Increased pCO_2 and temperature reveal ecotypic differences in growth and photosynthetic performance of temperate and Arctic populations of *Saccharina latissima*

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Abstract

Previous research demonstrated that warming and ocean acidification (OA) affect the biochemical composition of Arctic (Spitsbergen; SP) and cold-temperate (Helgoland; HL) *Saccharina latissima* differently, suggesting ecotypic differentiation. The present study analyses the responses to different pCO_2 (380, 800, 1500 µatm pCO_2) and temperature levels (SP population: 4°C, 10°C; HL population: 10°C, 17°C) on the photophysiology (O_2 production, pigment composition, D1-protein content) and carbon assimilation (Rubisco content, carbon concentrating mechanisms (CCMs), growth rate) of both ecotypes. Elevated temperatures stimulated O_2 production in both populations, and also led to an increase in pigment content and a deactivation of CCMs, as indicated by ^{13}C isotopic discrimination of algal biomass (ε_p) in the HL population, which was not observed in SP thalli. Generally, pCO_2 effects were less pronounced than temperature effects. High pCO_2 deactivated CCMs in both populations and produced a decrease in the Rubisco content of HL thalli while it was unaltered in SP population. As a result, the growth rate of the Arctic ecotype increased at elevated pCO_2 and higher temperatures and it remained unchanged in the HL population. Ecotypic differentiation was revealed by a significantly higher O_2 production rate and an increase in Chl *a*, Rubisco and D1-protein content in SP thalli, but a lower growth rate, in comparison to the HL population. We conclude that both populations differ in their sensitivity to changing temperatures and OA and that the Arctic population is more likely to benefit from the upcoming environmental scenario than its Atlantic counterpart.

Keywords

Arctic · Carbon concentrating mechanisms · Kelp · Ocean acidification · Photosynthesis · Pigment · Seaweed

Abbreviations

CCM Carbon concentrating mechanism

DPS De-epoxidation state

DW Dry weight

ε_P Isotopic fractionation of organic carbon production

HL Helgoland

OA Ocean acidification

pCO_2 Partial pressure of CO_2

PS Photosystem

RGR Relative growth rate

ROS Reactive oxygen species

SP Spitsbergen

VAZ Xanthophyll cycle pigment pool

Introduction

The rise of atmospheric pCO_2 causes changes in the seawater carbonate system (SWCS), resulting in an increase in CO_2, HCO_3^- and overall DIC, a decrease in CO_3^{2-} and a concomitantly lower pH. These changes are known as ocean acidification (OA) (Riebesell et al. 2010). During the last decades, OA-effects on marine algae got into the focus of marine research, revealing that the OA effects on photoautotrophs are species-specific, bearing positive, negative and neutral responses to elevated pCO_2 (e.g. Koch et al. 2013). It is becoming increasingly established that the outcome of OA-effects on growth and photosynthesis are dependent on the interaction with other environmental constraints, such as suboptimal temperatures, including global warming scenarios (Fu et al. 2007; Feng et al. 2008; ; Olischläger and Wiencke 2013; Sarker et al. 2013) and limited phosphorous availability (Xu et al. 2010). In some algae OA-effects are enhanced at limiting light conditions (Rokitta and Rost 2012), whereas other algae species are only sensitive towards OA at saturating light (Sarker et al. 2013) or under replete nitrogen conditions (Gordillo et al. 2001).

The long-term adaptation of an algal population to its local environment results in ecotypic differentiation possibly influencing also the response to OA. It was postulated that algae in cold seas are less dependent on active carbon uptake, since cold polar waters generally contain high concentrations of dissolved CO_2, which are considered as saturating for algal photosynthesis at low temperatures (Raven et al. 2002). However, high activities of external carbonic anhydrases (eCAs) were measured in all polar macroalgae tested (Gordillo et al. 2006), even in those lacking CCM such as *Phycodrys rubens*, pointing to a demand of continuous supply of CO_2 to the cells. In most of the analysed species, this high eCA activity was supposed to be involved in CCM operation, as suspected from the ^{13}C discrimination values obtained for polar seaweeds (Wiencke and Fischer 1990). For the OA-sensitivity of each species the question whether or to which extent an alga operates an active carbon concentrating mechanism (CCM) is considered to be crucial (Hepburn et al. 2011). Therefore, if polar algae are CO_2-saturated at present it is reasonable to assume that they are rather insensitive towards OA. On the other hand, if polar macroalgae need to express CCMs strongly, Arctic algae might be particularly sensitive towards OA, since a higher environmental pCO_2 facilitates passive

69 diffusion from the surrounding seawater towards Rubisco, allowing a down-regulation of the energy
70 demanding CCM and a reallocation of the resources used (Raven et al. 2012). Furthermore, a future
71 scenario with higher environmental CO_2 can compensate a low temperature-induced CCM inefficiency
72 (Olischläger and Wiencke 2013), which might be particularly important for species growing at the
73 edge of their physiological temperature range of tolerance. Such species might be Arctic macroalgae,
74 since the present marine flora reinvaded the Arctic after the ice-melt of the last glaciation event, less
75 than 18000 years ago from the North Atlantic and North Pacific (Lüning 1990). The Arctic
76 populations derive from the cold-temperate zones of the North-Atlantic and many species still grow in
77 cold temperate and polar habitats (Lüning 1990). The low degree of endemism in the Arctic relative to
78 the Antarctic can be explained by this small time interval. Nevertheless, indices of an ecotypic
79 differentiation were already reported in relation to zoospore germination and biochemical composition
80 of the cold-temperate and polar kelp *Saccharina latissima* (Müller et al. 2008; Olischläger et al. 2014).
81 The latter species occurs in cold-temperate and in polar environments (Lüning 1990) and, therefore, it
82 is particulary relevant to perform a comparison between both populations regarding its OA-response,
83 tackling the question whether or not polar macroalgae populations are less susceptible towards OA
84 than their temperate counterparts.

85 It was observed that the photosynthetic response to cultivation at elevated pCO_2 is often neutral or
86 positive (Harley et al. 2012; Olischläger et al. 2012; Olischläger and Wiencke 2013; Sarker et al.
87 2013), whereas the Rubisco and protein contents are often down-regulated in response to cultivation at
88 elevated pCO_2 (Andría et al. 2001, Gordillo et al. 2001). Hence, at elevated pCO_2, an improved
89 photosynthetic performance can be achieved by the same amount of Rubisco and pigments, if Rubisco
90 was not saturated at actual CO_2 concentrations (the majority of algae from temperate areas, Raven and
91 Beardall 2003), resulting in a more efficient resource-use. For algae with CCMs, increased pCO_2 could
92 lead to an unchanged photosynthetic rate but an increased growth rate due to CCM down-regulation
93 (Gordillo et al. 2001), as CCM activity have associated an energy cost (Raven and Beardall 2016). In
94 either case, this indicates a decreased amount of energy demand at high pCO_2, which might trigger a
95 re-arrangement of the light harvesting complex, consisting in the elimination of pigment molecules
96 that are in excess and would not participate efficiently in the light capture. Thus, a decrease in pigment

content at high CO_2 has been frequently observed as part of the acclimation process (e.g. García-Sanchez et al. 1994; Gordillo et al. 1999; Gordillo et al. 2001). The D1 protein is a key component of PS II reaction center, so that a change in the number of PS II should be reflected also in the amount of D1. However, this relation can be modified by different degradation and replacement rates during photoinhibition (Segovia et al. 2015).

Clearly, the physiological response to elevated pCO_2 is also temperature dependent (Fu et al. 2008; Olischläger and Wiencke 2013; Sarker et al. 2013). The reason for these findings might be that photosynthetic light reactions are independent of temperature (Raven and Geider 1988), whereas the enzymatic reactions involved in the photosynthesis and carbon acquisition are not (Raven and Geider 1988; Olischläger and Wiencke 2013). Hence, an effective avoidance of photoinhibition requires a balance between excitation, in the light harvesting reactions and the enzymatic carbon acquisition and assimilation. Therefore, the aim of the study was to link the effects of pCO_2 and temperature to light harvesting, carbon acquisition and fixation. Furthermore, we compared the results from cold-temperate and polar populations of *S. latissima* and tested if ecotypes differ in their response pattern to OA and temperature.

The following hypotheses were tested: (i) Arctic and temperate populations of *S. latissima* show ecotypic differentiation in their photophysiology and growth. (ii) Arctic and temperate populations of *S. latissima* show different sensitivity to OA, with a less sensitive response of the Arctic population towards OA in term of pigmentation, photosynthesis and growth. (iii) Pigmentation and Rubisco content are down-regulated synchronously at elevated pCO_2 in both populations, and (iv) OA effects are more pronounced at low temperatures in both populations.

Material and methods

Algal material and experimental conditions

As previously described by Olischläger et al. (2014), young vegetative sporophytes were raised from polar and temperate *S. latissima* gametophytes (Spitsbergen; AWI-culture number: ♂-gametophytes 3123, ♀-gametophytes 3124; Helgoland, North Sea; AWI-culture number: ♂-gametophytes 3094, ♀-

gametophytes 3096). Male and female gametophytes from the two populations were mixed, carefully fragmented with pestle and mortar and kept in dim white light (15-20 µmol photons m^{-2} s^{-1}) at 10 °C until experimental use. For the experiment, a photon fluence rate (PFR) of 70 ± 10 µmol photons m^{-2} s^{-1} at the bottom and 120 ± 10 µmol photons m^{-2} s^{-1} at the top of the beaker was adjusted, using a flat head cosine corrected quantum sensor attached to a radiometer (Li-185-B, flat head quantum sensor; LI-COR Biosciences, Lincoln, NE, USA). Fluorescent tubes were used as light source (OSRAM 58W /965 Biolux, Munich, Germany).

