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Fatty acid compositions associated with high-light tolerance in the intertidal rhodophytes *Mastocarpus stellatus* and *Chondrus crispus*

Kristina Koch[1*], Wilhelm Hagen[2], Martin Graeve[3] and Kai Bischof[1]

Abstract

The rhodophytes *Mastocarpus stellatus* and *Chondrus crispus* occupy the lower intertidal zone of rocky shores along North Atlantic coastlines, with *C. crispus* generally occurring slightly deeper. Consequently, *M. stellatus* is exposed to more variable environmental conditions, related to a generally higher stress tolerance of this species. In order to extend our understanding of seasonal modulation of stress tolerance, we subjected local populations of *M. stellatus* and *C. crispus* from Helgoland, North Sea, to short-term high-light stress experiments over the course of a year (October 2011, March, May and August 2012). Biochemical analyses (pigments, antioxidants, total lipids, fatty acid compositions) allowed to reveal mechanisms behind modulated high-light tolerances. Overall, *C. crispus* was particularly more susceptible to high-light at higher water temperatures (October 2011 and August 2012). Furthermore, species-specific differences in antioxidants, total lipid levels and the shorter-chain/longer-chain fatty acid ratio (C14 + C16/C18 + C20) were detected, which may enhance the tolerance to high-light and other abiotic stress factors in *M. stellatus*, so that this species is more competitive in the highly variable upper intertidal zone compared to *C. crispus*. Since the high-light tolerance in *C. crispus* seemed to be affected by water temperature, interactions between both species may be impacted in the future by rising mean annual sea surface temperature around the island of Helgoland.

Keywords: Antioxidants, *Chondrus crispus*, Helgoland, High-light stress, Fatty acid composition, Macroalgae, *Mastocarpus stellatus*

Introduction

Mastocarpus stellatus ((Stackhouse) Guiry, 1984; Phyllophoraceae, Gigartinales, Rhodophyta) and *Chondrus crispus* (Stackhouse, 1797; Gigartinaceae, Gigartinales, Rhodophyta) are morphologically similar red macroalgal species, both approximately 10 cm in size with numerous dichotomously branching blades arising from a flattened stipe [1–3]. In the lower intertidal zone of rocky shorelines along North Atlantic coastlines [4], *M. stellatus* and *C. crispus* are of significant ecological and economic importance, providing food and habitat to associated invertebrates [3, 5] and representing a source of carrageenan, which is used in food, cosmetic and pharmaceutical industries [6]. Additionally, the species are of commercial interest due to their high content of polyunsaturated fatty acids with 20 carbon atoms such as 20:4(n-6) (arachidonic acid) and 20:5(n-3) (eicosapentaenoic acid) [7]. Arachidonic acid has medical significance as precursor of prostaglandins, whereas eicosapentaenoic acid is an essential constituent in the feed of several mariculture species and this omega-3 fatty acid is suggested to reduce the risk of thrombosis, atherosclerosis and heart disease in humans [8, 9].

As inhabitants of the intertidal zone, *M. stellatus* and *C. crispus* alternate between periods of immersion in seawater and exposure to air, where they experience several

*Correspondence: krkoch@gmx.de
[1] Marine Botany, Bremen Marine Ecology - Center for Research and Education (BreMarE), University of Bremen, Leobener Str. NW2, 28359 Bremen, Germany
Full list of author information is available at the end of the article

potentially stressful environmental conditions such as intense photosynthetically active and ultraviolet radiation (PAR and UV), high or low temperatures (e.g. changes of 10 to 20 °C compared to seawater temperature in the Gulf of Maine, USA) [10], desiccation, osmotic stress and nutrient limitation [11]. To prevail in their particularly challenging, dynamic environment, intertidal macroalgae have generally developed effective ecophysiological acclimation mechanisms [e.g. 11]. Such mechanisms may include a high scavenging capacity for reactive oxygen species (ROS) [12, 13] and UV-screening substances, e.g. mycosporine-like amino acids (MAA), commonly found in red algae [14, 15]. Furthermore, the algae have to adjust their thylakoid membrane fluidity to the prevailing environmental conditions in order to maintain the integrity of these membranes, and thus, a proper operation of the photosynthetic machinery in a highly variable environment. Photosystem II is embedded in the thylakoid membrane, so that the rate of the D1 reaction center protein repair cycle, especially the re-integration of *de-novo* synthesized proteins via lateral diffusion through the membrane, depends strongly on membrane fluidity [16] and references therein]. Besides this, optimal membrane fluidities under variable environmental conditions are needed in order to stabilize membrane-associated proteins and to maintain electron transport chains and transmembrane proton gradients [17]. Membrane fluidity is mainly determined by the chain length of fatty acids and their saturation state. It is generally accepted that at low temperatures, biological membranes feature higher amounts of shorter-chain and unsaturated fatty acids with lower melting points, which compensate for low temperature-induced decreases in membrane fluidity. At high temperatures, vice versa, more longer-chain and saturated fatty acids with higher melting points are incorporated into biomembranes. These fatty acids increase rigidity and, thus, may prevent membrane leakage at elevated temperatures [18]. Some previous studies have already demonstrated that changes in temperature can lead to modifications of macroalgal fatty acid profiles [e.g. 19–22]. Becker et al. [16] reported, for example, that the Antarctic red alga *Palmaria decipiens* acclimated to different temperature regimes by adjusting the degree of fatty acid saturation. In addition, variations in light conditions were also shown to affect the membrane fatty acid composition of macroalgae, but they did not reveal consistent responses [e.g. 23–26]. Since marine macroalgae are poikilothermic organisms, the sensitivity of membrane fluidity and the change in fatty acid composition in response to temperature is plausible, but fluctuation in the fluidity with respect to light acclimation is less understandable [27]. However, due to the close connection between lipids of thylakoid membranes and the photosynthetic integral membrane protein complexes, light-induced variations in the photosynthetic performance might likely be mirrored in the thylakoid membrane fatty acid composition [e.g. 23]. Thereby, adjustments of fatty acid profiles can facilitate electron and ion transport across/within the thylakoid membranes [27] and enhance the stabilizing effect of lipids on the protein complexes during photosynthesis under variable light conditions [28, 29].

The frequency and duration of submersed periods during high tide and emersed periods during low tide depends on the vertical position of an alga on the shore. Species found higher on the coast are generally thought to be less susceptible to environmental stress than those inhabiting lower levels [12, 30, 31]. *M. stellatus* and *C. crispus* occupy different levels within the lower intertidal, with *C. crispus* generally occurring slightly deeper [4]. Along the south-western coast of the island of Helgoland in the North Sea, for example, the highest part of the lower intertidal is dominated by an almost monospecific zone of *M. stellatus*, whereas in the deeper part the two macroalgal species co-occur as mixed assemblages [32]. Consequently, *M. stellatus* is considered as being more tolerant with respect to the adverse effects of ultraviolet-B radiation [15], freezing [33, 34] and desiccation [35] than *C. crispus*. Interestingly, *M. stellatus* was not recorded on Helgoland before 1983, when the species was accidentally introduced to the island during scientific field experiments [3]. Afterwards, *M. stellatus* established and massively dispersed over the island, with drastic alterations of the native communities [36]. Differences in stress tolerances appear to be advantageous for *M. stellatus* over *C. crispus* in terms of competition and colonization of new habitats [15, 33–35].

The object of the present study was to extend our understanding of stress tolerance in the local populations of *M. stellatus* and *C. crispus* from Helgoland. As light exposure is a major factor controlling vertical distribution of algae on the shore, we selected high-light as abiotic variable in stress experiments. Our study should be considered as a rather general approach, since we refer to the overall light stress (frequency and duration), which the algae experience during the submersed periods at high tide as well as during the emersed periods at low tide. More specifically, we tackled the question, whether differences in high-light tolerance are species-specific or rather habitat-specific, with habitat being defined as vertical position on the shore. Further, we checked for the possible ecophysiological mechanisms behind different high-light tolerances. Besides measurements of pigment concentrations and antioxidant activities, we determined total lipid levels and fatty acid compositions. Since solar radiation strongly varies between seasons [37], we performed our study during four events over the course of one year.

