Journal of Experimental Botany, Vol. 64, No. 18, pp. 5587–5597, 2013
doi:10.1093/jxb/ert329 Advance Access publication 14 October, 2013

Journal of Experimental Botany
www.jxb.oxfordjournals.org

RESEARCH PAPER

Ocean acidification alleviates low-temperature effects on growth and photosynthesis of the red alga *Neosiphonia harveyi* (Rhodophyta)

Mark Olischläger* and Christian Wiencke

Alfred Wegener Institute for Polar and Marine Research, Section of Functional Ecology, Department of Seaweed Biology, Am Handelshafen 12, D-27570 Bremerhaven, Germany

* To whom correspondence should be addressed. E-mail: Mark.Olischlaeger@awi.de

Received 21 May 2013; Revised 26 August 2013; Accepted 30 August 2013

Abstract

This study aimed to examine interactive effects between ocean acidification and temperature on the photosynthetic and growth performance of *Neosiphonia harveyi*. *N. harveyi* was cultivated at 10 and 17.5 °C at present (~380 µatm), expected future (~800 µatm), and high (~1500 µatm) pCO_2. Chlorophyll *a* fluorescence, net photosynthesis, and growth were measured. The state of the carbon-concentrating mechanism (CCM) was examined by pH-drift experiments (with algae cultivated at 10 °C only) using ethoxyzolamide, an inhibitor of external and internal carbonic anhydrases (exCA and intCA, respectively). Furthermore, the inhibitory effect of acetazolamide (an inhibitor of exCA) and Tris (an inhibitor of the acidification of the diffusive boundary layer) on net photosynthesis was measured at both temperatures. Temperature affected photosynthesis (in terms of photosynthetic efficiency, light saturation point, and net photosynthesis) and growth at present pCO_2, but these effects decreased with increasing pCO_2. The relevance of the CCM decreased at 10 °C. A pCO_2 effect on the CCM could only be shown if intCA and exCA were inhibited. The experiments demonstrate for the first time interactions between ocean acidification and temperature on the performance of a non-calcifying macroalga and show that the effects of low temperature on photosynthesis can be alleviated by increasing pCO_2. The findings indicate that the carbon acquisition mediated by exCA and acidification of the diffusive boundary layer decrease at low temperatures but are not affected by the cultivation level of pCO_2, whereas the activity of intCA is affected by pCO_2. Ecologically, the findings suggest that ocean acidification might affect the biogeographical distribution of *N. harveyi*.

Key words: Carbonic anhydrase, CCM, climate change, CO_2, DIC, distribution, macroalgae, photosynthesis.

Introduction

Anthropogenic combustion of fossil fuels increases the atmospheric pCO_2. Predictions state that the current pCO_2 of 380 µatm will be exceeded more than twice by the year 2100 and reach about 800 µatm, or even higher (Doney *et al.*, 2009). The consequence will be elevated temperatures and, since about 20–30% of the human-released CO_2 is absorbed by the oceans, ocean acidification (OA) (Orr *et al.*, 2009). OA occurs by the dissolution of atmospheric CO_2 in sea water and the subsequent formation of carbonic acid from CO_2 and H_2O, which nearly completely dissociates into HCO_3^- and H^+.

Besides a lower pH, one impact of OA is an increase in the concentration of dissolved inorganic carbon, including

Abbreviations: α, photosynthetic efficiency; ANOVA, analysis of variance; AZ, acetazolamide; CCM, carbon-concentrating mechanism; DBL, diffusive boundary layer; E_k, light saturation point; exCA, external carbonic anhydrase; FSW, filtered sea water; FW, fresh weight; $\Delta F/F'_m$, effective quantum yield; intCA, internal carbonic anhydrase; net-PS, net photosynthesis; OA, ocean acidification; P-E curve, photosynthesis-irradiance curve; PES, Provasoli's enriched sea water; rETR(max), maximum relative electron transport rate; RGR, relative growth rate; RM-ANOVA, repeated-measurement analysis of variance; ROS, reactive oxygen species; SWCS, sea-water carbonate system.

HCO_3^- and CO_2, in parallel with a decrease in CO_3^{2-} (Doney *et al.*, 2009). Macroalgae acquire dissolved inorganic carbon for photosynthesis in the form of CO_2 and/or HCO_3^- (Maberly, 1990). Consequently, the increase in HCO_3^- and CO_2 might be of biological relevance for marine macroalgae, because many marine macroalgae are carbon-limited at present pCO_2 (Holbrook *et al.*, 1988) and/or need to express the energetically unfavourable uptake of HCO_3^- via a carbon-concentrating mechanism (CCM) to saturate their photosynthetic carbon demand (Giordano *et al.*, 2005). Marine algae have evolved different types of CCMs (Raven and Hurd, 2012). One widespread type of CCM involves the enzyme external carbonic anhydrase (exCA), which converts HCO_3^- and H^+ into CO_2 and H_2O. It is believed that the functioning of exCA is often facilitated by active acidification of the diffusive boundary layer (DBL) via local H^+ extrusion (Mercado *et al.*, 2006; Moulin *et al.*, 2011).

