Our most severe light treatments constituted a stress for all tested species, which presumably would have been lethal if
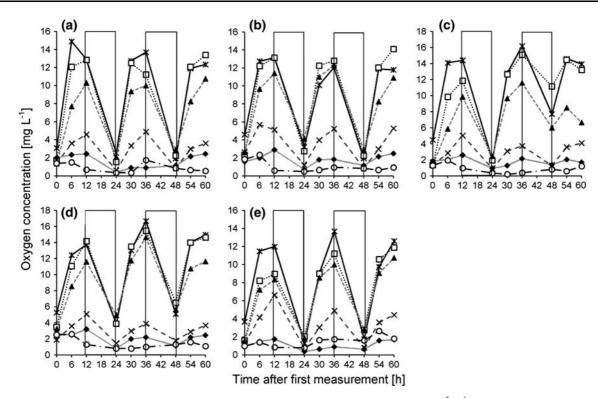
Fig. 1 Evaluation of the light compensation points of **a** *Codium decorticatum*, **b** *Osmundaria obtusiloba*, **c** *Pterocladiella capillacea*, **d** *Sargassum vulgare*, **e** *Stypopodium zonale* under experimental conditions with a 12:12 h light:darkness period. Shown are mean concentrations of dissolved oxygen at the different light levels over a period of 60 h. *Asterisks* 124–197 µmol photons m^{-2} s^{-1}, *squares* 66–112 µmol photons m^{-2} s^{-1}, *triangles* 30–44 µmol photons m^{-2} s^{-1}, *crosses* 7–12 µmol photons m^{-2} s^{-1}, *rhombs* 4–5 µmol photons m^{-2} s^{-1} and *circles* 1–2 µmol photons m^{-2} s^{-1}. Exact values of light intensity varied among species and replicates. Large, *transparent boxes* indicate phases of complete darkness. For reasons of clarity, variances are not shown

applied for a longer period. This is supported by the negligible biomass increase or even the loss of tissue we found at these levels during the course of the experiment. Though the light compensation points we observed for the different algal species were surprisingly low, indicating that they are well adapted to low light, only for *Pterocladiella capillacea* and *Sargassum vulgare* they were in the range of our lowest irradiation treatment (1–2 µmol photons m^{-2} s^{-1}). For all other species, the light compensation points were at higher light intensities. As the only species, *P. capillacea* did not grow at any irradiation level. This should be due to the fact that the fine, delicate thallus structure of *P. capillacea* led to a permanent loss of tissue during the experimental phase, which could have masked light effects on algal growth. However, all other species exhibited growth rates which increased conspicuously and significantly with irradiation intensity. This, in turn, indicates that laboratory conditions such as light supply, except at the intended low-light treatments, were not stressful for the test algae. While growth rates of most test algae increased linearly with irradiation, those of *C. decorticatum* peaked at an intermediate light level. Since oxygen production in *C. decorticatum* showed a linear increase with light level, photoinhibition was presumably not responsible for this unimodal pattern.

Instead, due to high biomass accrual, self-shading may have reduced the relative biomass increase at the highest irradiance levels.