Methods

Algal material and sampling site

Individuals of *M. stellatus* and *C. crispus* were collected during low tide at the south-western rocky shore of the island of Helgoland (German Bight, North Sea, 54°11′N, 7°53′E) during four sampling events (21 October 2011; 7 March, 14 May and 9 August 2012). The air temperatures on these days were within the typical range measured during the period 2001–2010 (Deutscher Wetterdienst; Table 1a) and can therefore be regarded as representative of the seasons. *M. stellatus* (hereafter isolate Mast-ex) was taken from higher levels of the lower intertidal, which were fully exposed to air during low tide. Additionally, *M. stellatus* (hereafter isolate Mast-ov) and *C. crispus* (hereafter isolate Chon-ov) were sampled from deeper levels of the lower intertidal, which were only exposed to air for limited times and not during each tidal cycle. In the latter position, both species occurred within an overlapping zone. Since *M. stellatus* and *C. crispus* are perennial species [4], we sampled individuals of the same size to ensure that algae of a similar age were used in the high-light stress experiments and for the ecophysiological analyses. In *C. crispus*, we did not discriminate between the gametophyte and tetrasporophyte stage. However, since we collected a great number of individuals, we feel confident to say that a representative mix of the two life cycle stages of the local *C. crispus* community was used in the present study. Collected algal individuals were directly placed in plastic bags with sufficient seawater to keep them moist. Afterwards, algal individuals were kept in darkness and immediately transported to the marine laboratory of the Biologische Anstalt Helgoland (BAH) of the Alfred Wegener Institute, where they were stored overnight in a flow-through seawater basin (approximately 100 l) at ambient water temperature (Table 1b). One day later, algal individuals were transported in coolers under dark, cool and moist conditions to the laboratory of the Department of Marine Botany at the University of Bremen, where the high-light stress experiment and the ecophysiological analyses were conducted.

Table 1 Environmental conditions at the study site

	(a) Period 2001–2010			
	October	**March**	**May**	**August**
Monthly mean air temperature (°C)	12.1 ± 1.8	4.4 ± 1.6	11.2 ± 1.0	18.1 ± 1.0
Monthly min air temperature (°C)	10.5 ± 1.8	2.9 ± 1.5	9.4 ± 0.9	16.1 ± 1.1
Monthly max air temperature (°C)	13.6 ± 1.6	6.0 ± 1.6	13.5 ± 1.1	20.0 ± 1.1
	(b) Sampling event			
	October 2011	**March 2012**	**May 2012**	**August 2012**
Air temperature (°C)	10.5	4.2	9.9	16.1
Light–dark cycle (h)	10:14	11:13	16:8	15:9
Water temperature (°C)	14	4	8	16
E_k (µmol photons m^{-2} s^{-1})				
Mast-ex	71.4 ± 7.7	21.5 ± 5.3	91.8 ± 6.2	56.0 ± 4.0
Mast-ov	75.7 ± 7.9	10.4 ± 2.6	129.9 ± 19.5	42.5 ± 4.6
Chon-ov	83.2 ± 9.1	7.8 ± 2.0	40.1 ± 10.4	56.6 ± 7.5
10× E_k (high-light stress; µmol photons m^{-2} s^{-1})				
Mast-ex	710	220	920	560
Mast-ov	760	100	1300	430
Chon-ov	830	80	400	570

(a) Monthly average, minimum and maximum air temperatures (°C) of the island of Helgoland (German Bight, North Sea) for October, March, May and August over the period from 2001 to 2010. Air temperatures were taken from the data base of Deutscher Wetterdienst and are given as mean ± SD (n = 28–31, depending on the days per month). (b) Air temperatures (°C) of the island of Helgoland (German Bight, North Sea) for the four sampling events (October 2011, March 2012, May 2012 and August 2012) as well as experimental conditions applied during the recovery from sampling stress (light–dark cycle, water temperature) and high-light stress exposure [water temperature, 10× saturating photon flux density of algal photosynthesis (E_k)] of algal isolates (Mast-ex, Mast-ov and Chon-ov) from the four sampling events. E_k-values were defined by P–E curve fitting after Jassby and Platt [39] and are given as mean ± SEM (n = 6). High-light stress was defined as 10× E_k, so that it was possible to expose the three algal isolates from four events to comparable stress conditions

Min, minimum; max, maximum; SD, standard deviation; Mast-ex, *Mastocarpus stellatus* from higher levels of the lower intertidal; Mast-ov, *Mastocarpus stellatus* from overlapping zone in deeper levels of lower intertidal; Chon-ov, *Chondrus crispus* from overlapping zone in deeper levels of lower intertidal; SEM, standard error of the mean

High-light stress experiment

Subsequently, algal individuals were cleaned of any visible epibionts and their holdfasts were removed, so that thallus branches of about 2 cm remained. For recovery from sampling and preparation stress, thallus branches were kept for 24 h in continuously aerated seawater at a relatively low photon flux density of approximately 30 µmol photons m^{-2} s^{-1} (provided by daylight fluorescence tubes) at light and dark cycles and temperatures matching environmental conditions in the field (Table 1b).

In order to test for differences in high-light susceptibility between the three algal isolates from different shore levels, short-term responses in maximum quantum yields (F_v/F_m) were monitored with a pulse amplitude-modulated fluorometer (PAM 2500; Walz, Effeltrich, Germany) during a high-light stress experiment. Maximum quantum yields were determined in dark adapted (5 min) thallus branches and calculated as:

$$F_v/F_m = (F_m - F_0)/F_m$$

with the variable fluorescence (F_v) representing the difference between the maximal fluorescence (F_m), when all photosystem II (PSII) reaction centers are reduced, and the dark adapted initial minimal fluorescence (F_0), when all PSII reaction centers are oxidized [38].

Based on experience, high-light stress was defined by us as 10× the saturating photon flux density of algal photosynthesis (also known as saturating irradiance, E_k), so that it was possible to expose the three algal isolates from four sampling events to comparable stress conditions (Table 1b). Prior to the experiment, electron transport rates (ETR; 6 replicates per isolate) were estimated from rapid photosynthesis versus photon flux density curves (also known as photosynthesis versus irradiance curves, P–E curves). Thallus branches were irradiated with a series of stepwise increasing actinic photon flux densities (approximately 20–1800 µmol photons m^{-2} s^{-1}) at 30 s intervals, provided by a red light-emitting diode (LED; [38]). Subsequently, the saturating photon flux density was defined by P–E curve fitting after Jassby and Platt [39], using an Excel macro (Table 1b).

For the experiment, thallus branches were placed in glass crystallizing dishes (diameter: 10 cm) filled with approximately 100 ml filtered (pore size: 0.2 µm) seawater at ambient temperature (Table 1b). Per isolate five crystallizing dishes were used. For feasibility reasons, thallus branches were exposed to high-light (10× E_k) for 120 min and subsequently, they were allowed to recover from the high-light treatment under dim light (approximately 3 µmol photons m^{-2} s^{-1}) for 120 min and finally over night (approximately for 16 h). High-light was provided by halogen lamps (400 W) and dim light by daylight fluorescence tubes (36 W). Experimental photon flux densities were measured with a LI-190 cosine corrected quantum sensor (LiCor, Lincoln, NB, USA) connected to a LI-189 radiometer (LiCor, Lincoln, NB, USA). Temperature-control was achieved by a cryostat (Model 1160S, VWR International GmbH, Darmstadt, Germany).

Measurements of F_v/F_m were carried out at the beginning of the experiment, after 15, 30, 60 and 120 min of high-light exposure as well as after 15, 30, 60 and 120 min and over-night recovery by using an individual thallus branch for each point in time. In addition, at the beginning of the high-light exposure, five individual thallus branch replicates per isolate were selected for the determination of the ecophysiological algal characteristics of Mast-ex, Mast-ov and Chon-ov in the field (for details see below).

Ecophysiological analyses

To determine differences in the ecophysiological characteristics and potential adaptive traits of the isolates Mast-ex, Mast-ov and Chon-ov in the field, the following response variables were measured at the beginning of the high-light stress experiment: pigment concentrations (chlorophyll, carotenoids and phycobilins), antioxidant activity, total lipid content and fatty acid compositions. For the different ecophysiological analyses, thallus branches were pooled to form a replicate of approximately 500 mg fresh weight. This algal material was carefully blotted dry with paper towels, shock frozen in liquid nitrogen and stored at −80 °C until further processing.

Pigments and phycobilins

Pigment determination was performed by reversed phase high-performance liquid chromatography (HPLC). The algal material was lyophilized for 24 h and pulverized at 4 m s^{-1} for 20 s in a high-speed benchtop homogenizer (FastPrep®-24; MP Biomedicals, Solon, OH, USA). Pigments from the algal material (approximately 125 mg dry weight) were extracted in 1 ml of ice-cold 90% acetone for 24 h at −20 °C in the dark. After centrifugation (5 min, 4 °C, 13,000*g*) and filtration through a 45 µm nylon syringe filter (Nalgene®; Nalge Nunc International, Rochester, NY, USA), HPLC analysis was performed on a LaChromElite® system equipped with a chilled autosampler L-2200 and a DAD detector L-2450 (VWR-Hitachi International GmbH, Darmstadt, Germany). A Spherisorb® ODS-2 column (25 cm × 4.6 mm, 5 µm particle size; Waters, Milford, MA, USA) with a LiChropher® 100-RP-18 guard cartridge was used for the separation of pigments, applying a gradient according to Wright et al. [40]. Peaks were detected at 440 nm and identified as well as quantified by co-chromatography with standards for chlorophyll *a* (Chl *a*), β-carotene and lutein (DHI

Lab Products, Hørsholm, Denmark) using the software EZChrom Elite ver. 3.1.3. (Agilent Technologies, Santa Clara, CA, USA). Pigment concentrations were expressed as mg per mg Chl *a* (except for Chl *a*, which was given as µg per mg dry weight).