Consequently, at future pCO_2 the increased availability of CO_2 and HCO_3^- could benefit algae in three synergistic ways. Firstly, the increased content of dissolved CO_2, which can diffuse passively into the cell (Raven and Hurd, 2012), could provide additional substrate for Rubisco and mitigate the inorganic carbon limitation for algae without a CCM. However, a CCM-operating alga would be unable to benefit from passive diffusion of CO_2 into the cell, because a CCM increases the CO_2 concentration around Rubisco above the CO_2 level reachable by passive diffusion (Raven *et al.*, 2012). Accordingly the passive diffusion of CO_2 would be directed outwards in these algae if no counteractive means to prevent CO_2 loss by passive diffusion would be taken (Raven *et al.*, 2012). Secondly, it could decrease the oxygenase reaction of Rubisco (Hepburn *et al.*, 2011) and the third possibility is that the higher concentration of CO_2 could decrease the energetic demand of the active carbon acquisition.

Inevitably, a CCM requires an energetic investment for expression and operation (Raven *et al.*, 2012). By decreasing the energetic demand of the CCM, OA could even benefit carbon-saturated macroalgae. Decreased CCM activity in response to experimentally enriched inorganic carbon has already been shown (Giordano *et al.*, 2005). Consequently, it is not surprising that the effects of OA on carbon acquisition, photosynthesis, and/or growth of many non-calcifying macroalgae are positive (e.g. Gordillo *et al.*, 2001; Olischläger *et al.*, 2012, 2013). Nevertheless, a pCO_2 elevated above present levels would not necessarily cause an increase in photosynthesis (Giordano *et al.*, 2005). Since the effect of OA on growth and photosynthetic performance seems to be species-specific, with some species benefitting and some others showing inhibition or no response (Israel and Hophy, 2002), it is suspected that OA might promote changes at the community level.

Temperature is a key factor regulating photosynthesis and pigmentation (Raven and Geider, 1988; Davison, 1991; Staehr and Weinberg, 2009). However, the combined effects of OA and temperature on carbon acquisition, pigmentation, and photophysiology are not well understood (Raven *et al.*, 2011). Both increasing temperature and increasing pCO_2 were shown to decrease the content of pigments (Gordillo *et al.*, 2001; Staehr and Wernberg, 2009) and both can affect photosynthesis (Davison, 1991). Consequently, it is reasonable to assume synergistic effects of OA and temperature on the physiology and growth of marine macroalgae.

Previous studies have already shown that elevated temperature and pCO_2 increased synergistically the specific growth rate and photosynthesis of the marine cyanobacterium *Synechococcus* sp. Nägeli and *Emiliana huxleyi* (Lohmann) Hay & Mohler (Fu *et al.*, 2007; Feng *et al.*, 2008). However, the combination of CO_2 and elevated temperatures only stimulated photosynthesis of the raphidophyte *Heterosigma akashiwa* (Hada) Hara & Chihara, but did not affect its growth rate (Fu *et al.*, 2008). Other species, like the cyanobacterium *Prochlorococcus* sp. S.W.Chisholm, S.L.Frankel, R.Goericke, R.J.Olson, B.Palenik, J.B.Waterbury, L.West-Johnsrud & E.R.Zettler (Fu *et al.*, 2007), the polar diatom *Navicula directa* (W.Smith) Ralfs and the dinoflagellate *Prorocentrum minimum* (Pavillard) J.Schiller did not respond to a combined elevation of temperature and pCO_2 (Fu *et al.*, 2008; Torstensson *et al.*, 2012). However, in a mesocosm experiment increased pCO_2 and elevated temperature enhanced synergistically the abundance of red turf algae (Connell and Russell, 2010). The latter might account for the findings of Sarker *et al.* (2013), who showed that elevated pCO_2 could compensate for the negative effects of sub-optimal high temperature on growth. In conclusion, temperature and OA synergistically affect at least some marine photoautotrophs, but the physiological background of this interaction needs clarification. Furthermore, most of the above-mentioned studies examined the effects of elevated temperature. Interactive effects between pCO_2 and low temperatures have not been systematically addressed. Temperature is fundamental for the biogeographic distribution of algae (Lüning, 1990) and if OA alleviated the harmful effects of low temperature on invasive warm-water species one prerequisite for the further poleward extension of their biogeographic distribution would be fulfilled. The red alga *Neosiphonia harveyi* (J.W.Bailey) M.-S. Kim, H.-G.Choi, Guiry & G.W.Saunders was chosen for these experiments because it is a widespread species in the North Atlantic that was introduced from Asia (Mathieson *et al.*, 2008).

Based on this knowledge three hypotheses were defined: (i) temperature and elevated pCO_2 interact to influence photosynthetic performance and growth of the red alga *N. harveyi*; (ii) effects of temperature and pCO_2 on growth are directly related to the potential effects on photosynthesis; and (iii) elevated pCO_2 and low temperature interact to decrease the activity of the CCM.

Materials and methods

To address the research questions two experiments were subsequently performed. Experiment 1 addressed the effects of elevated pCO_2 and temperature on growth and photosynthesis of *N. harveyi*. Accordingly the photosynthetic characteristics were measured after cultivation (experimental settings described below) at the respective temperatures in sea water adjusted to treatment pCO_2. Experiment 2 aimed to show the effects of elevated pCO_2 and temperature on the activity of the CCM. Therefore, the photosynthetic characteristics

and the activity of the CCM were measured at different temperatures but always in sea water adjusted to low (present) pCO_2. This procedure was necessary because an expected down-regulation of the CCM following cultivation at high pCO_2 might be masked by faster diffusion of CO_2 into cells, which might occur if the experimental response of the CCM is measured at high pCO_2.