The experiment was started by transferring 0.5 ± 0.1 g fresh weight of algae from the precultivation to 5 L beakers filled with filtered seawater (FSW; 0.2 µm), enriched with unbuffered nutrients after Provasoli (1968), including 2.0 mM NO_3^- and 0.05 mM PO_4^{3-}. Experimental beakers were aerated continuously with artificial air (20% oxygen, 80% nitrogen) with a target pCO_2 of 380 µatm, 800 µatm, or 1500 µatm generated by a gas mixing device (HTK GmbH, Hamburg, Germany). Subsequently, these pCO_2 treatments are called 'present', 'expected' and 'high' pCO_2. The media were aerated with the different gas mixtures described above for 24 h prior to experimental use and water was exchanged every 3-4 days. Thalli were moved continuously by aeration without tumbling. The experiment lasted 18 days, and was performed in temperature-controlled rooms adjusted to 17°C ± 1.5°C and 10°C ± 1.5°C for the Helgoland population and 10°C ± 1.5°C and 4°C ± 1.5°C for the Spitsbergen population. 10°C is chosen for the examination of the ecotypic differentiation, since the 10°C isotherme in August is considered to be the boundary between the marine cold-temperate and the Arctic zone (Lüning 1990) and the two populations could theoretically coexist in northern continental Norway (Lüning 1990).

Monitoring of the seawater carbonate system during the experiment

The seawater carbonate system (SWCS), including the pCO_2 of the FSW was monitored as described by Olischläger et al. (2014). The equilibrium constants for the dissociation of carbonic acid in seawater from Millero et al. (2006), and for sulfuric acid the constants of Dickson (1990), were applied in the calculations. The pH was monitored on a total scale, except for one measuring date in SP 10°C-treatment, for which the pH was measured on the National Bureau of Standards (NBS)-Scale

150 due to a technical failure. The SWCS-calculations of this measurement date used the dissociation
151 constants for carbonic acid from Takahashi et al. (1982), which are recommended for the NBS-scale.
152 Detailed values of the measured characteristics of the SWCS are presented in this accompanying
153 publication (Olischläger et al. 2014).

154 **Pigment analysis**

155 Determination of pigment content was performed by a reversed phase HPLC. Algal samples were
156 lyophilized for 24 h and pulverized at 4 m s^{-1} for 20 s in a high-speed benchtop homogenizer
157 (FastPrep®-24; MP Biomedicals, Solon, OH, USA). Pigments in the samples (approx. 30 mg dry
158 weight) were extracted in 1 mL of ice-cold 90% acetone for 24 h at -20°C in darkness. After
159 centrifugation (5 min, 4°C, 13000 *g*) and filtration through a 45 µm nylon syringe filter (Nalgene®,
160 Nalge Nunc International, Rochester, NY, USA), HPLC analyses were performed on a LaChromElite®
161 system equipped with a chilled autosampler L-2200 and a DAD detector L-2450 (VWR-Hitachi
162 International GmbH, Darmstadt, Germany). A Spherisorb® ODS-2 column (25 cm x 4.6 mm, 5 µm
163 particle size; Waters, Milford, MA, USA) with a LiChropher® 100-RP-18 guard cartridge was used for
164 the separation of pigments, applying a gradient according to Wright et al. (1991). Peaks were detected
165 at 440 nm and identified as well as quantified by co-chromatography with standards for chl *a* and c_2,
166 fucoxanthin, β-carotene, violaxanthin, antheraxanthin and zeaxanthin (DHI Lab Products, Hørsholm,
167 Denmark) using the software EZChrom Elite ver. 3.1.3. (Agilent Technologies, Santa Clara, CA,
168 USA). Pigment contents were expressed as µg per mg dry weight.

169 Further, the de-epoxidation state (DPS) of the xanthophyll cycle pigments was calculated as follows:

170 $DPS = ([Zea] + 0.5 \times [Ant]) / VAZ$

171 where Ant is antheraxanthin, Zea is zeaxanthin and VAZ is the xanthophyll cycle pigment pool (sum
172 of viola-, anthera- and zeaxanthin). The DPS represents the photoprotective state of the xanthophyll
173 cycle as ant- and zeaxanthin play an important role in the dissipation of excess excitation energy as
174 heat and thereby protecting the reaction centers against photoinhibition (Pfündel and Bilger 1994;
175 Colombo-Palotta et al. 2006).

Rubisco and D1 protein quantification

Protein extraction procedure was performed as explained by Olischlager et al. (2014). Proteins from extracts and Rubisco standard were resolved by SDS–PAGE (Laemmli 1970) in Mini-Protean Tetra Cell (Bio-Rad, Hercules, USA) at a constant voltage of 175 V during 60 min, using 12% polyacrylamide in the resolving gel. All lanes were loaded with 50 μg protein.

The amount of Rubisco was estimated by Coomassie Brilliant Blue polyacrylamide gel staining procedure (Neuhoff et al. 1988), using purified spinach Rubisco as standard (R-8000, Sigma-Aldrich, St. Louis, MO, USA). Stained gels were photographed, and bands identified as Rubisco large subunits were quantified with the Molecular Imaging Software (Eastman-Kodak, Rochester, NY, USA) based on both size and intensity.

Changes in the relative amounts of D1 protein were detected by subsequent blotting after electrophoresis using a D1-specific primary antibody (AS 01016 Chicken Anti PsbA, Agrisera, Sweden) at 1:10000 dilution, and anti-chicken IgY-HRP conjugated (Abcam, Cambridge, United Kingdom) as secondary antibody at 1:15000 dilution. The signal was detected by chemiluminescence (ECL-Plus; GE Healthcare, Buckinghamshire, UK) using a KODAK Gel Logic 1500 Imaging System (Eastman-Kodak, Rochester, NY, USA), and the relative intensity of cross-reactions was quantified as above.

Chlorophyll fluorescence

After 14 days of treatment the effective quantum yield (ΔF/Fm') was quantified by measuring the variable chlorophyll fluorescence of photosystem II (PSII), using a PAM-2100 (Walz-GmbH, Effeltrich, Germany), following Maxwell and Johnson (2000). The saturation pulse lasted 800 ms and had an irradiance of 8500 μmol photons m^{-2} s^{-1} (400-800 nm).

Net photosynthesis

Net photosynthesis was estimated by O_2 evolution after 15 and 16 days as described in Olischläger et al. (2012), at an irradiance of 120 μmol m^{-2} s^{-1}, at 17°C and 10°C for the HL population, and at 10°C

and at 4°C for the SP population. In order to obtain more information from this measurement, O_2 production rates were calculated with respect to DW, Chl *a* content and Rubisco content.

Stable carbon isotopic determination

The abundance of ^{13}C relative to ^{12}C in plant samples was determined by mass spectrometry using a DELTA V Advantage (Thermo Electron Corporation, USA) Isotope Ratio Mass Spectrometer (IRMS) connected to a Flash EA 1112 CNH analyser. The ^{13}C isotopic discrimination in the algal samples ($\delta^{13}C_{alga}$) was calculated as deviations from the $^{13}C/^{12}C$ ratio of the Pee-Dee Belemnite $CaCO_3$ (PDB).

Isotopic fractionation of organic carbon production (ε_p) was calculated from the $\delta^{13}C_{alga}$ value relative to the isotopic composition of dissolved CO_2 ($\delta^{13}C_{CO2}$) in the medium according to Freeman and Hayes (1992):

$$\varepsilon_p = (\delta^{13}C_{CO2} - \delta^{13}C_{alga})/(1 + (\delta^{13}C_{alga}/1000))$$

This correction of the $\delta^{13}C_{alga}$ with the $\delta^{13}C_{CO2}$ was needed, since the CO_2 source used in the experiment for the CO_2-enriched treatment came from previously fixed CO_2 which had already been subjected to discrimination relative to CO_2 dissolved in seawater.

To determine the isotopic composition of dissolved inorganic carbon ($\delta^{13}C_{DIC}$), 20 ml of FSW from each cylinder was filtered (Whatman GF/F) and stored at 2ºC in dark in septum-sealed glass vials without leaving a head-space until analysis. Measurements of $\delta^{13}C_{DIC}$ were taken with the same IRMS connected to a GasBench II (Thermo Electron Corporation) system. The isotopic composition of dissolved CO_2 ($\delta^{13}C_{CO2}$) in the medium was calculated from $\delta^{13}C_{DIC}$, following a mass-balance equation (Zeebe and Wolf-Gladrow 2001). Fractionation factors between CO_2 and HCO_3^- and between HCO_3^- and CO_3^{2-} were applied according to Mook et al. (1974) and Zhang et al. (1995), respectively.