Phycobilin concentrations were determined following the method of Beer and Eshel [41] with slight modifications. The algal material was lyophilized and pulverized as described above. Phycobilins from the algal material (approximately 80 mg dry weight) were extracted in 1 ml 0.1 M phosphate buffer, pH 6.8. After centrifugation (20 min, 10,000*g*), the absorbance of the supernatant was measured at 455, 564, 592, 618 and 645 nm using a spectrophotometer (UV-2401PC; Shimadzu, Duisburg, Germany). Concentrations of phycoerythrin (E) and phycocyanin (C) in mg ml^{-1} were calculated from the absorbance (A) at the respective wavelengths as follows:

$$E = [(A_{564} - A_{592}) - (A_{455} - A_{592})0.20]\,0.12$$
$$C = [(A_{618} - A_{645}) - (A_{592} - A_{645})0.51]\,0.15$$

Phycobilin concentrations were expressed as mg per mg Chl *a*.

Antioxidant activity

The antioxidant activity was measured by the free radical DPPH (2,2-diphenyl-1-picrylhydrazyl; Sigma-Aldrich, Seelze, Germany) scavenging method according to Cruces et al. [42] with slight modifications. Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid; Sigma-Aldrich, Seelze, Germany) was used as a standard. A 150 µM DPPH* stock solution was prepared in ethanol. The algal material was lyophilized and pulverized as described above. Antioxidants from the algal material (approximately 50 mg dry weight) were extracted in 1 ml of 70% acetone for 24 h at 4 °C while shaken in the dark. Afterwards, 22 µl of the supernatant and 200 µl of the DPPH* stock solution were directly mixed in a 96-well microplate. After 15 min, the absorbance was measured at 520 nm using a microplate reader (FLUOstar OPTIMA; BMG Labtech GmbH, Ortenberg, Germany). The antioxidant activity was estimated from triplicate subsamples, from which a mean was calculated, and expressed as mg Trolox equivalent (TE) per mg Chl *a*.

Total lipid content and fatty acid composition

The algal material was lyophilized for 48 h and pulverized at 1500 rpm for 1 min with liquid nitrogen in a homogenizer (Mikro-Dismembrator, Typ U; B. Braun Biotech International GmbH, Melsungen, Germany). Total lipids were extracted in dichloromethane:methanol (2:1, per volume) following the methods described by Folch et al. [43] and Bligh and Dyer [44]. Extracts were mixed and ultrasonicated and total lipid contents were determined gravimetrically after Hagen [45]. For the analysis of fatty acid composition, aliquots of the algal extracts were taken and converted to their methyl ester derivatives (FAMEs) by transesterification with methanol containing 3% concentrated sulfuric acid for 4 h at 80 °C. After extracting the FAMEs three times with hexane, their composition was analyzed using a HP 6890 gas chromatograph (Agilent Technologies, Waldbronn, Germany) equipped with a DB-FFAP column (60 m length, 0.25 mm inner diameter, 0.25 µm film thickness; Agilent Technologies, Waldbronn, Germany) operated with temperature-programming essentially after Kattner and Fricke [46]. FAMEs were identified by comparing their retention times with those derived from standards of known composition. Individual fatty acids were calculated as mass percentage of the total fatty acid content and grouped according to their degree of saturation and their chain length, with shorter-chain fatty acids being defined as fatty acids with 14 and 16 carbon atoms (C14 and C16, respectively) and longer-chain fatty acids as fatty acids with 18 and 20 carbon atoms (C18 and C20, respectively).

Statistical analysis

To test for differences in algal F_v/F_m (high-light stress and recovery phase) and ecophysiological characteristics (pigments, phycobilins, antioxidants, lipids and fatty acids) related to the factors isolate (Mast-ex, Mast-ov and Chon-ov) and sampling event (October 2011; March, May and August 2012), two-factorial analyses of variance (2-way ANOVA) were carried out. When the ANOVA revealed significant differences for main effects and/or the interaction, Fisher's least significant difference (LSD) procedure was applied, respectively. Prior to all statistical analyses, percentage data were arcsin-transformed. Further, all data were tested for normality and homogeneity of variances, using Kolmogorov–Smirnov's test and Levene's test, respectively. The software PASW Statistics 18 (SPSS; Armonk, NY, USA) was used for statistical analyses. Critical significance levels of 5% were applied.

Results

Ecophysiological characteristics of isolates

Over the consecutive sampling events, changes in the ecophysiological characteristics of the red algal isolates were detected. For a better comparability between the three isolates, Chl *a* was used as denominator for the calculation of pigment concentrations and antioxidant activity. The Chl *a* concentration was highest in Chon-ov, significantly lower in Mast-ex and again significantly lower in Mast-ov (Tables 2, 3). In contrast, the β-carotene and lutein concentrations did not show consistent isolate-specific differences between the four sampling

Table 2 Statistical evaluation of response variables of *M. stellatus* and *C. crispus*: amplitudes of the maximum quantum yield (F_v/F_m) for the high-light stress phase and the recovery phase as well as Chl *a* concentration, ratios of pigments (β-carotene/Chl *a*, lutein/Chl *a*, phycoerythrin/Chl *a*, phycocyanin/Chl *a*) and antioxidant (antioxidants (TE)/Chl *a*), total lipid content, saturation states of fatty acids [sum of saturated fatty acids (SFA), sum of monounsaturated fatty acids (MUFA), sum of polyunsaturated fatty acids (PUFA), saturated/unsaturated fatty acid ratio (SFA/UFA)] and shorter-chain/longer-chain fatty acid ratio (C14 + C16/C18 + C20) of initial thallus branches, using two-factorial analysis of variance, with the factors sampling event (October 2011, March 2012, May 2012 and August 2012) and isolate (Mast-ex, Mast-ov and Chon-ov) and their interaction

Source of variation	F_v/F_m amplitude (high-light stress phase)					F_v/F_m amplitude (recovery phase)				
	SS	*df*	MS	*F*-ratio	*p* value	SS	*df*	MS	*F*-ratio	*p* value
Isolate	0.052	2	0.026	8.193	**0.001**	0.029	2	0.015	3.003	0.059
Sampling event	0.276	3	0.092	29.233	**<0.001**	0.140	3	0.047	9.589	**<0.001**
Isolate × sampling event	0.091	6	0.015	4.799	**0.001**	0.063	6	0.011	2.167	0.063
Error	0.151	48	0.003			0.234	48	0.005		
Source of variation	**Chl *a***					**β-carotene/Chl *a***				
	SS	*df*	MS	*F*-ratio	*p* value	SS	*df*	MS	*F*-ratio	*p* value
Isolate	0.115	2	0.057	32.101	**<0.001**	3.0×10^{-5}	2	1.5×10^{-5}	0.545	0.583
Sampling event	0.016	3	0.005	2.985	**0.040**	2.2×10^{-4}	3	7.3×10^{-5}	2.646	0.060
Isolate × sampling event	0.019	6	0.003	1.786	0.122	0.001	6	1.3×10^{-4}	4.586	**0.001**
Error	0.086	48	0.002			0.001	48	2.8×10^{-5}		
Source of variation	**Lutein/Chl *a***					**Phycoerythrin/Chl *a***				
	SS	*df*	MS	*F*-ratio	*p* value	SS	*df*	MS	*F*-ratio	*p* value
Isolate	0.021	2	0.011	44.316	**<0.001**	0.273	2	0.137	0.115	0.891
Sampling event	0.011	3	0.004	14.937	**<0.001**	1.988	3	0.663	0.560	0.644
Isolate × sampling event	0.004	6	0.001	2.731	**0.023**	6.260	6	1.043	0.882	0.516
Error	0.012	48	2.4×10^{-4}			56.805	48	1.183		
Source of variation	**Phycocyanin/Chl *a***					**Antioxidants (TE)/Chl *a***				
	SS	*df*	MS	F-ratio	*p* value	SS	*df*	MS	*F*-ratio	*p* value
Isolate	0.188	2	0.094	2.790	0.071	106.940	2	53.470	49.380	**<0.001**
Sampling event	0.142	3	0.047	1.402	0.254	137.450	3	45.817	42.312	**<0.001**
Isolate × sampling event	0.081	6	0.014	0.401	0.875	30.891	6	5.149	4.755	**0.001**
Error	1.618	48	0.034			51.976	48	1.083		
Source of variation	**Total lipid**					**SFA**				
	SS	*df*	MS	*F*-ratio	*p* value	SS	*df*	MS	*F*-ratio	*p* value
Isolate	0.001	2	3.4×10^{-4}	44.667	**<0.001**	0.006	2	0.003	1.428	0.250
Sampling event	1.8×10^{-5}	3	6.1×10^{-6}	0.815	0.492	0.023	3	0.008	3.468	**0.023**
Isolate × sampling event	3.2×10^{-4}	6	5.3×10^{-5}	7.037	**<0.001**	0.028	6	0.005	2.071	0.074
Error	3.6×10^{-4}	48	7.5×10^{-6}			0.108	48	0.002		
Source of variation	**MUFA**					**PUFA**				
	SS	*df*	MS	*F*-ratio	*p* value	SS	*df*	MS	*F*-ratio	*p* value
Isolate	0.011	2	0.006	16.058	**<0.001**	0.001	2	3.4×10^{-4}	0.055	0.947
Sampling event	0.005	3	0.002	5.250	**0.003**	0.079	3	0.026	4.332	**0.009**
Isolate × sampling event	0.005	6	0.001	2.277	0.052	0.036	6	0.006	0.987	0.445
Error	0.017	48	3.8×10^{-4}			0.293	48	0.006		