Algal material and culture conditions

For both experiments the experimental material was obtained from a stock culture obtained from isolated female thalli of *N. harveyi* from Heligoland, North Sea, and cultured at 10 °C and a photon fluence rate of 30 µmol m^{-2} s^{-1} until experimental use. During the experiment the thalli were cultured in 5 l beakers filled with filtered (0.2 µm) sea water (FSW) enriched with nutrients [Provasoli's enriched sea water (PES)], following a modified, buffer-free recipe of Provasoli (1968). The beakers were inoculated with 0.5 ± 0.1 g of fresh weight (FW) at the start of the experiment. One difference in the experimental settings between experiment 1 and 2 has to be stated. In experiment 1 the adjustment of the sea-water carbonate system (SWCS) was started with the inoculation of the beaker, whereas in experiment 2 the thalli were placed in sea water that had already been pre-aerated for 24 h with artificial air. Generally, the pH and other parameters of the SWCS were adjusted to treatment conditions by aerating the beakers with 0.5 l min^{-1} artificial air containing 20% O_2/80% N_2 and 380, 800, or 1500 µatm CO_2 representing present, expected future, and high pCO_2 conditions. The artificial air was generated by a gas-mixing device (HTK, Hamburg, Germany). Due to the aeration the thalli were continuously and gently circulating within the beakers. The PES was exchanged in both experiments every 3 or 4 days, with the new PES being aerated for 24 h with treatment air before exchange.

The photon fluence rate was set to 100 µmol m^{-2} s^{-1} photons at the bottom and 150 µmol m^{-2} s^{-1} photons at the surface of the PES in the beaker and supplied by white fluorescent lamps (Biolux; Osram, München, Germany). Preliminary experiments revealed that this irradiance is saturating for growth (data not shown). The experiment was performed in two temperature-controlled rooms at 10 ± 1.5 and 17.5 ± 1.5 °C. Pilot studies have shown that the relative growth rate (RGR) increased from 5 up to 17.5 °C and became stable between 17.5 and 25 °C. Accordingly, 17.5 °C was considered to be within the optimal temperature range of this species (data not shown).

The SWCS was controlled every 3–4 days according to Olischläger *et al.* (2012). The characteristics of the SWCS are presented in Table 1.

Photosynthesis

In experiment 1 the effective quantum yield ($\Delta F/F'_m$) was measured after 2 weeks of culture under different CO_2 and temperature conditions by use of an Imaging-PAM (Walz, Effeltrich, Germany). Thalli were examined in pre-aerated treatment water at 17.5 ± 1.5 or 10 ± 1.5 °C. Immediately after transfer $\Delta F/F'_m$ and a rapid photosynthesis-irradiance (P-E) curve were determined. For the rapid P-E curve the thalli were exposed to stepwise increases of actinic light (0–590 µmol photons m^{-2} s^{-1} at 400–800 nm provided by halogen lamp). To measure changes in $\Delta F/F'_m$ every 20 s a saturation pulse lasting 800 ms (~2000 µmol photons m^{-2} s^{-1}, 400–800 nm) was applied. After the saturation pulse the actinic light was set to the next level. Relative electron transport rates were calculated using equation 1:

$$\text{rel.ETR} = \frac{\Delta F}{F'_m} \cdot PAR \cdot 0.5 \quad (1)$$

rel.ETR is relative electron transport rate, PAR is photosynthetically active radiation, and 0.5 is the factor assuming an equal contribution between photosystem I and photosystem II.

P-E curves were calculated according to Jassby and Plat (1976) and maximal relative electron transport rate [rETR(max); µmol e^- m^{-2} s^{-1}] and photosynthetic efficiency (α; e^- $photons^{-1}$) were determined accordingly. The light saturation point, E_k (µmol photons m^{-2} s^{-1}), was calculated as rETR(max)/α.

In experiment 1 net O_2 production (net photosynthesis, net-PS) of the thalli was measured in magnetically stirred photosynthetic

Table 1. *Mean±standard deviation of parameters of the SWCS over the entire experimental period*

Experiment 1 at 10 °C: *n*=6 for the first 2 weeks and *n*=3 in the last week. Experiment 1 at 17.5 °C: *n*=6 over the entire experimental period. Experiment 2: *n*=4 at 10 and 17.5 °C over the entire experimental period. All replicates were analysed every 3–4 days prior to water exchange. n.d., not determined; SW, sea water; TA, total alkalinity. See text for details.