Growth

Relative growth rates (RGR) were measured according to Olischläger et al. (2013). The fresh weight was determined to a precision of 1 mg (LA 310S, Sartorius, Göttingen, Germany) after having blotted

the thalli carefully between tissue paper until all superficial water was absorbed. RGR was calculated after Lüning (1990):

$$RGR\ (\%\ day^{-1}) = \frac{100 * Ln(W1\ /\ W2)}{T1 - T2}$$

W1 = Fresh weight (g) in time 1, W2 = Fresh weight (g) in time 2, T1 and T2 = Time in days

Statistics

Two-factorial designs were analyzed with two-way ANOVA ($P < 0.05$) after the homogeneity of variances was confirmed using the Cochran's-test. Post hoc comparisons were performed by Fisher's LSD test ($P < 0.05$). The analyses were performed using Statistica software v.7 (StatSoft Inc, Tulsa, USA). Ecotypic differences were tested at 10°C using a two-factorial ANOVA. The outcome of the statistical analyses testing the effects of elevated pCO_2 and temperature on the SP and the HL population are summarized in Table 1 and 2; the statistical analyses testing the effects of ecotype and pCO_2 at 10°C are summarized in Table 3.

Results

Overall, both populations of *S. latissima* were significantly more affected by temperature than by pCO_2 or the interaction between the two factors (Table 1 and 2), although the responses were different between both populations cultured at 10ºC in 70% of those variables (Table 3).

The effects of pCO_2 and temperature on growth and the photosynthetic performance of the Helgoland population

The majority of the photosynthetic pigments contents were, on a DW basis, significantly higher at 17°C ($P < 0.001$, two-factorial ANOVA; Table 1, Fig. 1 and 2), but only Chl *c*, β-carotene and the xanthophyll cycle pigment pool (VAZ) were significantly altered by pCO_2 ($P < 0.05$, two-factorial ANOVA), showing a general decrease at future pCO_2 conditions, although none of them were influenced by the interaction between both factors. The only one not affected by temperature was antheraxanthin, while zeaxanthin was significantly reduced at 17ºC ($P < 0.01$, two-factorial ANOVA), producing a lower DPS of the xanthophyll cycle pigments ($P < 0.001$, two-factorial ANOVA, Fig. 2).

When the pigments contents are calculated per Chl *a,* the only significantly temperature-sensitive accessory pigments were fucoxanthin and the xanthophyll cycle pigments ($P < 0.001$, two-factorial ANOVA; Table 2), showing a reduction of zeaxanthin Chl a^{-1} at 17ºC ($P < 0.001$, two-factorial ANOVA), and an enhancement of antheraxanthin Chl a^{-1} and violaxanthin Chl a^{-1} at 17ºC ($P < 0.01$, two-factorial ANOVA). The latter also showed a significant interaction between temperature and pCO_2 ($P < 0.001$, two-factorial ANOVA). However, the sum of all accessory pigments per Chl *a*, which is an indicator of the antenna size, was not influenced by any of the factors.

The ΔF/Fm' was not significantly affected, either by pCO_2, temperature or the interaction of the two ($P > 0.05$, two-factorial ANOVA; Table 1; Fig. 3). Nevertheless, the photosynthetic O_2 evolution (either referred to DW or to Rubisco content) was significantly higher at the highest tested temperature ($P < 0.001$, two-factorial ANOVA; Fig. 4). However, when the O_2 evolution was referred to the Chl *a* content, there was no significant effect. Elevated pCO_2 did not significantly influence the O_2 evolution referred to DW or Chl *a*, but when the O_2 evolution was referred to Rubisco content, a stimulating influence of elevated pCO_2 was shown, although it was only significant at 17ºC ($P < 0.05$, Fisher's LSD test).

The content of Rubisco per g of DW and per g of total protein was significantly affected by temperature ($P < 0.01$, two-factorial ANOVA, Table 1; Fig. 5), showing a decrease at higher temperature and current pCO_2 conditions ($P < 0.05$, Fisher's LSD test). Also, elevated pCO_2 produced a decrease in Rubisco content ($P < 0.05$, two-factorial ANOVA), although it was not statistically significant at 17ºC ($P > 0.05$, Fisher's LSD test). The content of the D1 protein, a core component of PS II that is involved in PS II damage and repair in photoinhibition, was only significantly altered by pCO_2 ($P < 0.01$, two-factorial ANOVA), showing a decrease at elevated pCO_2 conditions at both temperatures ($P > 0.05$, Fisher's LSD test).

The ^{13}C isotopic fractionation of organic carbon production (ε_p) is primarily controlled by the acquisition and intracellular transport of inorganic carbon and the rate of carbon fixation (Rau et al. 1997); thus, it is used as an indicator of the CCMs operation, since the increase in the utilization of isotopically heavier HCO_3^- respect to isotopically lighter CO_2 produce a decrease in ε_p. Our results

showed a significant increase in ε_p at higher temperature ($P < 0.001$, two-factorial ANOVA) and increased pCO_2 conditions ($P < 0.001$, two-factorial ANOVA; Table 1, Fig. 6).

Neither temperature, nor pCO_2, nor the interaction of both factors significantly influenced the RGR ($P > 0.05$, two-factorial ANOVA; Fig. 7).

The effects of pCO_2 and temperature on growth and the photosynthetic performance of the Spitsbergen population

Very few significant differences in the photosynthetic pigments contents between treatments were obtained, and most of them were due to temperature but not pCO_2. β-carotene, anthera-, viola-, and zeaxanthin, on a DW basis, were significantly altered by temperature ($P < 0.05$, two-factorial ANOVA; Table 1, Fig. 1 and 2), although the differences were rather small. Thus, DPS was reduced at 10ºC ($P < 0.001$, two-factorial ANOVA). Zeaxanthin and VAZ were the only ones affected by pCO_2 ($P < 0.05$, two-factorial ANOVA), showing a decrease at future pCO_2 conditions, but this decrease was only significant at 10ºC ($P < 0.05$; Fisher's LSD test). If the pigment contents are referred to Chl *a*; anthera-, viola-, zeaxanthin and VAZ were significantly altered by temperature ($P < 0.05$, two-factorial ANOVA), and Chl *c* was influenced by the interaction of both factors ($P < 0.05$, two-factorial ANOVA). Nevertheless, the sum of all accessory pigments per Chl *a* was not altered.

Both temperature and pCO_2 ($P < 0.05$, two-factorial ANOVA) affected ΔF/Fm' significantly, although there was only a significant minor decrease at 4ºC combined with future pCO_2 conditions, in comparison with the result obtained at 10ºC and present pCO_2 ($P < 0.05$; Fisher's LSD test, Fig. 3). The net photosynthetic rate (referred to DW, Rubisco content or Chl *a* content) was significantly altered by temperature ($P < 0.05$, two-factorial ANOVA; Fig. 4), showing an increase at 10ºC at current pCO_2 conditions ($P < 0.05$; Fisher's LSD test); this difference being stronger when the photosynthetic rate was corrected by Rubisco content. Moreover, the latter was also influenced by the interaction between pCO_2 and temperature ($P < 0.05$, two-factorial ANOVA). Rubisco content (referred to DW or to protein content) was significantly reduced at 10ºC ($P < 0.01$, two-factorial ANOVA), although a significant decrease at higher pCO_2, as described above for the HL population,

was not found ($P > 0.05$; Fisher's LSD test; Fig. 5). However, D1 content was not altered by temperature, pCO_2 or by the interaction between them.

ε_p showed a significant influence of pCO_2 ($P < 0.001$, two-factorial ANOVA; Table 1, Fig. 6), with a statistically significant increase at high but not at expected pCO_2 ($P < 0.05$, Fisher's LSD test).

Moreover, temperature and pCO_2 significantly affected RGR ($P < 0.05$, two-factorial ANOVA; Fig. 7), showing higher values at 10ºC, and also at increased pCO_2 compared to current pCO_2 ($P < 0.05$, Fisher's LSD test).

Ecotypic variation and the effects of pCO_2 on growth and the photosynthetic performance between the populations

Chl *a* content was significantly higher in the SP population than in the HL population at 10ºC, revealing an ecotypic differentiation ($P < 0.001$, two-factorial ANOVA; Table 3, Fig. 1). The content of all accessory pigments were not ecotype-specific, except violaxanthin and antheraxanthin, which showed lower values for the SP population ($P < 0.01$, two-factorial ANOVA; Fig. 2). However, apart from the xanthophyll cycle pigments, the rest of the accessory pigments were altered by pCO_2 ($P < 0.001$, two-factorial ANOVA), following a trend of a decreased content at high pCO_2. Thus, the ratio of the majority of the accessory pigments to Chl *a* was lower in the SP population compared to the HL population, including the sum of all accessory pigments per Chl *a*. However, antheraxanthin Chl a^{-1} was higher in the SP population and zeaxanthin Chl a^{-1} showed no response. There was no population-specific difference in the DPS.

While there was no ecotypic differentiation for the $\Delta F/Fm'$ ($P > 0.05$, two-factorial ANOVA; Fig. 3), the net photosynthetic rate by O_2 evolution (referred either to DW, Rubisco content or Chl *a* content) was significantly higher in the SP population than in the HL population ($P < 0.001$, two-factorial ANOVA; Fig. 4).