Table 2 continued

Source of variation	SFA/UFA					C14 + C16/C18 + C20				
	SS	*df*	MS	*F*-ratio	*p* value	SS	*df*	MS	*F*-ratio	*p* value
Isolate	0.002	2	0.001	0.801	0.455	3.4×10^{-5}	2	1.7×10^{-4}	4.073	**0.023**
Sampling event	0.177	3	0.059	55.952	**<0.001**	0.001	3	3.9×10^{-4}	93.871	**<0.001**
Isolate × sampling event	0.018	6	0.003	2.857	**0.019**	3.9×10^{-5}	6	6.4×10^{-6}	1.550	0.183
Error	0.050	47	0.001			2.0×10^{-4}	47	4.1×10^{-6}		

p values in bold highlight significant differences at $p < 0.05$

Mast-ex, *Mastocarpus stellatus* from higher levels of the lower intertidal; Mast-ov, *Mastocarpus stellatus* from overlapping zone in deeper levels of lower intertidal; Chon-ov, *Chondrus crispus* from overlapping zone in deeper levels of lower intertidal

events (Tables 2, 3). The concentrations of the phycobilins phycoerythrin and phycocyanin also did not differ significantly with respect to the factors isolate and sampling event (Tables 2, 3). For the majority of the sampling events, the antioxidant activity was significantly higher in the two *M. stellatus* isolates than in *C. crispus* (Fig. 1 and Table 2). In contrast, the total lipid content was significantly lower in Mast-ex and Mast-ov compared to Chon-ov (Tables 2, 3).

The sum of saturated fatty acids (SFA) and the sum of polyunsaturated fatty acids (PUFA) did not differ significantly between the three algal isolates within each sampling event (Tables 2, 3). Contrarily, the sum of monounsaturated fatty acids (MUFA) exhibited significant isolate-specific differences, with highest contents in Mast-ov, followed by those in Mast-ex and lowest contents in Chon-ov (Tables 2, 3). Following the differences in the various saturation states of fatty acids, the saturated/unsaturated fatty acid ratio (SFA/UFA) showed no consistent pattern with respect to algal isolate over the course of one year (Tables 2, 3). However, the shorter-chain/longer-chain fatty acid ratio (C14 + C16/C18 + C20) was significantly higher in Mast-ex and Mast-ov compared to Chon-ov within each of the four sampling events (Tables 2, 3). In total, nine different fatty acids were identified in the algal isolates (Table 4). The saturated fatty acid 16:0 and the three unsaturated fatty acids 18:1(n-9), 20:4(n-6) and 20:5(n-3) comprised almost 90% of the total fatty acids in the algae. Other fatty acids, detected only in minor amounts, were 14:0, 16:1(n-7), 18:0, 18:1(n-7) and 18:2(n-6). Significant isolate-specific differences were found for four single fatty acids [16:1(n-7), 18:0, 18:1(n-7) and 18:2(n-6)]. Within each sampling event, both *M. stellatus* isolates contained higher concentrations of the fatty acid 16:1(n-7) and lower concentrations of the fatty acids 18:0 and 18:2(n-6) compared to *C. crispus*. The amount of the fatty acid 18:1(n-7) was highest in Mast-ex, followed by Mast-ov and lowest in Chon-ov, whereas the concentration of fatty acid 14:0 did not differ significantly between the three algal isolates. In contrast, the concentrations of the fatty acids 16:0, 18:1(n-9), 20:4(n-6) and 20:5(n-3) did not show consistent isolate-specific differences between the various sampling events (Tables 4, 5).

Short-term responses in maximum quantum yield (F_v/F_m) of isolates to high-light stress

Ecophysiological changes during the high-light stress experiment in F_v/F_m were calculated as percentage of initial values to enable a better comparability between the three isolates (Fig. 2). Furthermore, since the photoinhibition and recovery phase are very different processes, amplitudes were estimated for each phase separately. For this, differences between the beginning and end of the high-light stress phase (beginning of experiment and 120 min of high-light exposure) and the recovery phase (120 min of high-light exposure and 120 min of recovery) were calculated from absolute F_v/F_m values for the three algal isolates from the four sampling events, respectively (Table 6). The changes in F_v/F_m of the algal isolates with respect to high-light stress and subsequent recovery differed between the various sampling events (Fig. 2; Tables 2, 6). In March and May 2012, the responses during the high-light stress exposure of the algal isolates were very similar (Fig. 2b, c; Tables 2, 6), whereas they showed significant isolate-specific differences in October 2011 and August 2012 (Fig. 2a, d; Tables 2, 6). In March 2012, there was almost no decrease in F_v/F_m after 120 min of high-light exposure in the *M. stellatus* and *C. crispus* isolates and the values returned quickly to the initial values during the recovery period (Fig. 2b). In May 2012, F_v/F_m declined to approximately 60% of initial values in all three isolates after the high-light stress (120 min; Fig. 2c) and was able to increase again to above 90% of the initial values after over-night recovery (data not shown). In October 2011, the decrease of F_v/F_m during the high-light stress differed significantly between the three algal isolates (Fig. 2a; Tables 2, 6). It was strongest and fastest in Chon-ov (to 70 and 50% of initial values after 15 and 120 min, respectively), followed by Mast-ov (to 95 and 60% of initial values

Table 3 Pigment and lipid composition of *M. stellatus* and *C. crispus*: chlorophyll *a* concentration, ratios of pigments to chlorophyll *a* (β-carotene/Chl *a*, lutein/Chl *a*, phycoerythrin/Chl *a*, phycocyanin/Chl *a*), total lipid content, saturation states of fatty acids [sum of saturated fatty acids (SFA), sum of monounsaturated fatty acids (MUFA), sum of polyunsaturated fatty acids (PUFA), saturated/unsaturated fatty acid ratio (SFA/UFA)] and shorter-chain/longer-chain fatty acid ratio (C14 + C16/C18 + C20) of initial thallus branches collected on four sampling events (October 2011, March 2012, May 2012 and August 2012)