Treatment/ parameter	10 °C			17.5 °C		
	380 µatm pCO_2	800 µatm pCO_2	1500 µatm pCO_2	380 µatm pCO_2	800 µatm pCO_2	1500 µatm pCO_2
Experiment 1						
pH	8.06 ± 0.02	7.82 ± 0.02	7.57 ± 0.03	8.14 ± 0.03	7.86 ± 0.04	7.64 ± 0.06
pCO_2 (µatm)	410 ± 22	770 ± 38	1418 ± 85	348 ± 27	733 ± 64	1315 ± 144
CO_2 (µmol kg SW^{-1})	18 ± 1	34 ± 2	60 ± 4	12 ± 1	26 ± 2	46 ± 5
HCO_3^- (µmol kg SW^{-1})	2032 ± 22	2172 ± 16	2262 ± 13	1941 ± 47	2148 ± 26	2269 ± 44
CO_3^- (µmol kg SW^{-1})	145 ± 7	86 ± 4	53 ± 4	205 ± 9	118 ± 10	77 ± 11
Dissolved inorganic carbon (µmol kg SW^{-1})	2195 ± 19	2292 ± 15	2375 ± 14	2159 ± 45	2292 ± 27	2392 ± 46
TA (µmol kg SW^{-1})	2386 ± 18	2381 ± 14	2392 ± 15	2435 ± 40	2432 ± 33	2453 ± 58
Experiment 2						
pH	8.01 ± 0.03	n.d.	7.55 ± 0.02	8.06 ± 0.06	n.d	7.60 ± 0.03
pCO_2 (µatm)	473 ± 32	n.d.	1511 ± 77	439 ± 63	n.d.	1422 ± 80
CO_2 (µmol kg SW^{-1})	22 ± 1	n.d.	69 ± 3	15 ± 2	n.d.	50 ± 3
HCO_3^- (µmol kg SW^{-1})	2145 ± 57	n.d.	2344 ± 59	2051 ± 100	n.d.	2332 ± 79
CO_3^- (µmol kg SW^{-1})	125 ± 7	n.d.	46 ± 2	182 ± 19	n.d.	76 ± 6
Dissolved inorganic carbon (µmol kg SW^{-1})	2291 ± 51	n.d.	2460 ± 62	2249 ± 93	n.d.	2454 ± 84
TA (µmol kg SW^{-1})	2451 ± 62	n.d.	2458 ± 62	2493 ± 81	n.d.	2507 ± 88

chambers with a volume of 26 ml on days 15 and 16 of the experiment at 18.5 ± 1.5 and 12 ± 1.5 °C with micro-optodes and TX3 control units (PreSens, Regensburg, Germany). Net-PS was measured at a photon fluence rate of 110 µmol photons m^{-2} s^{-1}. The thalli were allowed to acclimate to the light conditions in the photosynthetic chamber for 5 min before the measurements of net-PS were started. The chambers were filled with CO_2-enriched FSW according to the culture conditions [mean pCO_2 were 440 ± 2, 849 ± 6, and 1482 ± 19 µatm (n=2) in the 10 °C treatment and 433 ± 8, 827 ± 6, and 1416 ± 1 µatm (n=2) in the 17.5 °C treatment at present, expected, and high pCO_2 values]. The mean pO_2 values at the start of the net-PS measurements were 0.24 ± 0.03, 0.24 ± 0.07, and 0.25 ± 0.02 atm at present, expected, and high pCO_2 at 18.5 °C and 0.22 ± 0.07, 0.23 ± 0.02, and 0.24 ± 0.02 atm in the respective pCO_2 treatments at 12 °C. After 10 min of a stable linear increase in the pO_2 the final pO_2 ranged between 0.28 and 0.41 atm at 18.5 °C and between 0.24 and 0.33 atm at 12 °C. Blank values without algae were determined for each treatment condition. Blanks were not significantly affected by the pCO_2 and were therefore pooled at each temperature. Thalli were weighted after the measurements to avoid the possibility that stress during the weighting procedure might affect the measurements. FW of the thalli was 0.4 ± 0.15 g.

CCM

In order to prove the presence of a CCM a pH-drift experiment was performed on day 24 of experiment 1. Thalli of 60 mg FW were placed into closed containers filled with 20 ml of FSW, sealed, and placed on a shaker. The relevance of the carbonic anhydrases and the change in their contribution to the carbon acquisition after cultivation at different pCO_2 values was addressed in a parallel experimental assay, which was performed in 20 ml of FSW with 0.2 mM ethoxyzolamide, an inhibitor of both exCA and internal carbonic anhydrase (intCA). The temperature was 15 ± 1 °C. The test tubes were continuously illuminated at 150 µmol photons m^{-2} s^{-1}. The pH was measured at the start and after 2, 4, 24, and 25.5 h. After 24 h the pH was constant. There were no significant differences in the pH measured at 24 and 25.5 h (P>0.05, t test). Therefore the measured pH values after 2, 4, and 24 h were used in the analysis of the pH-drift experiments. This experiment was performed with thalli cultured at 10 °C only.

In experiment 2 photosynthetic activity was measured in the same manner and experimental set-up as described for experiment 1. Measurements were performed at 10 ± 1.5 or 17.5 ± 1.5 °C on days 14 and 15 following cultivation at treatment pCO_2. Photosynthesis was measured for 10 min with and without exCA inhibitor, and with inhibitors of both exCA and DBL-acidification together, in FSW with a pCO_2 of 498 ± 24 µatm at 10 °C (n=2) and 462 ± 43 µatm (n=2) at 17.5 °C and a pO_2 of 0.22 ± 0.06 and 0.23 ± 0.08 atm, respectively. The final pO_2 ranged between 0.26 and 0.31 atm. After the determination of net-PS the medium was exchanged and exCA inhibited by adding acetazolamide (AZ; Sigma-Aldrich, Munich, Germany) from a stock solution of 20 mM AZ dissolved in 50 mM NaOH to a final concentration of 0.1 mM AZ. Subsequently, the thalli were rinsed with FSW and the photosynthetic chamber was filled with fresh medium. Again AZ was added and additionally Tris buffer [Tris-(hydroxymethyl)-aminomethanhydrochloride; Carl Roth, Karlsruhe, Germany] was added to a final concentration of 50 mM from a stock solution of 2 M Tris, with pH adjusted to pH 8.7. Tris buffer inhibits the acidification of the DBL by H^+ extrusion (Mercado *et al.*, 2006). The inhibitor concentrations were chosen according to Moulin *et al.* (2011).