Rubisco content per g DW was significantly different between populations ($P < 0.05$, two-factorial ANOVA; Table 3), showing a decrease of Rubisco in the SP population at present pCO_2 compared

with the HL population ($P < 0.05$, Fisher's LSD test; Fig. 5), and a significant interaction between ecotype and pCO_2 was also revealed ($P < 0.05$, two-factorial ANOVA). However, the Rubisco content per g of protein was only influenced by pCO_2 ($P < 0.05$, two-factorial ANOVA) and not by ecotype, although it was only significantly reflected in the HL population ($P < 0.05$, Fisher's LSD test). D1 content was significantly higher in the SP population ($P < 0.001$, two-factorial ANOVA) and decreased at elevated pCO_2 ($P < 0.05$, two-factorial ANOVA), but again it was only significantly reflected in the HL population ($P < 0.05$, Fisher's LSD test).

ε_p showed a significant influence of ecotype and pCO_2 ($P < 0.001$, two-factorial ANOVA; Table 3), with lower values for the HL population and increased values for high pCO_2 ($P < 0.05$, Fisher's LSD test; Fig. 6). Moreover, ecotype and pCO_2 affected RGR ($P < 0.05$, two-factorial ANOVA), showing higher values for the HL population in comparison with the SP population, and increased values at expected pCO_2 only for the SP population ($P < 0.05$, Fisher's LSD test; Fig. 7).

Discussion

Our results provide evidences of a different susceptibility of both *S. latissima* populations toward ocean acidification and temperature, indicating ecotypic differentiation, which is reflected in the growth rate response. However, temperature exerted stronger effects in the physiology of both populations than ocean acidification, in agreement with the results obtained by Olischläger et al. (2014) for its biochemical composition.

The effects of temperature and pCO_2 on growth and the photosynthetic performance of the Helgoland population of *Saccharina latissima*

The unchanged ratios between accessory pigments and Chl *a* suggest that the antenna size of each photosystems (PS) remained constant (Fig. 1), whereas the decrease in Chl *a* content (based on DW) at lower temperatures might indicate a reduction in the quantity of PS. The former is reflected in the temperature insensitivity of the effective quantum yield of the HL population (Fig. 3), showing that the same relative proportion of the incident light was processed at the PS. Consequently, nearly the same amount of light energy per PS was processed, but the total amount of processed energy per g

355 DW was lower. This result could reflect an adaptation to lower temperatures, which led to lower
356 enzymatic reaction rates of carbon acquisition and fixation (Raven and Geider 1988). Therefore, a
357 decrease in the number of PS must be part of the cold-acclimation in the Atlantic *S. latissima*. This
358 conclusion is further supported from the xanthophyll-data, as the total content of xanthophylls (based
359 on DW) decreased at low temperatures in the HL population (Fig. 2), whereas the content of
360 xanthophylls per Chl *a* only changed at high pCO_2, indicating an interaction between both factors.
361 However, the higher antheraxanthin content and the unchanged zeaxanthin content (based on DW) at
362 10ºC, which produced an enhancement of DPS values, suggest that, per PS, more light was dissipated
363 as heat at low temperatures.

364 The lower pigment contents and the evidence for a lower amount of PS II measured at 10ºC explain
365 the reduced rate of O_2 production per DW (Fig. 4). Moreover, there was no temperature-dependent
366 difference if the O_2 production rate is referred to Chl *a*, supporting the above conclusion that the
367 produced O_2 per PS II was equal at both temperatures but there was a lower amount of PS.
368 Nevertheless, the temperature sensitivity is revealed if we calculate the amount of O_2 per unit of
369 Rubisco. Assuming that one molecule of O_2 is equivalent to one fixed CO_2 (Al-Najar et al. 2012), the
370 reason for the lower O_2 production rate referred to Rubisco content was the decreased activity of the
371 enzyme at low temperatures (Raven and Geider 1988), which could be partially compensated by the
372 higher Rubisco content observed at 10ºC (based on DW or protein content, Fig. 5). Further factors in
373 algae decreasing the amount of produced O_2 could be the oxygenase reaction of Rubisco (the so called
374 photorespiration) and the pseudocyclic electron transport (also called water-water cycle) (Sültemeyer
375 et al. 1993; Leboulanger et al. 1998). According to Olischläger and Wiencke (2013), photorespiration
376 at low temperatures can occur when the carbon acquisition mechanisms are operating at its limits and
377 the alga is unable to further enhance its CCM capacity. A shortage in the CO_2 supply would also
378 hamper the electron drain from the photosynthetic electron transport chain and favor the O_2 fixation
379 via pseudocyclic electron transport, unavoidably leading to an increased formation of reactive oxygen
380 species (ROS; Sültemeyer et al. 1993). However, the ^{13}C isotopic fractionation of organic carbon
381 production (ε_p) for the HL population decreased at 10ºC (Fig. 6). This result suggests a preference for
382 bicarbonate use as inorganic carbon source, which is enriched in ^{13}C compared to CO_2, leading to a

383 higher fixation of ^{13}C by Rubisco. Thus, the decreased ε_p indicated an increased activation of CCMs at
384 10ºC, which could partially compensate the decreased catalytic activity of Rubisco. This enhanced
385 activation might be fuelled by an increased cyclic electron flow around PS I (Giordano et al. 2005),
386 which is thought to act as a photoprotection mechanism at low temperatures (Clarke and Johnson
387 2001). Moreover, the suspected reduced number of PS at 10ºC was not significantly reflected in a
388 reduced D1 content (Fig. 5). The content of D1 protein must be the result of a balance between
389 damage of the D1 protein and replacement of the damaged protein by a newly synthesized D1
390 polypeptide, which have an energetic cost (Raven 2011). Thus, our results might indicate a higher
391 turnover of the protein at 10ºC, which can be related to an increased investment in photoprotection
392 mechanisms at low temperatures, as mentioned above.

393 Despite the higher photosynthetic rates indicated by O_2 evolution measurements at 17ºC, the growth
394 rates were unaltered by temperature (Fig. 7). This could suggest a change of the photosynthetic
395 quotient (PQ) between both temperatures due to different metabolic requirements, indicating a higher
396 investment of photosynthetic energy into processes other than carbon fixation at higher temperatures
397 (Iñiguez et al. 2015). Another explanation for the differences between photosynthetic O_2 evolution and
398 growth rate could be an increase of carbon accumulation and excretion at 17ºC. Our data show that, in
399 contrast to slightly lower contents of carbohydrates, lipids and proteins (Olischläger et al. 2014), the
400 content of soluble phlorotannins (Fig. S1) was higher at elevated temperature, indicating that
401 secondary metabolites, such as phlorotannins, may be the final sink of the additionally fixed C which
402 was not invested into growth. This could be related also to an increase in the release of dissolved
403 organic carbon (DOC) at higher temperatures, which has been also observed in other species
404 (Borchard and Engel 2012; Iñiguez et al. 2015).

405 The impact of elevated pCO_2 on the physiology of the HL population is lower than the impact of
406 increased temperature. The only pigment contents affected by elevated pCO_2 were Chl *c*, VAZ and β-
407 carotene, although there were no change in these pigment contents corrected by Chl *a* (Fig. 1 and 2).

408 The lower β-carotene content at elevated pCO_2 could occur in response to a decreased need to detoxify
409 ROS. ROS production might be favored at low pCO_2 (Vardi et al. 1999; García-Gómez et al. 2014) in

410 response to a CO_2-shortage, and algal β-carotene might participate in the antioxidant mechanisms
411 (Holdt and Kraan 2011). A shortage of CO_2 can result in a shortage of $NADP^+$, the final oxidant of the
412 photosynthetic electron transport chain, which in turn would increase the excitation state of the
413 photosynthetic electron transport chain and would favour the production of ROS (Sitte et al. 2002).
414 Thus, the higher β-carotene content at present pCO_2 compared to high pCO_2 might prevent cells from
415 photoinhibition, the latter being reflected in a higher degradation of the D1 protein by increased
416 production of ROS (Lesser 2006, Fig. 5) and, consequently, in a lower effective quantum yield; a
417 response which was not observed in our data.

418 At 10ºC, Chl *a* content decreased at high pCO_2 without a change in the ratio accessory pigments Chl a^{-1}
419 , indicating a lower content of PS, which suggest a decrease in photosynthetic energy demand due
420 presumably to deactivation of CCMs, as shown by ε_p data (Fig. 6). This results are in agreement with
421 the reduction observed in the D1 content at high pCO_2. Furthermore, at 17ºC, the energy saved by
422 deactivation of CCMs at increased pCO_2 might have been invested in an increased accumulation of
423 carbohydrates (Olischläger et al. 2014), as the unchanged content of Chl *a* and the ratio accessory
424 pigment Chl a^{-1} suggest that the content of PS was not altered. However, the decrease in the D1
425 content observed at 17ºC and elevated pCO_2 might reflect a higher rate of degradation or a slower rate
426 of reposition than at present pCO_2.

427 Elevated pCO_2 decreased the amount of Rubisco per DW and per protein content, although at 17ºC,
428 this reduction was not statistically significant (Fig. 5). Since elevated pCO_2 does not change the O_2
429 production rate on a DW basis, the significantly higher photosynthetic O_2 production per mg of
430 Rubisco obtained could reflect that, at present pCO_2, Rubisco was not C-saturated despite CCM
431 functioning. Thus, a lower quantity of this enzyme was able to maintain the same photosynthetic rate
432 at high pCO_2. Lower protein content at increased CO_2 has been observed earlier in the chlorophyte
433 seaweed *Ulva rigida* (Gordillo et al. 2001). Other studies have shown that CCMs in Laminariales are
434 weak compared to other intertidal brown algae (Surif and Raven 1989; Haglund et al. 1992), and it
435 might be possible that CCM function increases Rubisco activity but does not completely saturate the
436 enzyme.