Response variables of initial thallus branches	Sampling event			
	October 2011	March 2012	May 2012	August 2012
Chl *a* (μg mg^{-1} dry weight)●				
Mast-ex	0.31 ± 0.01	0.26 ± 0.01	0.30 ± 0.01	0.31 ± 0.02
Mast-ov	0.23 ± 0.02	0.26 ± 0.01	0.28 ± 0.01	0.22 ± 0.01
Chon-ov	0.32 ± 0.03	0.33 ± 0.01	0.40 ± 0.04	0.35 ± 0.03
β-carotene/Chl *a*				
Mast-ex	0.11 ± 0.00[a]	0.11 ± 0.00[a]	0.12 ± 0.00[a]	0.11 ± 0.00[b]
Mast-ov	0.11 ± 0.00[a]	0.11 ± 0.00[a]	0.12 ± 0.00[a]	0.11 ± 0.00[b]
Chon-ov	0.11 ± 0.00[a]	0.11 ± 0.00[a]	0.11 ± 0.00[b]	0.12 ± 0.00[a]
Lutein/Chl *a*				
Mast-ex	0.21 ± 0.01[a]	0.23 ± 0.00[a]	0.25 ± 0.01[a]	0.23 ± 0.00[a]
Mast-ov	0.19 ± 0.01[b]	0.20 ± 0.01[b]	0.24 ± 0.01[a]	0.23 ± 0.00[a]
Chon-ov	0.17 ± 0.01[b]	0.19 ± 0.01[b]	0.18 ± 0.01[b]	0.20 ± 0.01[b]
Phycoerythrin/Chl *a*				
Mast-ex	1.48 ± 0.29	1.72 ± 0.12	2.05 ± 0.23	2.11 ± 0.72
Mast-ov	1.70 ± 0.30	2.82 ± 0.95	1.48 ± 0.28	2.01 ± 0.78
Chon-ov	2.24 ± 0.33	1.76 ± 0.36	1.47 ± 0.19	2.10 ± 0.47
Phycocyanin/Chl *a*				
Mast-ex	0.27 ± 0.05	0.26 ± 0.02	0.30 ± 0.04	0.37 ± 0.10
Mast-ov	0.30 ± 0.04	0.41 ± 0.12	0.24 ± 0.04	0.39 ± 0.15
Chon-ov	0.45 ± 0.08	0.47 ± 0.09	0.32 ± 0.03	0.50 ± 0.11
Total lipid (% of dry weight)				
Mast-ex	1.37 ± 0.15[b]	1.21 ± 0.14[b]	1.19 ± 0.05[b]	1.62 ± 0.24[a]
Mast-ov	1.11 ± 0.08[b]	1.01 ± 0.07[b]	1.21 ± 0.03[b]	1.12 ± 0.04[b]
Chon-ov	1.69 ± 0.10[a]	1.87 ± 0.06[a]	1.70 ± 0.04[a]	1.31 ± 0.12[b]
SFA (mass% of total fatty acids)				
Mast-ex	35.6 ± 0.7	30.4 ± 0.2	32.0 ± 0.2	37.6 ± 0.7
Mast-ov	34.4 ± 0.3	30.6 ± 0.2	32.1 ± 0.1	37.1 ± 0.8
Chon-ov	36.3 ± 1.4	30.4 ± 0.4	32.0 ± 0.3	34.4 ± 0.4
MUFA (mass% of total fatty acids)●				
Mast-ex	10.4 ± 1.5	13.2 ± 0.2	11.3 ± 0.1	9.5 ± 1.3
Mast-ov	13.7 ± 0.7	13.5 ± 0.1	10.6 ± 0.1	10.4 ± 0.2
Chon-ov	9.7 ± 0.4	8.6 ± 0.3	9.9 ± 0.2	9.4 ± 0.2
PUFA (mass% of total fatty acids)				
Mast-ex	54.0 ± 0.9	56.4 ± 0.1	56.7 ± 0.2	53.0 ± 0.7
Mast-ov	52.0 ± 0.4	55.9 ± 0.2	57.3 ± 0.2	52.5 ± 1.0
Chon-ov	54.1 ± 1.2	61.0 ± 0.4	58.1 ± 0.3	56.2 ± 0.2
SFA/UFA				
Mast-ex	0.55 ± 0.02[ab]	0.44 ± 0.00[a]	0.47 ± 0.00[a]	0.60 ± 0.02[a]
Mast-ov	0.52 ± 0.01[b]	0.44 ± 0.00[a]	0.47 ± 0.00[a]	0.59 ± 0.02[a]
Chon-ov	0.57 ± 0.03[a]	0.44 ± 0.01[a]	0.47 ± 0.01[a]	0.52 ± 0.01[b]
C14 + C16/C18 + C20●				
Mast-ex	0.58 ± 0.02	0.46 ± 0.00	0.49 ± 0.00	0.62 ± 0.02
Mast-ov	0.56 ± 0.01	0.47 ± 0.00	0.49 ± 0.00	0.60 ± 0.02

Table 3 continued

Response variables of initial thallus branches	Sampling event			
	October 2011	March 2012	May 2012	August 2012
Chon-ov	0.52 ± 0.01	0.42 ± 0.01	0.46 ± 0.01	0.50 ± 0.01

Table shows mean ± SEM (n = 5). Dark circles indicate isolate-specific (Mast-ex, Mast-ov and Chon-ov) differences within all four sampling events that are significant at $p < 0.05$ (significant isolate effect of 2-way ANOVA followed by a Fisher's LSD test). Different letters (a and b) indicate significant differences among algal isolates within one of the four sampling events (significant interaction isolate × sampling event of 2-way ANOVA followed by a Fisher's LSD test, $p < 0.05$)

Mast-ex, *Mastocarpus stellatus* from higher levels of the lower intertidal; Mast-ov, *Mastocarpus stellatus* from overlapping zone in deeper levels of lower intertidal; Chon-ov, *Chondrus crispus* from overlapping zone in deeper levels of lower intertidal; SEM, standard error of the mean

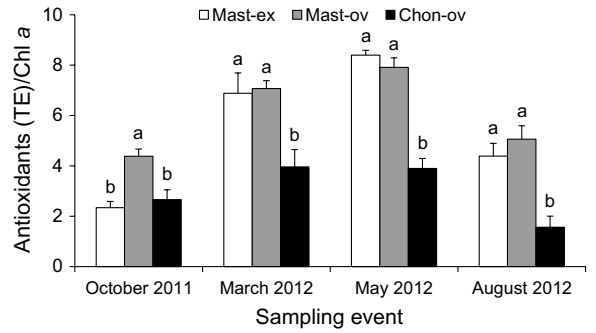

Fig. 1 Antioxidant content of *M. stellatus* and *C. crispus*: molar ratio of antioxidants (TE) to chlorophyll *a* (antioxidants (TE)/Chl *a*) of initial thallus branches collected on four sampling events (October 2011; March, May and August 2012). *Bars* are mean ± SEM (n = 5). *Different letters* (*a* and *b*) indicate significant differences among algal isolates within one of the four sampling events (significant interaction isolate × sampling event of 2-way ANOVA followed by a Fisher's LSD test, $p < 0.05$). TE, Trolox equivalent; Mast-ex, *Mastocarpus stellatus* from higher levels of the lower intertidal; Mast-ov, *Mastocarpus stellatus* from overlapping zone in deeper levels of lower intertidal; Chon-ov, *Chondrus crispus* from overlapping zone in deeper levels of lower intertidal; SEM, standard error of the mean

after 15 and 120 min, respectively) and Mast-ex (almost no decrease and to 75% of initial values after 15 and 120 min, respectively; Fig. 2a). During the recovery phase, the maximum quantum yields of Mast-ex and Mast-ov recovered quickly from high-light stress (Fig. 2a), whereas F_v/F_m of Chon-ov only reached 75% of the initial values even after over-night recovery (data not shown), but these differences were not considered to be significant in the F_v/F_m amplitudes for the recovery phase (Tables 2, 6). In August 2012, the response of F_v/F_m to high-light exposure showed again significant differences between Mast-ex, Mast-ov and Chon-ov (Fig. 2d; Tables 2, 6). After the recovery period over-night, all three algal isolates were able to reach 90–100% of their initial F_v/F_m values (data not shown).

Discussion

Overall, the results of the present study revealed that the local populations of *M. stellatus* and *C. crispus* from Helgoland differ in their high-light tolerance, with *M. stellatus* generally being less sensitive to this stress factor. Further, we found that the algal isolates exhibited significant differences in a number of ecophysiological characteristics (antioxidants, pigments, total lipids, fatty acid composition) tested, which seem to be species-specific rather than habitat-specific. Please note that *M. stellatus* is an invasive species on Helgoland and potential founder effects cannot be excluded, hence, the results of this study may not apply to this species in general. Furthermore, our results should be interpreted with the understanding that not only abiotic stress factors (e.g. light, temperature or desiccation), but also biotic interactions are responsible for the development of distinct vertical algal zonation patterns in the rocky intertidal. Examples for biotic interactions are herbivory, symbiosis or endophytism [47] and references therein]. *C. crispus* is host to a range of endophytic pathogens, like filamentous green algae of the genus *Acrochaete* [48]. For example, *Acrochaete operculata* is able to infect sporophytes of *C. crispus*, causing disintegration of the host thallus and secondary infections with bacteria [49] and references therein]. Pathogen attacks are known to induce the *de-novo* formation of oxylipins, which are generated by oxygenation of PUFA. Oxylipins form part of the defense mechanism against negative effects of endophytic pathogens in *C. crispus* [50–52]. This aspect might further contribute to the here observed differences in the fatty composition between *C. crispus* and *M. stellatus*.