Growth

Thalli were cultured for 24 days. At the beginning of the experiment thalli were gently blotted with tissue paper and weighed (LA 310S; Sartorius, Göttingen, Germany). The inoculum was 0.5 ± 0.1 g FW. Measurements were repeated after 2 weeks. On day 17 the biomass in the beakers was reduced to 1 g FW to prevent biomass effects on the treatment conditions and to gain a better comparison of growth rates of thalli in the CO_2-treatment-acclimated state. Due to lower biomass in the 10 °C treatment and use and storage of algal material for further analysis it was necessary to pool two replicates to reach 1 g FW. The RGR (% day^{-1}) of the thalli was calculated according to Lüning (1990) using equation 2:

$$\mathrm{RGR} = \frac{100 * \ln\left(\frac{FW_1}{FW_2}\right)}{T_2 - T_1} \quad (2)$$

FW_1 and FW_2 are FW in grams at times 1 and 2, respectively; T_1 and T_2 are time in days.

Statistics

Percentage data were arcsin transformed prior to statistical analysis as recommended by Sokal and Rohlf (1995). Unpaired t tests were used for direct comparisons of pairs of data sets (P<0.05). Two-factor designs (CO_2 and temperature) were analysed using two-way analysis of variance (ANOVA; P<0.05). The homogeneity of variances was confirmed using the Cochrans test (P<0.05). *Post hoc* comparisons were performed by Fisher's LSD test (P<0.05). Parameters that were repeatedly measured were analysed with a repeated-measurement ANOVA (RM-ANOVA) and subsequent *post hoc* analysis by Fisher's LSD test (P<0.05). The analyses were performed using Statistica software version 7 (StatSoft, Tulsa, OK, USA).

Results

CO_2 and temperature affected both the growth and the photosynthetic performance of *N. harveyi* and some interactive effects were revealed.

Chlorophyll fluorescence

$\Delta F/F'_m$ was significantly influenced by the CO_2 treatments (P<0.001, two-way ANOVA) but not by temperature (P>0.05, two-way ANOVA; Fig. 1a). At 10 and 17.5 °C $\Delta F/F'_m$ rose with increasing pCO_2 (Fig. 1a) and the $\Delta F/F'_m$ values measured at 1500 µatm pCO_2 at both temperatures were significantly different from those at both lower CO_2 conditions (P<0.05, Fisher's LSD test; Fig. 1a).

Overall temperature, CO_2 treatment, and their interaction significantly influenced α (all P<0.001, two-way ANOVA). At 10 °C α (e^- photons $^{-1}$) rose significantly with each tested pCO_2 (P<0.05, Fisher's LSD test), whereas at 17.5 °C it was not affected by pCO_2 (P>0.05, Fisher's LSD test). Remarkably, there was no significant difference between the α measured at 1500 µatm pCO_2 and 10 °C and the values obtained for the same pCO_2 at 17.5 °C (P>0.05, Fisher's LSD test; Fig. 1b).

The E_k values (µmol photons m^{-2} s^{-1}) were significantly influenced by temperature, pCO_2, and the interaction of temperature and pCO_2 (all P<0.05, two-way ANOVA; Fig. 1c). At 10 °C the E_k values of the thalli cultivated at 380 µatm pCO_2 were significantly higher than the E_k values at 800 and 1500 µatm pCO_2 at 10 and 17.5 °C (P<0.05, Fisher's LSD