437 All this physiological pCO_2 and temperature driven re-arrangement led to an unchanged RGR, which
438 was apparently at its maximum and potentially limited by the meristematic capacity, as it was neither
439 temperature nor pCO_2-sensitive (Fig. 7, see Koch et al. 2013; Harley et al. 2012; Gao et al. 2012)

440 The effects of pCO_2 and temperature on growth and the photosynthetic performance of
441 the Spitsbergen population of *Saccharina latissima*

442 According to the Chl *a* and accessory pigments content, comparable amounts of photons were
443 harvested at both temperatures tested. Only β-carotene and the xanthophyll cycle pigments
444 individually, were significantly affected by temperature with a rather small effect (Fig. 1). Hence,
445 neither the number of PS nor the photosystem-antenna size was strongly affected in the tested
446 temperature range. Consequently, comparable amounts of accessory pigments might have transported
447 similar amounts of photons towards the reaction centers of the PS at both temperatures (Fig. 1, Fig. 2),
448 despite an enhanced energy dissipation due to an increased anthera- and zeaxanthin content at lower
449 temperature, which is translated in a higher DPS.

450 The small but significant decrease of the effective quantum yield at low temperature in the SP
451 population could indicate a higher non-photochemical dissipation of energy in PS II (Fig. 3), as shown
452 by the significantly higher value of DPS (Fig. 2). However, the temperature effect on light harvesting
453 and effective quantum yield cannot explain the proportion of decrease in O_2 production rate at 4°C
454 (irrespective of it being expressed on the basis of DW, Chl *a*, or Rubisco content). This might be a
455 consequence of increased photorespiration, an enhancement of the water-water cycle and/or an
456 activation of cyclic electron flow around PS II, as all these processes may alter net O_2 production rate
457 without affecting the effective quantum yield of PS II. Cyclic electron flow around PS II could act as
458 part of a photoprotection strategy, dissipating excess of excitation energy even at subsaturating
459 irradiances at low temperatures, when the metabolic activity is reduced such that its capacity is
460 exceeded by the absorption of light by the photosynthetic apparatus. This mechanism was also
461 suggested for *U. rigida* by Gordillo et al. (2003) and for the diatom *Phaeodactylum tricornutum* by
462 Feikema et al. (2006). Moreover, the water-water cycle and the cyclic electron flow around PS I have
463 also been proposed as photoprotection mechanisms for the consumption of photons under conditions

where the rate of photosynthetic electron transport exceeds the capacity of the C metabolism, leading to increased thylakoid acidification required for the de-epoxidation of violaxanthin to zeaxanthin and the non-photochemical dissipation of excess excitation energy (Horton et al. 1996). However, unlike the Mehler reaction, cyclic electron flow around PS I does not have the associated problem of harmful free-radical production, as the enzymes for detoxification of radicals would be slowed down at low temperature (Clarke and Johnson 2001). Thus, an increase in the photoprotection mechanisms at low temperatures would help to dissipate excess of photons and, unavoidably, decrease the carbon fixation (and growth rate) by producing an excess of ATP over that needed in CO_2 assimilation. This increased ATP production might support the maintenance of a similar CCM activity at low temperature (Fig. 6). Furthermore, an enhanced production of intra-chloroplastic ROS, as a consequence of photoinhibition at low temperatures, is not supported by a higher content of antioxidants, such as β-carotene and phlorotannins (see Fig. 1S), or by a change in the D1 protein content. An additional reason for the decreased growth rate at 4ºC could be a higher accumulation of lipids, as shown in Olischläger et al. (2014), serving as an energetic reserve for growth during the polar night (Bartsch et al. 2008).

As has been shown for HL population, the impact of pCO_2 on the physiology of the SP population was also lower than the impact of temperature. The effective quantum yield at 4°C was slightly lower at elevated pCO_2, which could suggest a photochemical stress, a higher heat dissipation or a reduced light harvesting. However, the assumption of a photochemical stress is not supported by the CO_2-insensitive O_2 evolution, the CO_2-stimulated growth or the unchanged D1 content. Moreover, a higher heat dissipation is not supported either by the decrease in the zeaxanthin content at high CO_2 or the unchanged DPS values, and there was no significantly effect in the amount of PS or the PS antenna size that could further point to an increased light harvesting. Despite the unchanged net photosynthetic rate, the increased growth at elevated pCO_2 could suggest a reduced loss of carbon, mainly by decreasing respiration and/or DOC excretion, as proposed by Gordillo et al. (2001) for *U. rigida* and Iñiguez et al. (2015) for the Arctic kelp *Alaria esculenta*. This indicates that the cell division rate of the meristem is apparently not maximal. According to Körner (2012), under those conditions the meristematic growth could be stimulated by the provision of additional resources. This response has been previously reported at elevated pCO_2 (de Castro Araújo and Tarvano García 2005; Olischläger

and Wiencke 2013; Sarker et al. 2013). The reason why high pCO_2 (1500 µatm pCO_2) did not stimulate growth at 10°C remains unclear, but it is a frequent phenomenon reported in previous studies (Kübler et al. 1999; Diaz-Pulido et al. 2011). An hypothesis for that fact could be a different sensitivity to changes in external pH between the studied temperatures, leading to a higher DOC excretion and/or a reallocation of the energy saved due to CCM deactivation (as suggested by ε_p data, see Fig. 6). Potentially, the difference in growth between 800 µatm and 1500 µatm pCO_2 can be linked to a stimulated Rubisco content at 10°C and 800 µatm pCO_2, which was not observed at high pCO_2 (Fig. 5). In contrast to the HL population, Rubisco content of the Arctic ecotype was not decreased at high pCO_2, and the net photosynthetic rate per mg of Rubisco was not altered, indicating that Rubisco was already CO_2-saturated at present CO_2 concentrations.

Ecotypic variation and the effect of pCO_2 on both populations

When the physiology of the SP and the HL populations are compared under equal conditions (10°C), pronounced differences between their physiological performance could be observed. The Chl *a* content of the SP population was higher (Fig. 1), whereas the total content of accessory pigments was not different, giving a lower ratio of accessory pigments Chl a^{-1}. Thus, the number of PS of the Arctic population should be higher and the antenna size smaller than in the cold-temperate population (Fig. 1), which might reflect an adaptation that avoids photoinhibition in cold environments. Hence, the excitation pressure on the PS was lower in the SP population, a finding which might be reflected in the lower investment in protective means against excessive excitation, such as the xanthophyll cycle (Fig. 2). Remarkable is that, despite the lower antenna size and xanthophyll content and the reduced investment in active carbon acquisition, the O_2 evolution was clearly higher in the SP population, in parallel to a lower content of Rubisco per g DW. The latter suggests that, at 10°C, the carbon fixation rate was more efficient in the SP population, suggesting changed kinetic characteristics between Rubisco of the two populations (Morgan-Kiss et al. 2006). Conclusively, the physiological data reveal that the photosynthetic performance of SP population at 10°C is better than that of the HL population, clearly indicating shifted temperature sensitivity between both populations and revealing ecotypic differentiation. However, this improved physiological performance was not reflected in higher growth

519 rates for SP population; contrastingly, the growth of the HL population was even higher at 10°C. This
520 might be a consequence of higher contents of storage metabolites, such as lipids and carbohydrates in
521 the SP population, as it has been shown in Olischläger et al. (2014). As previously reported, the Arctic
522 population is adapted to store a higher fraction of the produced photosynthates during nutrient-
523 depleted summer, likely serving as energy reserve for the nutrient-repleted long polar night, when
524 most of the growth takes place (Bartsch et al. 2008).

525 pCO_2 drove some similar responses in both ecotypes. Most of the pigments contents per DW analysed
526 tend to decrease at elevated pCO_2, even though Chl *a* and accessory pigments Chl a^{-1} were not
527 significantly affected, and also ε_p values suggest that CCMs seemed to be at least partially deactivated
528 at high CO_2 in both ecotypes. However, deactivation in both populations at 10ºC occurred only at high
529 CO_2 concentrations (1500 µatm) but it was not appreciated at 800 µatm, although the HL population
530 already showed a deactivation at 800 µatm CO_2 at 17ºC. This could indicate a higher need of CCM
531 activity at low temperatures, as suggested before by Gordillo et al. (2016).

532 There were very few significant interactions between ecotypes and pCO_2. In the HL population,
533 expected and future pCO_2 levels decreased the content of Rubisco per DW, whereas in the SP
534 population, the content of Rubisco increased at predicted pCO_2, but not at high pCO_2. It is known that
535 the algal response to elevated pCO_2 is dependent on the applied pCO_2, and bell-shaped responses in
536 growth rates were previously reported (Kübler et al. 1999; Diaz-Pulido et al. 2011). This is
537 remarkable, since the presented bell-shaped pattern for the Rubisco content in the SP population at
538 10°C was mirrored by the growth pattern, whereas the growth-pattern of the HL-population
539 (insensitive to elevated pCO_2) did not reflect the significantly lower Rubisco content. This is
540 potentially attributable to the degree of activation of the enzyme Rubisco, since in polar algae,
541 Rubisco appears to be almost completely activated and at near CO_2 saturation, achieving the high
542 carbon fixation rates observed (Young et al. 2015), whereas the temperate population could have a
543 higher degree of Rubisco deactivation in some circumstances. Also, this fact could be related to the
544 previously discussed ecotype-specific differences in the degree of utilization of the meristems at 10°C

and/or an ecotypic difference in the allocation of the photosynthates towards storage metabolites or growth (Bartsch et al. 2008; Olischläger et al. 2014).