During the high-light stress experiments, we observed the typical pattern of photoinhibition (decrease of F_v/F_m) and subsequent recovery after stress exposure, with the completeness of recovery depending on the algal isolate and season [e.g. 15, 30]. In line with our results, previous studies found that the sensitivity of photoinhibition towards abiotic stress differs with the vertical position of red algae on the shore [15, 30, 31]. Dring et al. [30] assumed that the sensitivity to UV radiation of red algae occurring around the island of Helgoland varies amongst other factors with growth depth of algae. In their study, the rate of the initial decline of F_v/F_m during UV exposure was greatest and the extent of recovery was less pronounced in species from greater water depths, like *Delesseria sanguinea* and *Plocamium cartilagineum*,

Table 4 Fatty acid compositions of *M. stellatus* and *C. crispus*: single fatty acids (mass% of total fatty acids) of initial thallus branches collected on four sampling events (October 2011, March 2012, May 2012 and August 2012)

Fatty acid	Sampling event			
	October 2011	March 2012	May 2012	August 2012
14:0				
Mast-ex	2.8 ± 0.1	1.5 ± 0.0	1.8 ± 0.0	3.4 ± 0.1
Mast-ov	2.9 ± 0.1	1.8 ± 0.0	1.9 ± 0.0	3.2 ± 0.1
Chon-ov	3.2 ± 0.2	1.8 ± 0.1	2.4 ± 0.1	3.4 ± 0.1
16:0				
Mast-ex	30.5 ± 0.5^{a}	26.8 ± 0.1^{a}	28.2 ± 0.1^{a}	32.0 ± 0.7^{a}
Mast-ov	29.0 ± 0.3^{b}	26.7 ± 0.1^{a}	28.2 ± 0.1^{a}	31.2 ± 0.6^{a}
Chon-ov	30.4 ± 0.6^{a}	27.2 ± 0.3^{a}	28.2 ± 0.2^{a}	29.5 ± 0.6^{b}
16:1(n-7)				
Mast-ex	2.4 ± 0.0^{a}	2.3 ± 0.0^{a}	1.8 ± 0.0^{a}	2.0 ± 0.1^{a}
Mast-ov	2.6 ± 0.1^{a}	2.3 ± 0.0^{a}	1.7 ± 0.0^{a}	1.8 ± 0.1^{a}
Chon-ov	0.4 ± 0.2^{b}	0.6 ± 0.0^{b}	0.7 ± 0.1^{b}	0.4 ± 0.0^{b}
18:0●				
Mast-ex	1.2 ± 0.1	1.0 ± 0.1	0.9 ± 0.0	1.3 ± 0.2
Mast-ov	1.1 ± 0.0	1.0 ± 0.1	1.1 ± 0.1	1.4 ± 0.2
Chon-ov	2.2 ± 0.6	1.3 ± 0.2	1.1 ± 0.1	1.3 ± 0.1
18:1(n-7)●				
Mast-ex	2.2 ± 0.0	2.4 ± 0.1	2.4 ± 0.1	2.0 ± 0.0
Mast-ov	1.9 ± 0.1	2.2 ± 0.1	2.1 ± 0.0	1.4 ± 0.1
Chon-ov	1.2 ± 0.2	1.6 ± 0.1	1.5 ± 0.1	0.9 ± 0.0
18:1(n-9)				
Mast-ex	5.6 ± 1.4^{b}	8.0 ± 0.1^{ab}	6.7 ± 0.0^{a}	5.3 ± 1.3^{b}
Mast-ov	8.6 ± 0.6^{a}	8.5 ± 0.1^{a}	6.5 ± 0.0^{a}	6.9 ± 0.2^{ab}
Chon-ov	8.0 ± 0.1^{a}	6.3 ± 0.2^{b}	7.6 ± 0.2^{a}	8.0 ± 0.2^{a}
18:2(n-6)●				
Mast-ex	1.1 ± 0.1	1.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.0
Mast-ov	1.5 ± 0.2	1.1 ± 0.0	0.9 ± 0.0	1.0 ± 0.0
Chon-ov	1.4 ± 0.2	2.3 ± 0.3	1.6 ± 0.1	1.6 ± 0.6
20:4(n-6)				
Mast-ex	25.3 ± 0.9^{b}	16.5 ± 0.5^{b}	13.8 ± 0.4^{c}	25.0 ± 0.4^{ab}
Mast-ov	24.4 ± 0.5^{b}	13.4 ± 0.7^{c}	15.9 ± 0.3^{b}	23.7 ± 0.4^{b}
Chon-ov	27.4 ± 0.4^{a}	25.8 ± 0.9^{a}	24.8 ± 1.0^{a}	25.9 ± 0.4^{a}
20:5(n-3)				
Mast-ex	25.9 ± 0.5^{a}	37.0 ± 0.6^{b}	40.0 ± 0.4^{a}	25.8 ± 0.4^{b}
Mast-ov	24.1 ± 0.4^{a}	39.4 ± 0.9^{a}	38.7 ± 0.4^{a}	26.0 ± 0.7^{b}
Chon-ov	24.5 ± 1.5^{a}	32.8 ± 1.0^{c}	31.1 ± 0.7^{b}	28.1 ± 0.1^{a}

Table shows mean ± SEM (n = 5, with exception of n = 4 for Chon-ov collected in August 2012). The nomenclature of fatty acids (a:b(n-x)) is defined as follows: a = number of C-atoms (chain length), b = number of double bonds and (n-x) = position of the first double bond relative to the methyl-end. Dark circles indicate isolate-specific (Mast-ex, Mast-ov and Chon-ov) differences within all four sampling events that are significant at $p < 0.05$ (significant isolate effect of 2-way ANOVA followed by a Fisher's LSD test). Different letters (a, b and c) indicate significant differences among algal isolates within one of the four sampling events (significant interaction isolate × sampling event of 2-way ANOVA followed by a Fisher's LSD test, $p < 0.05$)

Mast-ex, *Mastocarpus stellatus* from higher levels of the lower intertidal; Mast-ov, *Mastocarpus stellatus* from overlapping zone in deeper levels of lower intertidal; Chon-ov, *Chondrus crispus* from overlapping zone in deeper levels of lower intertidal; SEM, standard error of the mean

than in intertidal or shallow subtidal species. Sagert et al. [31] observed a similar response in *C. crispus* from various growth depths (3.5 to 8.5 m below high-tide level) on the western Atlantic coast of Brittany, France, when those plants were exposed to irradiation of PAR and UV. The latter finding might indicate an acclimation to the radiation regime at the respective growth depths of this species.

The intensity of solar radiation not only differs with respect to vertical zonation on the shore, but also deviated strongly with respect to season [37], so that we expected differences in the responses of F_v/F_m of the algal isolates to the high-light stress between the four sampling events. In particular, we thought that isolate-specific differences should be distinct in months with higher levels of solar radiation (April to September with an overall monthly mean of 1600 µmol photons m^{-2} s^{-1}) and should be lower in months with less solar PAR (October to March with an overall monthly mean of 570 µmol photons m^{-2} s^{-1}) [37]. Actually, we found clear isolate-specific differences during the high-light exposure in August 2012 and October 2011, whereas in March and May 2012 the responses of the algal isolates were very similar. These findings did not correlate very well with the seasonal pattern of solar radiation. However, in the present study, the sensitivity of *C. crispus* to the high-light stress seemed to be influenced by the prevailing water temperature. *C. crispus* is able to grow over a wide temperature range from 5 to 20 °C [11], with maximal growth and photosynthetic rates at 15 °C [19, 53]. Further, thermal acclimation to growth temperature exists in this algal species, so that individuals acclimated to summer seawater temperatures (20 °C) can better tolerate brief exposures to extremely high temperatures than those acclimated to winter seawater temperatures (5 °C) [11]. Nevertheless, our findings indicated that high-light tolerance of *C. crispus* is less pronounced than that of *M. stellatus* in late summer and autumn (August 2012 and October 2011 with water temperatures of 16 °C and 14 °C, respectively) at higher water temperatures as compared to the other sampling events (May and March 2012 with water temperatures of 8 °C and 4 °C, respectively). This is consistent with findings for *C. crispus* from Maine, USA by Kübler and Davison [11], showing that light has a profound effect on the response of this species to high temperature. In their study, the photosynthesis of algae, acclimated to a temperature of 20 °C, was not inhibited by the exposure to 30 °C at moderate light levels (70–100 µmol photons m^{-2} s^{-1}), but inhibition did occur, when those algae were exposed to high light levels (600 µmol photons m^{-2} s^{-1}). Since air temperature during tidal emersion may be 10–20 °C higher (or lower) than water temperature [10], temperatures

Table 5 Statistical evaluation of fatty acid compositions of *M. stellatus* and *C. crispus*: single fatty acids of initial thallus branches, using two-factorial analysis of variance, with the factors sampling event (October 2011, March 2012, May 2012 and August 2012) and isolate (Mast-ex, Mast-ov and Chon-ov) and their interaction