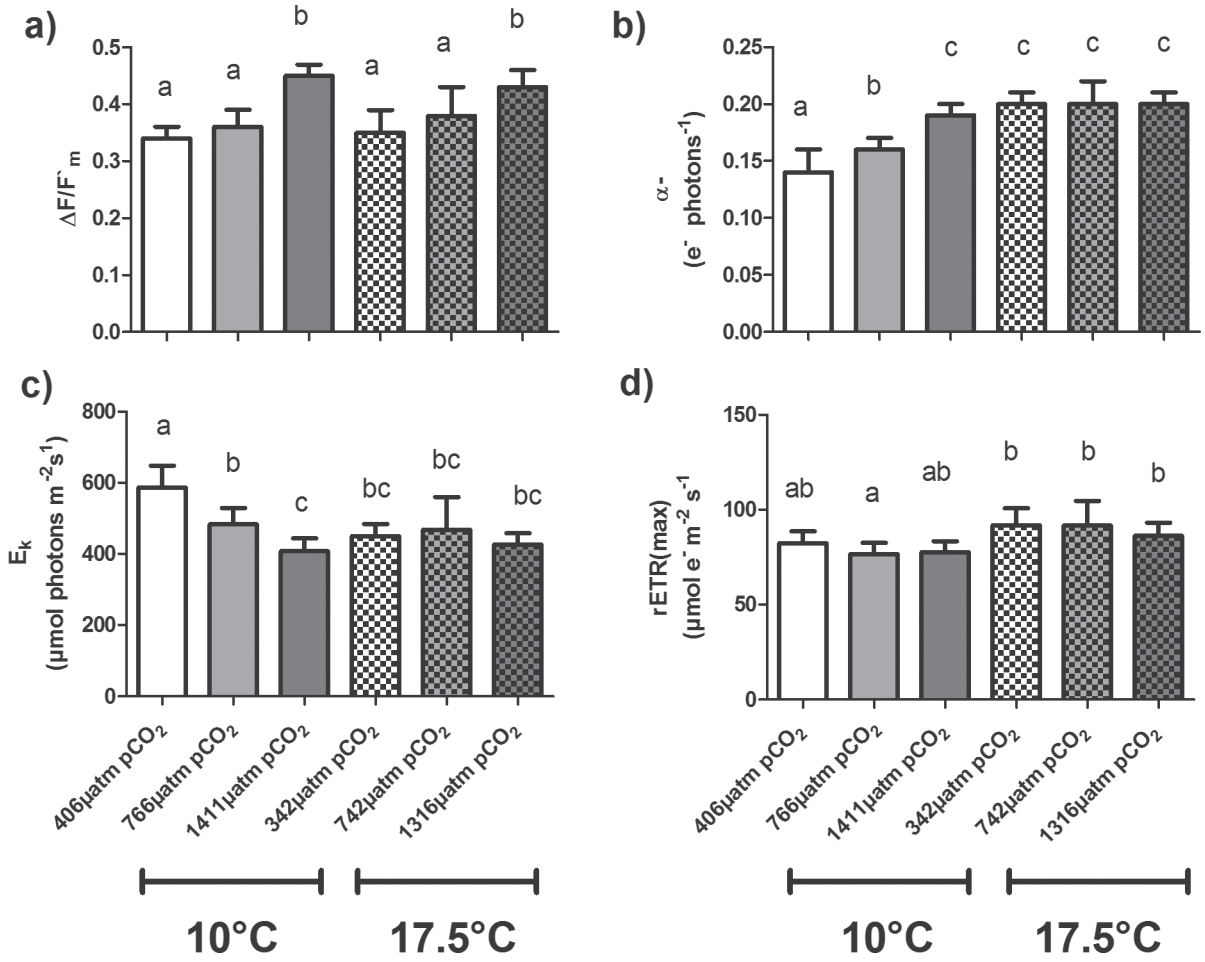

Fig. 1. Chlorophyll *a* fluorescence characteristics of *N. harveyi*. Means+SD (n=6) are shown, measured submerged in accordance with the treatment characteristics in aerated sea water after 2 weeks of cultivation at the indicated pCO_2 and temperature: (a) $\Delta F/F'_m$; (b) α; (c) E_k; (d) rETR(max). Squared pattern indicates the 17.5 °C treatment. Different letters indicate significant differences revealed by *post hoc* comparisions with Fisher's LSD test.

test), whereas at 17.5 °C no significant differences between the E_k values of the different CO_2 treatments were found (P>0.05, Fisher's LSD test).

rETR(max) (µmol e^- m^{-2} s^{-1}) was significantly affected by temperature (P<0.01, two-way ANOVA) but not by pCO_2 or the interaction of temperature and pCO_2 (P>0.05, two-way ANOVA; Fig. 1d). Values at 17.5 °C were generally higher than at 10 °C, although significant differences were only found at the intermediate CO_2 level.

O_2 evolution

In experiment 1 net-PS measured at growth-saturating irradiances was significantly influenced by temperature, pCO_2, and the interaction of the two (all P<0.05, two-way ANOVA). The net O_2 production of thalli cultured at 10 °C rose significantly with increasing pCO_2 (P<0.05, Fisher LSD-test; Fig. 2). The lower the CO_2 level the higher the temperature-enhancing effect, the value being lowest when measured at low CO_2 after cultivation at 10 °C and the highest after cultivation at 17.5 °C and 1500 µatm pCO_2. This was statistically different from all other measured net-PS, except from the values measured with thalli cultured at the same pCO_2 at 10 °C (P>0.05, Fisher's LSD test).

CCM

The final pH values of the pH-drift experiments were pH 9.3±0.1 and 9.4±0.1 (mean±SD) for thalli cultured at present and high pCO_2, respectively (Fig. 3a). Accordingly, *N. harveyi* has a CCM of low effectiveness (Maberly, 1990). The outcome of the pH-drift experiment in FSW was significantly influenced by CO_2 treatment, time, and the interaction of both factors (all P<0.05, RM-ANOVA). Thalli cultivated at 1500 µatm pCO_2 caused significantly higher pH values after 2 and 4 h (P<0.05, Fisher's LSD test). The values obtained after 24 h were not significantly different between CO_2 treatments (P>0.05, Fisher's LSD test).