Conclusion

The Arctic and the cold-temperate populations of *S. latissima* represent different ecotypes with striking differences in their physiology. Both populations were sensitive to changing temperatures and OA, but to different extent. Changes in temperature seems to exert a stronger effect than pCO_2 in the physiology of both populations. Concerning growth, the Arctic-ecotype was more sensitive to pCO_2, particularly at low, apparently suboptimal temperatures, compared to the Atlantic ecotype; although the biochemical composition of the Arctic-ecotype was more CO_2-insensitive, as shown by Olischläger et al. (2014) and data from pigment, D1 and Rubisco content shown in the present study. This fact reflects different acclimation strategies for both populations. The growth rate response of the Arctic population at increased temperature is most likely attributable to an incomplete adaptation to the cold environment, which *S. latissima* reinvaded after the last deglaciation event approximately 18000 years ago (Lüning 1990). Our findings show that the Arctic ecotype might benefit from global warming and, in a lesser extent, ocean acidification, with potentially large consequences for the current Arctic phytobenthic community and the associated fauna, as it has been suggested by other studies (Gordillo et al. 2015); while *S. latissima* from temperate latitudes, with an unaltered growth rate, is suspected not to be strongly affected by ocean acidification and increased temperature.

Author contribution

MO, CI planned the experiments; MO and CI conducted the experiments and did the required measurements. MO and CI did the statistical analysis of the data and wrote most parts of the manuscript with the assistance and the discussion of all coauthors. All authors contributed to the writing process and all authors read and approved the manuscript.

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Figures

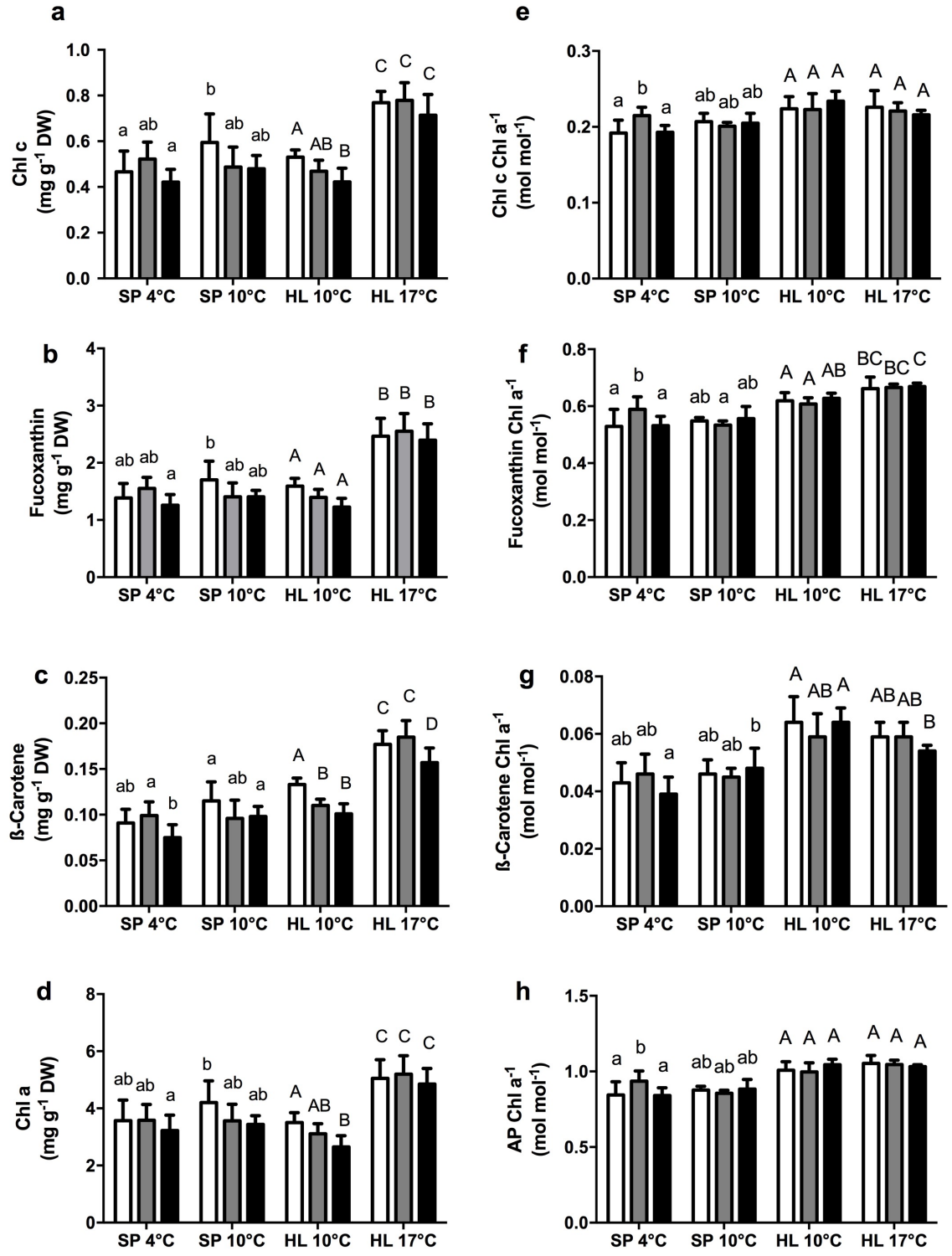

Fig. 1 Effects of pCO_2 (white: present pCO_2; grey: expected future pCO_2; black: high pCO_2) and temperature on the accessory pigment content of *Saccharina latissima*, from Spitsbergen (SP) and Helgoland (HL). Different letters indicate significant differences. AP, accessory pigments

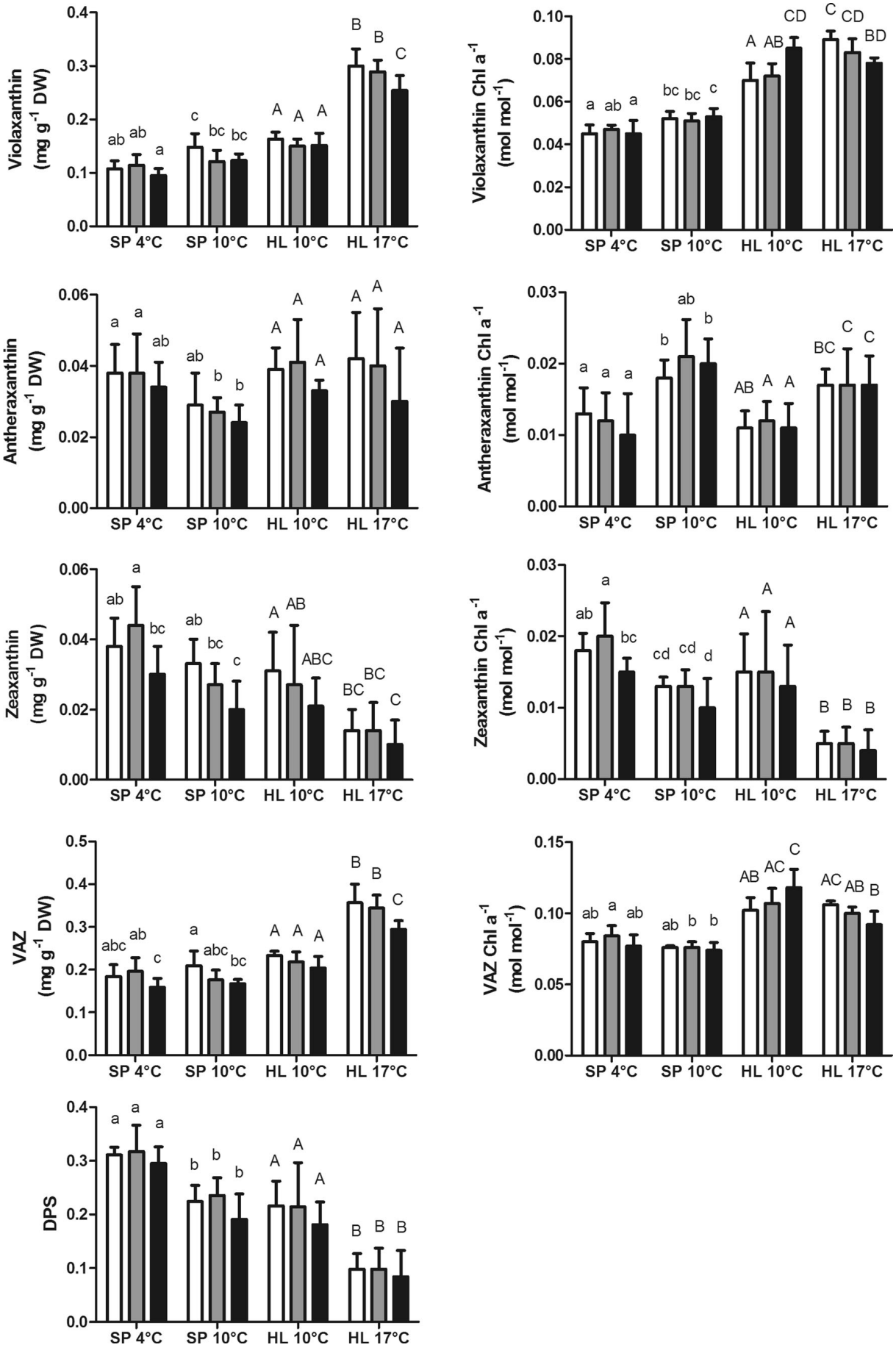
Violaxanthin (mg g-1 DW)
Violaxanthin Chl a-1 (mol mol-1)
Antheraxanthin (mg g-1 DW)
Antheraxanthin Chl a-1 (mol mol-1)
Zeaxanthin (mg g-1 DW)
Zeaxanthin Chl a-1 (mol mol-1)
VAZ (mg g-1 DW)
VAZ Chl a-1 (mol mol-1)
DPS
SP 4°C
SP 10°C
HL 10°C
HL 17°C

Fig. 2 Effects of pCO_2 (white: present pCO_2; grey: expected future pCO_2; black: high pCO_2) and temperature on the xanthophyll-content of *Saccharina latissima*, from Spitsbergen (SP) and Helgoland (HL). Different letters indicate significant differences. VAZ, xanthophyll cycle pigment pool (sum of viola-, anthera- and zeaxanthin).