Reduced irradiation did not lead to an increase in algal palatability. This indicates that algal defences against herbivory were not impaired by short-term low-light stress. Our findings therefore contradict the predictions of the EST, but previous works report similar observations: in a marine study by Cronin and Hay (1996a) and a study with freshwater macrophytes by Cronin and Lodge (2003) low-light stress did not influence the level of algal palatability, even though in most cases the concentrations of secondary metabolites decreased with lower light. Pansch et al. (2009), working on temperate seaweeds in a set-up very similar to ours, showed that light limitation affected tissue properties, such as toughness, but not chemical defences. The observation that a shortening of energy input does only exceptionally affect algal defences might be explained by four non-exclusive models: (a) the defence is either constitutive (cannot be regulated) or absent; (b) a reduction of defences by low-light stress may have been masked by a concurrent decrease of the nutritional value of the tissue; (c) the seaweeds maintained their level of defence by allocating resources from other life processes or by mobilizing
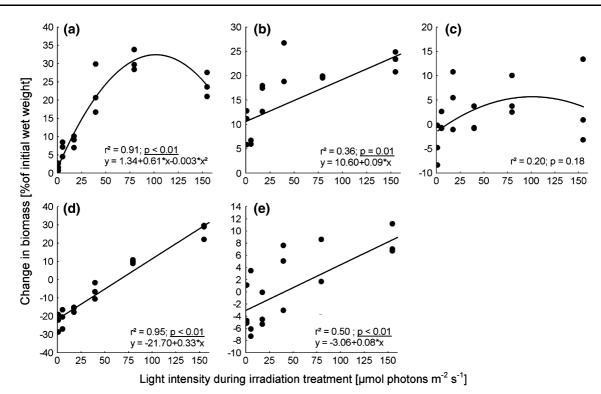
Fig. 2 Relative growth of **a** *Codium decorticatum*, **b** *Osmundaria obtusiloba*, **c** *Pterocladiella capillacea*, **d** *Sargassum vulgare*, **e** *Stypopodium zonale* during the irradiation treatment phase across the light gradient. Statistically significant *P*-values are *underscored*. All *r*² and *P*-values marked with a *subscript* "T" were derived from transformed data
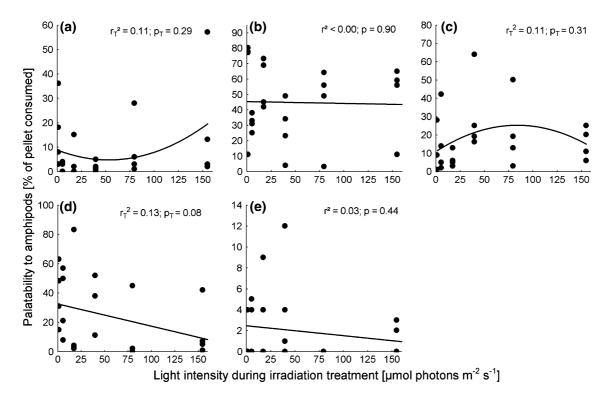

Fig. 3 Palatability of algal tissue (i.e. artificial food pellets) of **a** *Codium decorticatum*, **b** *Osmundaria obtusiloba*, **c** *Pterocladiella capillacea*, **d** *Sargassum vulgare*, **e** *Stypopodium zonale* during the irradiation treatment phase across the light gradient. All *r*² and *P*-values marked with a *subscript* "T" were derived from transformed data
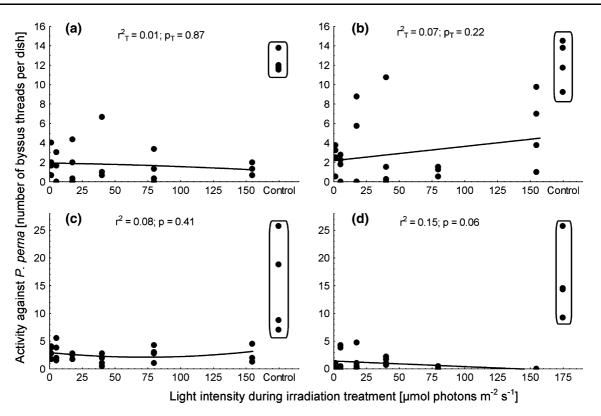
Fig. 4 Activity of algal extracts against *Perna perna* as a function of previously induced low-light stress of **a** *Codium decorticatum*, **b** *Osmundaria obtusiloba*, **c** *Pterocladiella capillacea*, **d** *Stypopodium zonale* during the irradiation treatment phase across the light gradient.

All r^2 and *P*-values marked with a *subscript* "T" were derived from transformed data. Data points enclosed in separate *box* show amounts of byssus threads in control dishes not containing algal extracts

storage compounds (i.e. an effect would have become apparent after a longer treatment); (d) defensive traits in these species are not energy dependent and were therefore not affected by the stress regimes imposed.

Which of these models applies best to our study organisms? First of all, we need to ask whether the tested algae are defended at all. For antifouling defences, this assumption is supported by the fact that we found significant differences between controls and filter papers loaded with extracts in all assays. This clearly indicates the presence of an activity against *P. perna* in all seaweeds sampled. High mussel mortality in tests with extracts from *P. capillacea* even indicates the presence of a toxin. Since the extracts were not confined to surface-bound metabolites, we cannot exclude that substances not active in defence against mussel attachment in nature may have caused the repulsion and mortality of *P. perna*. However, a previous study has shown the mussel test to be a relevant measure for algal defence against foulers (da Gama et al. 2003). Controlling for constitutive (or absent) antifeeding defences is more difficult, because a comparison with consumption of other algae is often confounded by further properties such as consistence and nutritional quality. If we accept that the observed antifouling effect is real, then its insensitivity to a

two-week-energy shortage indicates that resources either from other processes (e.g. growth) or from a storage pool were allocated towards defence or that the antifouling defence is not costly.