The addition of the exCA and intCA inhibitor ethoxyzolamide lowered the starting pH by 0.18. If exCA and intCA were inhibited, the CO_2 cultivation and time alone and their interaction significantly influenced the course of the pH values (P<0.05, RM-ANOVA). Values after 2 and 4 h were significantly higher following high-pCO_2 cultivation (P<0.05,

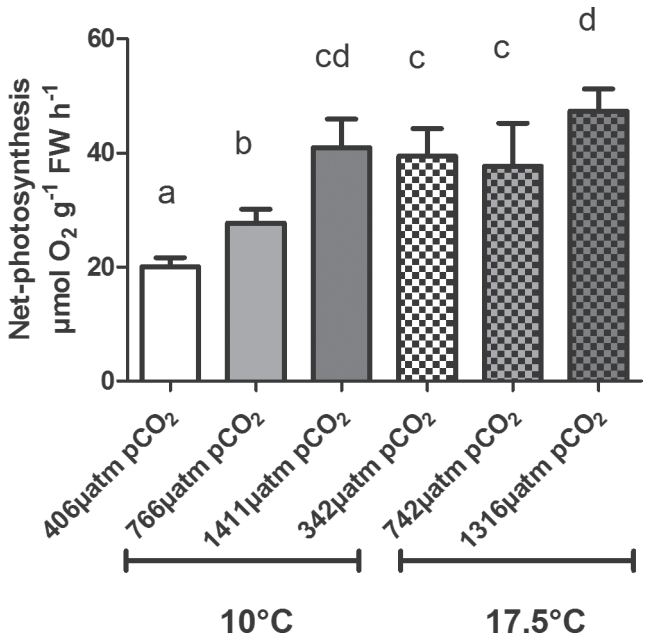

Fig. 2. Net-PS of *N. harveyi* (means+SD, *n*=4–5) measured after 15 and 16 days after cultivation at the indicated pCO_2 and temperatures. Squared pattern indicates cultivation at 17.5 °C. Net-PS measurements were performed at 12 ± 1.5 and 18.5 ± 1.5 °C. Different letters indicate significant differences revealed by *post hoc* comparisions with Fisher's LSD test. For potential further data interpretation the FW/chlorophyll *a* ratios are presented as supplementary material (Fig. S2).

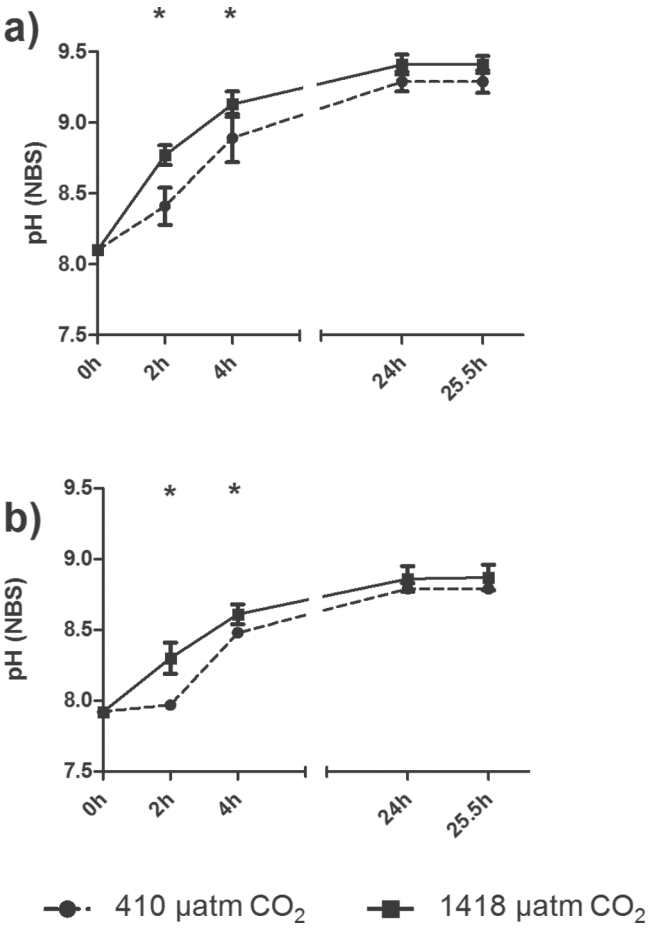

Fig. 3. Effects of cultivation at low and high pCO_2 in pH-drift experiments performed with *N. harveyi*. (a) pH values (means±SD, *n*=3) measured in the pH-drift experiments performed in sea water with algae cultivated at 410 or 1418 µatm pCO_2, at the indicated times. (b) pH values (means±SD, *n*=3) of measurements performed in sea water with 0.2 mM ethoxyzolamide. Significant differences revealed by *post hoc* comparisions with Fisher's LSD test are marked with asterisks. NBS, National Bureau of Standards.

Fisher's LSD test; Fig. 3b). The insignificantly different final pH values after addition of ethoxyzolamide were pH 8.8 ± 0.03 and 8.9 ± 0.09 (mean±SD) respectively for thalli cultivated at present and high pCO_2, respectively (P>0.05, Fisher's LSD test).

The inhibitor studies of experiment 2 revealed that the contribution of the exCA to the photosynthetic carbon supply was significantly higher at 17.5 °C (P<0.05, two-factorial ANOVA; Fig. 4) and that exCA is not involved in carbon acquisition at 10 °C. This temperature effect was also evident in an experiment in which exCA and the DBL acidification were inhibited simultaneously (P<0.05, two-factorial ANOVA). The inhibiting effect of TRIS and AZ was stronger compared to that achieved by AZ alone (P>0.05, t-tests at both temperatures). However, the cultivation at different pCO_2 did not significantly affect the contribution of exCA and DBL acidification to net-PS (P>0.05, two-factorial ANOVA) nor was a significant interaction of cultivation pCO_2 and temperature on the carbon acquisition verifiable (P>0.05, two-factorial ANOVA).