Source of variation	FA 14:0					FA 16:0					FA 16:1(n-7)				
	SS	*df*	MS	*F*	*p*	SS	*df*	MS	*F*	*p*	SS	*df*	MS	*F*	*p*
Isolate	2.7×10^{-5}	2	1.3×10^{-5}	1.992	0.148	0.001	2	3.3×10^{-4}	3.255	**0.047**	0.003	2	0.001	175.034	**<0.001**
Sampling event	0.002	3	0.001	84.675	**<0.001**	0.016	3	0.005	51.590	**<0.001**	1.1×10^{-4}	3	3.6×10^{-5}	4.188	**0.010**
Isolate × sampling event	1.6×10^{-5}	6	2.7×10^{-6}	0.409	0.869	0.002	6	3.8×10^{-4}	3.710	**0.004**	2.5×10^{-4}	6	4.1×10^{-5}	4.808	**0.001**
Error	3.2×10^{-4}	47	6.7×10^{-6}			0.005	47	1.0×10^{-4}			4.0×10^{-4}	47	8.5×10^{-6}		
Source of variation	**FA 18:0**					**FA 18:1(n-7)**					**FA 18:1(n-9)**				
	SS	*df*	MS	*F*	*p*	SS	*df*	MS	*F*	*p*	SS	*df*	MS	*F*	*p*
Isolate	2.3×10^{-4}	2	1.1×10^{-4}	4.199	**0.021**	4.8×10^{-4}	2	2.4×10^{-4}	21.566	**<0.001**	0.002	2	0.001	4.239	**0.020**
Sampling event	5.9×10^{-5}	3	2.0×10^{-5}	0.720	0.545	3.1×10^{-4}	3	1.0×10^{-4}	9.365	**<0.001**	0.001	3	1.8×10^{-4}	0.929	0.434
Isolate × sampling event	2.9×10^{-4}	6	4.8×10^{-5}	1.787	0.122	1.3×10^{-4}	6	2.2×10^{-5}	2.007	0.084	0.004	6	0.001	3.850	**0.003**
Error	0.001	47	2.7×10^{-5}			0.001	47	1.1×10^{-5}			0.009	47	1.9×10^{-4}		
Source of variation	**FA 18:2(n-6)**					**FA 20:4(n-6)**					**FA 20:5(n-3)**				
	SS	*df*	MS	*F*	*p*	SS	*df*	MS	*F*	*p*	SS	*df*	MS	*F*	*p*
Isolate	0.001	2	2.9×10^{-4}	12.315	**<0.001**	0.053	2	0.027	125.717	**<0.001**	0.013	2	0.007	22.987	**<0.001**
Sampling event	3.5×10^{-5}	3	1.2×10^{-5}	0.493	0.689	0.075	3	0.025	118.942	**<0.001**	0.191	3	0.064	221.290	**<0.001**
Isolate × sampling event	2.7×10^{-4}	6	4.5×10^{-5}	1.916	0.098	0.028	6	0.005	22.157	**<0.001**	0.029	6	0.005	16.761	**<0.001**
Error	0.001	47	2.4×10^{-5}			0.010	47	2.1×10^{-4}			0.014	47	2.9×10^{-4}		

p values in bold highlight significant differences at $p < 0.05$. The nomenclature of fatty acids (a:b(n-x)) is defined as follows: a = number of C-atoms (chain length), b = number of double bonds and (n-x) = position of the first double bond relative to the methyl-end

Mast-ex, *Mastocarpus stellatus* from higher levels of the lower intertidal; Mast-ov, *Mastocarpus stellatus* from overlapping zone in deeper levels of lower intertidal; Chon-ov, *Chondrus crispus* from overlapping zone in deeper levels of lower intertidal

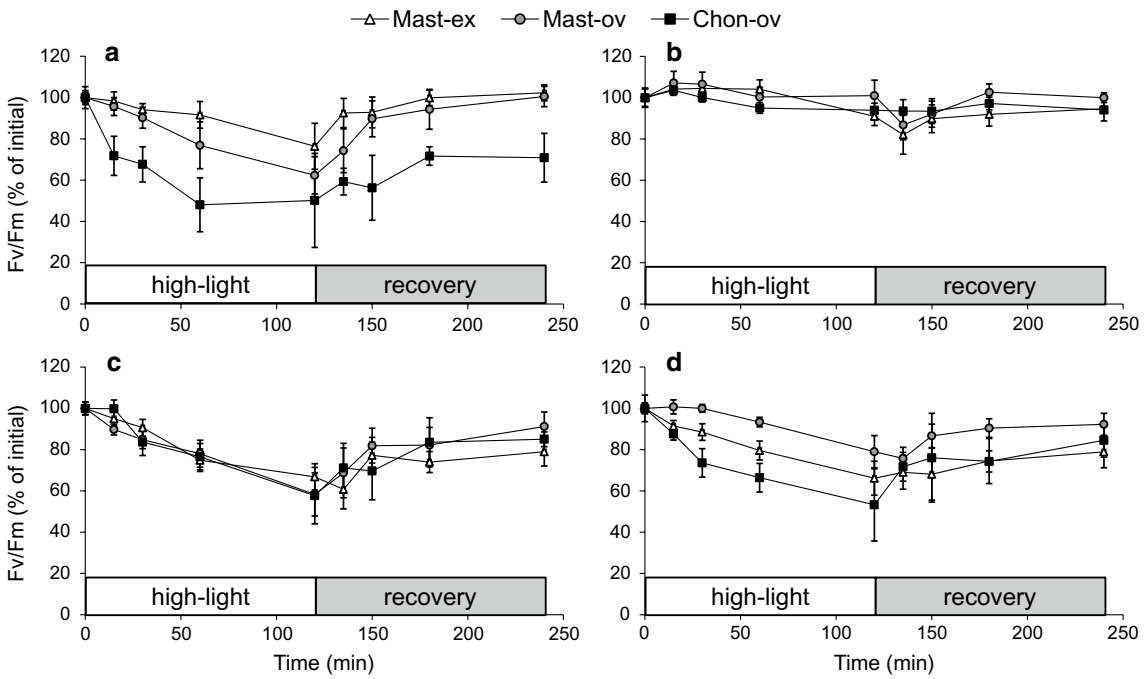

Fig. 2 Maximum quantum yield (F_v/F_m) of *M. stellatus* and *C. crispus*: F_v/F_m (% of initial) of thallus branches during exposure to high light (10× E_k; 0 to 120 min) and recovery from the high-light treatment under dim light (approximately 3 µmol photons m^{-2} s^{-1}; 120 to 240 min) in **a** October 2011, **b** March 2012, **c** May 2012 and **d** August 2012. Measurements of F_v/F_m were carried out at the beginning of the experiment (0 min), after 15, 30, 60 and 120 min of high-light exposure as well as after 15, 30, 60 and 120 min of recovery. To allow a better comparability between the three algal isolates (Mast-ex, Mast-ov, Chon-ov), F_v/F_m was calculated as percentage of initial values. Data points are means ± 95% confidence intervals (n = 5). Mast-ex, *Mastocarpus stellatus* from higher levels of the lower intertidal; Mast-ov, *Mastocarpus stellatus* from overlapping zone in deeper levels of lower intertidal; Chon-ov, *Chondrus crispus* from overlapping zone in deeper levels of lower intertidal

Table 6 Photosynthetic responses of experimental specimens of *M. stellatus* and *C. crispus*: amplitudes of the maximum quantum yield (F_v/F_m) for the high-light stress phase and the recovery phase of thallus branches collected on four sampling events (October 2011, March 2012, May 2012 and August 2012)

F_v/F_m amplitudes of thallus branches	Sampling event			
	October 2011	March 2012	May 2012	August 2012
High-light stress phase				
Mast-ex	0.124 ± 0.026^{c}	0.059 ± 0.014^{a}	0.181 ± 0.023^{a}	0.193 ± 0.026^{b}
Mast-ov	0.197 ± 0.024^{b}	0.044 ± 0.017^{a}	0.234 ± 0.027^{a}	0.112 ± 0.026^{c}
Chon-ov	0.292 ± 0.033^{a}	0.038 ± 0.011^{a}	0.219 ± 0.027^{a}	0.270 ± 0.035^{a}
Recovery phase				
Mast-ex	0.135 ± 0.028	0.024 ± 0.013	0.069 ± 0.022	0.080 ± 0.036
Mast-ov	0.200 ± 0.024	0.035 ± 0.013	0.188 ± 0.028	0.081 ± 0.025
Chon-ov	0.128 ± 0.063	0.024 ± 0.007	0.145 ± 0.024	0.191 ± 0.048

For this, differences between the beginning and end of the high-light stress phase (beginning of experiment and 120 min of high-light exposure) and the recovery phase (120 min of high-light exposure and 120 min of recovery) were calculated from absolute F_v/F_m values, respectively. Table shows mean ± SEM (n = 5). Different letters (a, b and c) indicate significant differences among algal isolates within one of the four sampling events (significant interaction isolate × sampling event of 2-way ANOVA followed by a Fisher's LSD test, $p < 0.05$)

Mast-ex, *Mastocarpus stellatus* from higher levels of the lower intertidal; Mast-ov, *Mastocarpus stellatus* from overlapping zone in deeper levels of lower intertidal; Chon-ov, *Chondrus crispus* from overlapping zone in deeper levels of lower intertidal; SEM, standard error of the mean

around 30 °C can easily be reached on Helgoland during summer and autumn.

Differences in ecophysiological characteristics might contribute to the generally higher stress tolerance of *M. stellatus* compared to *C. crispus* [15, 33–35]. We were able to show that, regardless of the position on the shore, *M. stellatus* possessed a higher antioxidant activity than *C. crispus* during the majority of the sampling events. This is in line with a study by Collén and Davison [12], who reported about a generally higher efficiency of the reactive oxygen metabolism and resistance to oxidative stress in *M. stellatus* (higher levels of ascorbate and β-carotene and higher activities of catalase and glutathione reductase) in comparison to *C. crispus*. However, this generality could not be confirmed in another investigation on the seasonal acclimatization of antioxidants in the same two red algal species [13]. These authors found that *M. stellatus* only had higher ascorbate contents, whereas the activities of the enzymes superoxide dismutase and ascorbate peroxidase were higher in *C. crispus*. We suggest, that the higher antioxidant activity, found in our study, may allow *M. stellatus* to exist at higher positions on the shore. Algal organisms living in those habitats are in particular exposed to several environmental stress factors, which are known to stimulate the formation of ROS. Thus, an effective defense system against ROS is necessary for their survival [54]. Generally, we detected higher antioxidant activities in the three algal isolates at colder water temperatures (March 2012 and May 2012), which might also emphasize the importance of this defense system during coldness. Those cold-induced increases in antioxidants are thought to compensate for the effect of lower temperatures on their activities and for the generation of ROS, which is particularly high, when chilling and freezing events occur [13].