For several of the species used here, antifeeding defences have also already been reported. *Pterocladiella capillacea* possesses an inducible defence against the amphipod community used in our study (Weidner et al. 2004). The same study also reported that amphipod grazers were killed or paralysed by a substance extracted from *S. zonale* and that *C. decorticatum* has a constitutive defence against these mesograzers. Many *Sargassum* species from the same (Pereira and Yoneshigue-Valentin 1999) and other biogeographical regions (Taylor et al. 2002; Ceh et al. 2005) are defended against grazing by amphipods. Recent chemical analyses have extracted a series of sulphated compounds from *O. obtusiloba*, which may prove to be active in chemical defence (e.g. de Carvalho et al. 2006). These observations and the fact that the grazers used are known to feed on all of the investigated macrophytes (Weidner et al. 2004) gives us reason to assume that the general absence of significant results cannot be explained by a lack of anti-herbivore defences in the investigated seaweeds.

Could changes in the nutritional value of the algal tissue have overridden effects of light limitation on defensive traits? Nitrogen content in the brown seaweed *Fucus gardneri* P. C. Silva is reduced by shading (Edwards et al. 2006). Such a change in the C:N ratio should influence grazer consumption. If nutritive value and defence strength both decrease with diminishing light, the two contrasting effects could neutralized each other with regard to net grazing. However, low food quality can also lead to enhanced, compensatory consumption in situations where the consumer cannot switch to higher value food (Cruz-Rivera and Hay 2003). Since food quality was not measured in this study, these questions cannot be answered.

The seaweeds may also have diverted energy away from growth or reproduction to keep chemical defence at a constant level. To date, most authors suggest the inverse re-allocation under resource limitation (Herms and Mattson 1992; Cronin 2001; Stamp 2003). In alga–herbivore interactions, both "strategies" can limit grazer damage with chemical defences reducing consumption and growth compensating it. However, though this is true for antifeeding defences, reducing antifouling defences as a response to light limitation would initiate a dangerous feedback, since enhanced shading as a consequence of less controlled fouling would amplify the low light stress. This may explain why antifouling defences seem to be less jeopardized by environmental stress than antifeeding defenses (M. Wahl et al. Defense ecology of the bladder wrack (*Fucus vesiculosus*): 1. patterns of microfouling and antimicrobial protection, submitted; F. Weinberger et al. Defense ecology of the bladder wrack (*Fucus vesiculosus*): 2. Effects of temperature and light on antigrazing defense, submitted). The observation that in most algae investigated here, growth (but not defences) was reduced under low light stress suggests that: (1) defence is more important and (2) any carbohydrate storage capacity, if extent, is not large enough to fuel growth and defences simultaneously. Since tropical macroalgae do not experience seasonal fluctuations in light availability, however, they may not possess a storage capacity comparable to those of seaweeds from higher latitudes (e.g. Lehvo et al. 2001). In future studies following a similar approach, the irradiation treatment phase should be extended in order to assure that the remobilisation of stored carbohydrates is not responsible for the upkeep of defences. Additionally, the concentration of storage compounds should be monitored throughout the course of the manipulation. These measures can serve to decide whether stored resources play a role in the maintenance of defences in the face of environmental stress.

Though the concept of metabolic costs for plant defences is long-standing and widely accepted (reviewed by Cronin 2001; Stamp 2003; Pavia and Toth 2008), it also has been questioned (e.g. Mole 1994) and was repeatedly tested in empirical studies: Two extensive reviews of 58 (Bergelson and Purrington (1996) and 33 (Strauss et al. (2002) studies detected defence costs in 33–76% of the interactions. Putative costs of antifouling defences have only been reported once to date (Dworjanyn et al. 2006). It is difficult to decide whether this reflects the scarcity of costs or a lack of research effort.

Conclusions

A 10–14 day application of low-light stress reduced algal growth rates but did not affect their defences as indicated by an absence of a change in palatability and settlement of macrofoulers. The impression that under energy limitation growth is of lower priority to the algae than is defence is intriguing and warrants further and more detailed research.

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