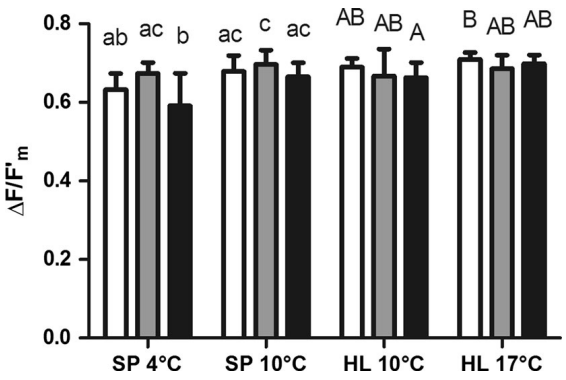

Fig. 3 Effects of pCO_2 (white: present pCO_2; grey: expected future pCO_2; black: high pCO_2) and temperature on the ΔF/Fm' of *Saccharina latissima*, from Spitsbergen (SP) and Helgoland (HL). Different letters indicate significant differences.

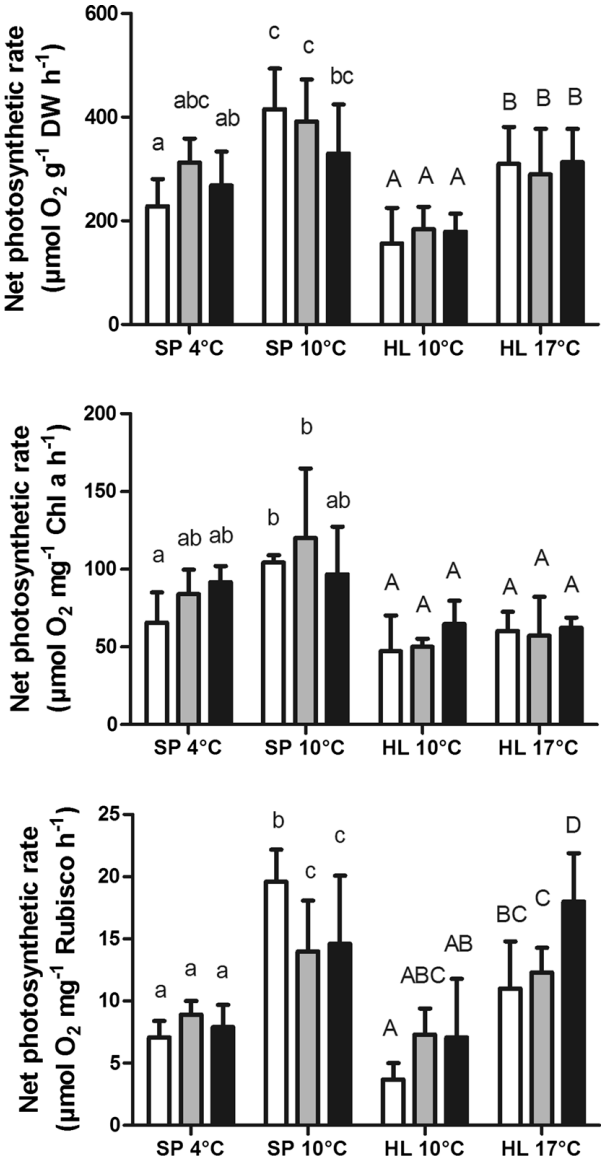

Fig. 4 Effects of pCO_2 (white: present pCO_2; grey: expected future pCO_2; black: high pCO_2) and temperature on the photosynthetic rate measured as O_2 evolution of *Saccharina latissima*, from Spitsbergen (SP) and Helgoland (HL). Different letters indicate significant differences.

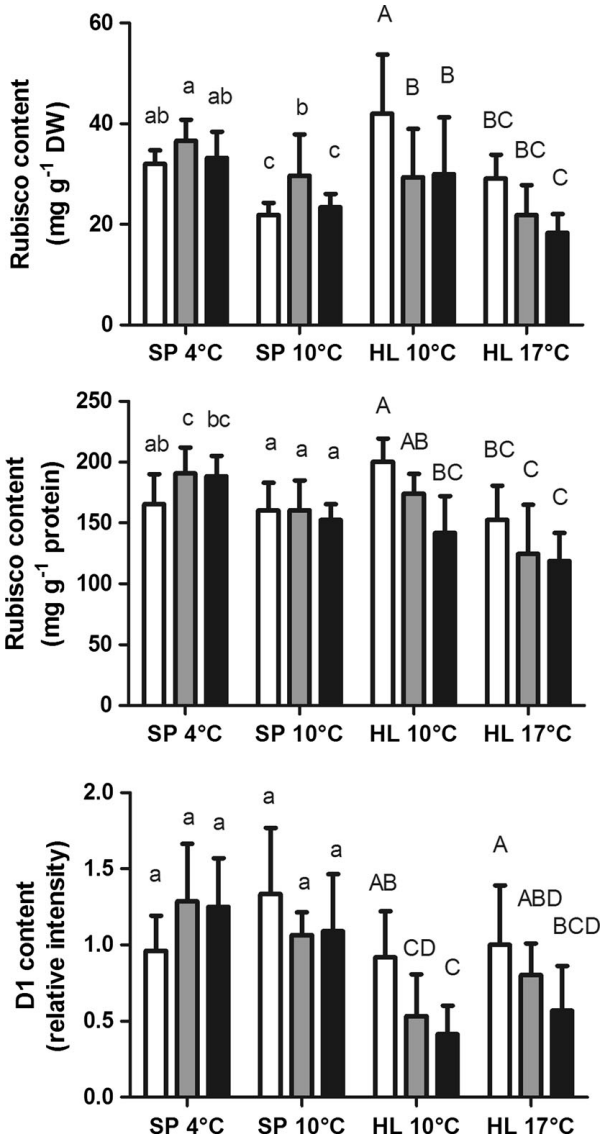

Fig. 5 Effects of pCO_2 (white: present pCO_2; grey: expected future pCO_2; black: high pCO_2) and temperature on the Rubisco and D1 protein content of *Saccharina latissima*, from Spitsbergen (SP) and Helgoland (HL). Different letters indicate significant differences.

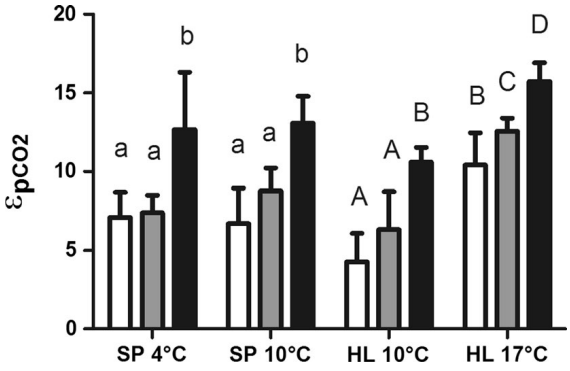

Fig. 6 Effects of pCO_2 (white: present pCO_2; grey: expected future pCO_2; black: high pCO_2) and temperature on the ε_p values of *Saccharina latissima*, from Spitsbergen (SP) and Helgoland (HL). Different letters indicate significant differences.

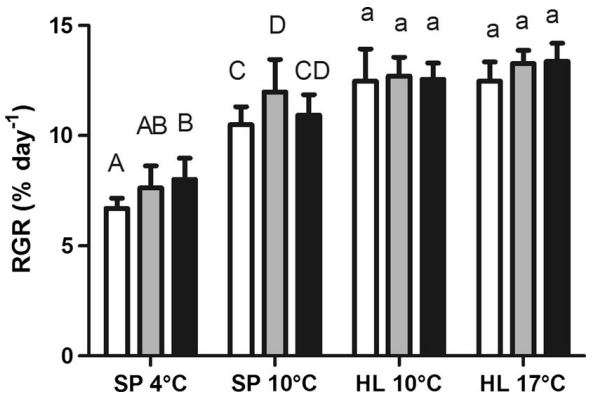

Fig. 7 Effects of pCO_2 (white: present pCO_2; grey: expected future pCO_2; black: high pCO_2) and temperature on the relative growth rate (RGR) in % day^{-1} of *Saccharina latissima*, from Spitsbergen (SP) and Helgoland (HL). Different letters indicate significant differences.