As was the case for the antioxidants, we observed that the red algal isolates also differed in their Chl *a* contents, with highest contents in *C. crispus*. This is part of a well-known photoacclimatory adjustment found in algal species from different shore levels. By increasing the concentration of chlorophyll, the utilization of solar radiation becomes more efficient for *C. crispus* in low light environments at greater water depths. Vice versa, excessive absorption of light is avoided in *M. stellatus* (particularly in Mast-ex) by lower chlorophyll amounts in shallower waters. Additionally, respective acclimations in antenna pigments (e.g. phycobilins), which result in further adjustments of light harvesting to various light climates, were also frequently observed [16, 55]. Why those pigments did not show clear species- or habitat-specific differences in our study remains to be resolved.

Overall, total lipid contents in *M. stellatus* and *C. crispus* were relatively low (approximately 1.5% of dry weight) in the present investigation. This agrees with a study on five macroalgal species by Herbreteau et al. [56], who also propose that very low total lipid levels appear to be characteristic for plants living in marine environments. We observed species-specific differences in total lipids during most of the sampling events, usually with higher contents in *C. crispus* than in *M. stellates*. Previous studies detected higher amounts of total lipids in individuals of the red macroalgae *Grateloupia turuturu* [57] and *Tichocarpus crinitus* [26] as well as of the red microalga *Porphyridium cruentum* [27] growing at low solar radiation compared to those being exposed to high light intensities. Thus, differences in total lipid levels in *M. stellatus* and *C. crispus*, found in our study, may also be due to variations in the light climates along the vertical gradient on the shore, with decreasing levels of solar PAR with depth.

In this study, major fatty acids found in the three algal isolates were 16:0, 18:1(n-9), 20:4(n-6) and 20:5(n-3), which agrees with the fatty acid compositions of many other red algae [e.g. 19, 58–62]. It is already known that the fatty acid composition of *C. crispus* varies with respect to the phase of the life cycle [60] and with respect to environmental conditions, such as light [23] and temperature [19]. However, to our knowledge, a comparative study of the fatty acid compositions between *M. stellatus* and *C. crispus* was not yet conducted. Please note that the method we used [43–45] extracts all fatty acids of the algal cells, i.e. free fatty acids and those being incorporated into polar lipids of membranes or neutral lipids of storage compounds. However, since up to 94% of total lipids in green, brown and red algae were found to be polar lipids, which indicated that they are structurally bound in membranes [63], we feel confident to make statements about changes in membrane compositions (fatty acid saturation state and chain length) based on our fatty acid data. We found higher contents of MUFA in the two isolates of *M. stellatus* compared to those of *C. crispus*. Further, we detected species-specific differences in the C14 + C16/C18 + C20 ratio, with higher values in *M. stellatus*. This means that *M. stellatus* exhibited a higher degree of unsaturation and more shorter-chain fatty acids than *C. crispus*, with both characteristics resulting in a higher fluidity of their biomembranes [18]. Previous studies highlighted differences in fatty acid compositions of green, brown and red macroalgae with respect to growth depth on the shore, with a higher degree of unsaturation in shallower compared to deeper waters [16, 64]. Apparently, in some red algae, fatty acid unsaturation is stimulated by an increase in light intensity [23, 65]. Since those high-light conditions exist in shallower waters around Helgoland, we propose that they might contribute to the higher contents

of monounsaturated fatty acids in this habitat, which we observed in *M. stellatus*. Shallower waters are characterized by extremely variable environmental conditions, including fluctuations in PAR and UV radiation as well as temperature, which is probably quite stressful for algae living there [11]. Generally, a high amount of unsaturated fatty acids is thought to be favorable in unsteady habitats. Unsaturated fatty acids are more responsive to environmental changes than saturated ones, so that they can adequately react to changes in the abiotic environment [63]. Under these conditions, the formation of ROS is known to increase, which in turn might promote the degradation of the D1 reaction center protein of PSII. A higher membrane fluidity facilitates the D1 protein repair cycle [16] and references therein] and supports the ion and electron transport between the two photosystems [27]. Therefore, our findings indicate that higher levels of fatty acid unsaturation may help *M. stellatus* to maintain biomembranes, especially thylakoid membranes containing the photosynthetic apparatus, operative in a wide range of light conditions in shallower water depths.

Conclusions

Our study on rhodophytes from Helgoland showed that local populations of *M. stellatus* have a higher tolerance towards high-light stress than those of *C. crispus*. Furthermore, our findings provided new insights into potential adaptive mechanisms of stress tolerance, indicated by differences in several ecophysiological characteristics (antioxidants, pigments, total lipids, fatty acid compositions) between the algal isolates. In this regard, the two *M. stellatus* isolates from two shore levels differed from *C. crispus* with respect to the antioxidants, total lipids and the C14 + C16/C18 + C20 ratio. These differences appear to be genetically determined and hence species-specific, since they are not masked by responses to various environmental settings along the depth gradient (habitat-specific differences). Such differences in ecophysiology may enhance the tolerance to different abiotic stress factors, but may also allow rapid recovery from this stress in *M. stellatus*. It may explain, why this species is more competitive in the highly variable upper intertidal compared to *C. crispus*. Since we assumed that high-light tolerance in *C. crispus* is negatively affected by higher water temperatures, interactions between both species around the island of Helgoland could be impacted in the future by rising mean annual sea surface temperatures [66]. To elucidate such interactions between the two species, future studies should determine the tolerance to high-light stress at various temperature levels. Further, more detailed studies should focus on changes in fatty acid composition within different polar and neutral lipid classes of the two species. This would allow to identify, which specific membranes (e.g. thylakoid membranes) are primarily affected by the observed differences in fatty acids.

Abbreviations

2-way ANOVA: two-factorial analysis of variance; BAH: Biologische Anstalt Helgoland; C14, C16, C18, C20: fatty acids with 14, 16, 18, 20 carbon atoms; C14 + C16/C18 + C20: shorter-chain/longer-chain fatty acid ratio; Chl *a*: chlorophyll *a*; Chon-ov: *Chondrus crispus* from overlapping zone in deeper levels of lower intertidal; DPPH: 2,2-diphenyl-1-picrylhydrazyl; E_k: saturating photon flux density; ETR: electron transport rate; F_0: dark adapted initial minimal fluorescence; FAME: fatty acid methyl ester; F_m: maximal fluorescence; F_v: variable fluorescence; F_v/F_m: maximum quantum yield; HPLC: high-performance liquid chromatography; LED: light-emitting diode; LSD: least significant difference; MAA: mycosporine-like amino acid; Mast-ex: *Mastocarpus stellatus* from higher levels of the lower intertidal; Mast-ov: *Mastocarpus stellatus* from overlapping zone in deeper levels of lower intertidal; max: maximum; min: minimum; MUFA: sum of monounsaturated fatty acids; PAM: pulse amplitude modulation; PAR: photosynthetically active radiation; P–E curve: photosynthesis versus photon flux density curve; PSII: photosystem II; PUFA: sum of polyunsaturated fatty acids; ROS: reactive oxygen species; SD: standard deviation; SEM: standard error of the mean; SFA: sum of saturated fatty acids; SFA/UFA: saturated/unsaturated fatty acid ratio; TE: Trolox equivalent; Trolox: 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid; UV: ultraviolet radiation.

Author details

[1] Marine Botany, Bremen Marine Ecology - Center for Research and Education (BreMarE), University of Bremen, Leobener Str. NW2, 28359 Bremen, Germany. [2] Marine Zoology, Bremen Marine Ecology - Center for Research and Education (BreMarE), University of Bremen, Leobener Str. NW2, 28359 Bremen, Germany. [3] Ecological Chemistry, Alfred Wegener Institute Helmholtz Centre for Polar and Marine Research (AWI), Am Handelshafen 12, 27570 Bremerhaven, Germany.

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Authors' contributions

KK collected the algae, performed the high-light stress experiments and the majority of the laboratory analyses, carried out the data processing and the statistical analyses and drafted the manuscript. WH participated in the interpretation of data and the critical revision of the manuscript. MG was responsible for the determination of the algal fatty acid profiles. KB developed the experimental design of this study and helped with data interpretation as well as manuscript improvement and revision. All authors read and approved the final manuscript.

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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