Table 1 Results of the testing for significant influences of temperature, cultivation-pCO_2 and their interaction on the measured characteristics of *Saccharina latissima* from cold-temperate latitudes (Helgoland, HL, grey) and Spitsbergen (SP, white) by a two-way ANOVA. One asterisk indicates $P < 0.05$, two asterisks indicate $P < 0.01$ and three asterisks indicate $P < 0.001$.

Variable	Temperature	pCO_2	Temperature * pCO_2
Growth rate (HL)	n.s.	n.s.	n.s.
Growth rate (SP)	***	*	n.s.
Net photosynthesis (Chl a^{-1}) (HL)	n.s.	n.s.	n.s.
Net photosynthesis (Chl a^{-1}) (SP)	*	n.s.	n.s.
Net photosynthesis (DW^{-1}) (HL)	***	n.s.	n.s.
Net photosynthesis (DW^{-1}) (SP)	***	n.s.	n.s.
Net photosynthesis (mg^{-1} Rubisco) (HL)	***	*	n.s.
Net photosynthesis (mg^{-1} Rubisco) (SP)	***	n.s.	*
D1 protein (HL)	n.s.	**	n.s.
D1 Protein (SP)	n.s.	n.s.	n.s.
Rubisco (mg^{-1} proteins) (HL)	***	**	n.s.
Rubisco (mg^{-1} proteins) (SP)	**	n.s.	n.s.
Rubisco (mg^{-1} DW) (HL)	**	*	n.s.
Rubisco (mg^{-1} DW) (SP)	***	*	n.s.
ΔF/Fm' (HL)	n.s.	n.s.	n.s.
ΔF/Fm' (SP)	**	*	n.s.
Chl *a* (mg^{-1} DW) (HL)	***	n.s.	n.s.
Chl *a* (mg^{-1} DW) (SP)	n.s.	n.s.	n.s.
Chl *c* (mg^{-1} DW) (HL)	***	*	n.s.
Chl *c* (mg^{-1} DW) (SP)	n.s.	n.s.	n.s.
Fucoxanthin (mg^{-1} DW) (HL)	***	n.s.	n.s.
Fucoxanthin (mg^{-1} DW) (SP)	n.s.	n.s.	n.s.
Violaxanthin (mg^{-1} DW) (HL)	***	n.s.	n.s.
Violaxanthin (mg^{-1} DW) (SP)	**	n.s.	n.s.
Antheraxanthin (mg^{-1} DW) (HL)	n.s.	n.s.	n.s.
Antheraxanthin (mg^{-1} DW) (SP)	**	n.s.	n.s.
Zeaxanthin (mg^{-1} DW) (HL)	**	n.s.	n.s.
Zeaxanthin (mg^{-1} DW) (SP)	**	*	n.s.
β-carotene (mg^{-1} DW) (HL)	***	**	n.s.
β-carotene (mg^{-1} DW) (SP)	*	n.s.	n.s.
VAZ (mg^{-1} DW) (HL)	***	*	n.s.

VAZ (mg^{-1} DW) (SP)	n.s.	*	n.s.
DPS (HL)	***	n.s.	n.s.
DPS (SP)	***	n.s.	n.s.
ε_p (HL)	***	***	n.s.
ε_p (SP)	n.s.	***	n.s.

Table 2 Results of the testing for significant influences of temperature, cultivation-pCO_2 and their interaction on the pigment-content of *Saccharina latissima* relative to the Chl *a* from cold-temperate latitudes (Helgoland, HL, grey) and Spitsbergen (SP, white) by a two-way ANOVA. One asterisk indicates $P < 0.05$, two asterisks indicate $P < 0.01$ and three asterisks indicate $P < 0.001$.

Variable	Temperature	pCO_2	Temperature * pCO_2
Chl *c* Chl a^{-1} (mol mol^{-1}) (HL)	n.s.	n.s.	n.s.
Chl *c* Chl a^{-1} (mol mol^{-1}) (SP)	n.s.	n.s.	*
Fucoxanthin Chl a^{-1} (mol mol^{-1}) (HL)	***	n.s.	n.s.
Fucoxanthin Chl a^{-1} (mol mol^{-1}) (SP)	n.s.	n.s.	n.s.
β-carotene Chl a^{-1} (mol mol^{-1}) (HL)	n.s.	n.s.	n.s.
β-carotene Chl a^{-1} (mol mol^{-1}) (SP)	n.s.	n.s.	n.s.
Sum accessory pigments Chl a^{-1} (HL)	n.s.	n.s.	n.s.
Sum accessory pigments Chl a^{-1} (SP)	n.s.	n.s.	n.s.
Antheraxanthin Chl a^{-1} (mol mol^{-1}) (HL)	***	n.s.	n.s.
Antheraxanthin Chl a^{-1} (mol mol^{-1}) (SP)	***	n.s.	n.s.
Zeaxanthin Chl a^{-1} (mol mol^{-1}) (HL)	***	n.s.	n.s.
Zeaxanthin Chl a^{-1} (mol mol^{-1}) (SP)	***	*	n.s.
Violaxanthin Chl a^{-1} (mol mol^{-1}) (HL)	**	n.s.	***
Violaxanthin Chl a^{-1} (mol mol^{-1}) (SP)	***	n.s.	n.s.
VAZ Chl a^{-1} (mol mol^{-1}) (HL)	***	n.s.	***
VAZ Chl a^{-1} (mol mol^{-1}) (SP)	*	n.s.	n.s.

Table 3 Results of testing for significant differences in the measured characteristics between Arctic and cold-temperate populations of *Saccharina latissima* at 10°C (ecotypes) and the effects exerted by pCO_2 and its interaction with ecotype by a two-factorial ANOVA. One asterisk indicates $P < 0.05$, two asterisks indicate $P < 0.01$ and three asterisks indicate $P < 0.001$.

Variable	**Ecotype**	**pCO_2**	**Ecotype * pCO_2**
Growth rate	***	*	n.s.
Net photosynthesis (Chl a^{-1})	***	n.s.	n.s.
Net photosynthesis (DW^{-1})	***	n.s.	n.s.
Net photosynthesis (mg $Rubisco^{-1}$)	***	n.s.	n.s.
D1 Protein	***	*	n.s.
Rubisco (mg $proteins^{-1}$)	n.s.	*	n.s.
Rubisco (mg DW^{-1})	**	n.s.	*
ΔF/Fm’	n.s.	n.s.	n.s.
Chl *a* (mg DW^{-1})	***	n.s.	n.s.
Chl *c* (mg DW^{-1})	n.s.	*	n.s.
Chl *c* Chl a^{-1} (mol mol^{-1})	***	n.s.	n.s.
Fucoxanthin (mg^{-1} DW)	n.s.	**	n.s.
Fucoxanthin Chl a^{-1} (mol mol^{-1})	***	n.s.	n.s.
Violaxanthin (mg^{-1} DW)	**	n.s.	n.s.
Violaxanthin Chl a^{-1} (mol mol^{-1})	***	*	*
Antheraxanthin (mg^{-1} DW)	***	n.s.	n.s.
Antheraxanthin Chl a^{-1} (mol mol^{-1})	***	n.s.	n.s.
Zeaxanthin (mg^{-1} DW)	n.s.	n.s.	n.s.
Zeaxanthin Chl a^{-1} (mol mol^{-1})	n.s.	n.s.	n.s.
β-carotene (mg^{-1} DW)	n.s.	**	n.s.
β-carotene Chl a^{-1} (mol mol^{-1})	***	n.s.	n.s.
Sum accessory pigments Chl a^{-1}	***	n.s.	n.s.
DPS	n.s.	n.s.	n.s.
VAZ (mg^{-1} DW)	***	*	n.s.
VAZ Chl a^{-1} (mol mol^{-1})	***	n.s.	n.s.
ε_p	***	***	n.s.

Supplements

List of supplements: Figure S1 (content of phlorotannins)

Supplement S1

The content of Phlorotannins was quantified as described in Olischläger et al. (2012)

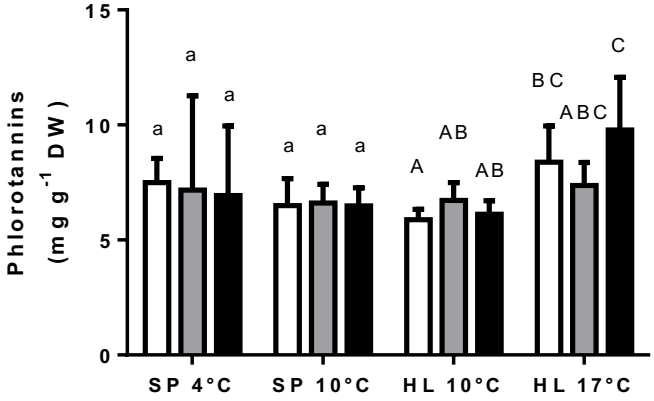

Figure S1: Content of phlorotannins (mean +SD) in *Saccharina latissima* macrothalli from cold-temperate latitudes (Helgoland, HL) and Spitsbergen (SP) after cultivation at present pCO_2 (white bar), expected future pCO_2 (gray bar) and high pCO_2 (black bar) at different temperatures.