Growth

During the first 2 weeks the RGRs were significantly influenced by temperature, pCO_2, and the interaction of both (all P<0.05, two-way ANOVA). At 10 and 17.5 °C the RGRs at present pCO_2 did not significantly differ (P>0.05, Fisher's LSD test) from the RGRs at expected future pCO_2 but both the RGRs of present and expected future pCO_2 were significantly lower than the RGRs at high pCO_2 (P<0.05, Fisher's LSD test; Fig. 5a). Irrespective of pCO_2 treatment, all measured growth rates at 10 °C were significantly lower than their counterparts at 17.5 °C (P<0.05, Fisher's LSD test) but the RGR measured at high pCO_2 at 10 °C was not significantly different from the RGR measured at present and expected future pCO_2 at 17.5 °C (P>0.05, Fisher's LSD test).

In the last week of the experiment (days 17–24) the RGRs were significantly affected by pCO_2, temperature, and their interaction (P<0.05, two-way ANOVA). The RGRs at present pCO_2 and 10 °C were significantly lower than the RGRs at expected future and high pCO_2 (P<0.05, Fisher's LSD test; Fig. 5b). There was no significant difference (P>0.05, Fisher's LSD test) between the RGRs for days 17–24 measured at high CO_2 and 10 °C and the RGRs measured at present and expected future pCO_2 at 17.5 °C. The RGR obtained at high pCO_2 and 17.5 °C was significantly different from all others

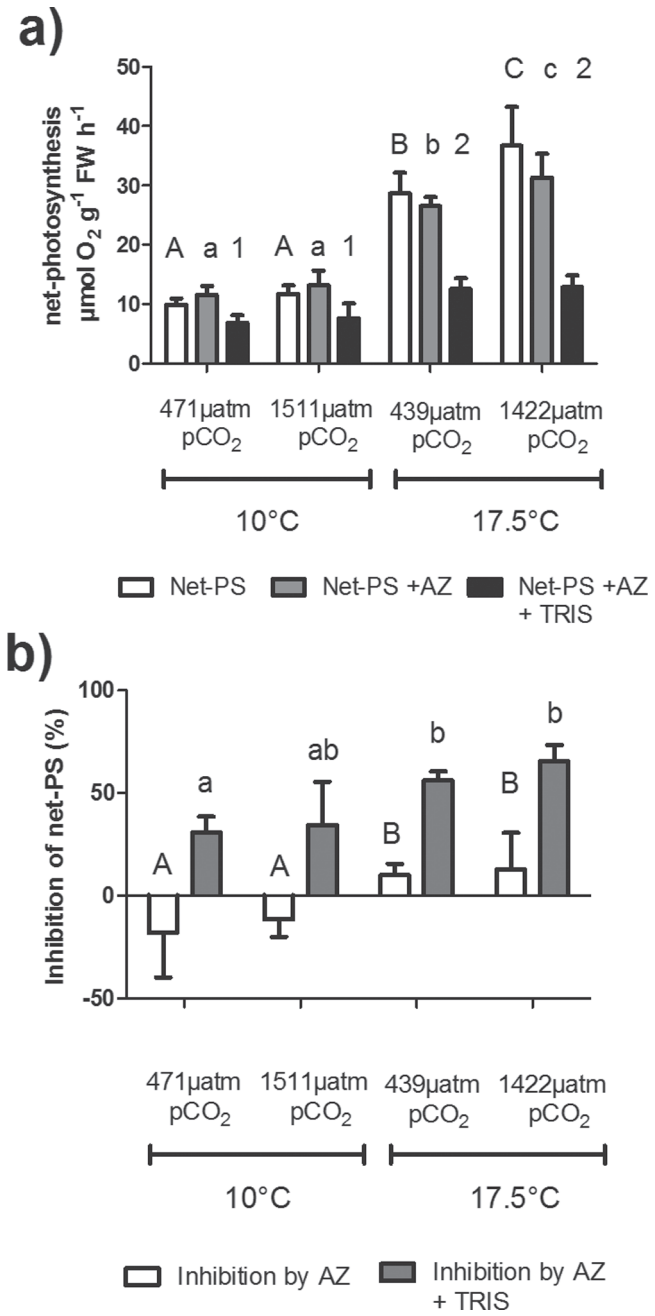

Fig. 4. (a) Net-PS of *N. harveyi* (means+SD, *n*=3–4) measured in sea water adjusted to present pCO_2 after 2 weeks of cultivation at the indicated pCO_2 and temperatures without and with addition of AZ and AZ+Tris. (b) Effect of the addition of AZ and AZ+Tris expressed in percentage values of the measured net-PS. Significant differences revealed by *post hoc* comparisions with Fisher's LSD test are presented in (a) by capital letters for net-PS, by small letters for net-PS+AZ, and by numbers for net-PS+AZ+Tris; in (b) capital letters indicate significant differences revealed with Fisher's LSD test for the inhibition of net-PS by AZ and small letters indicate significant differences in the inhibitive effect of AZ+Tris.

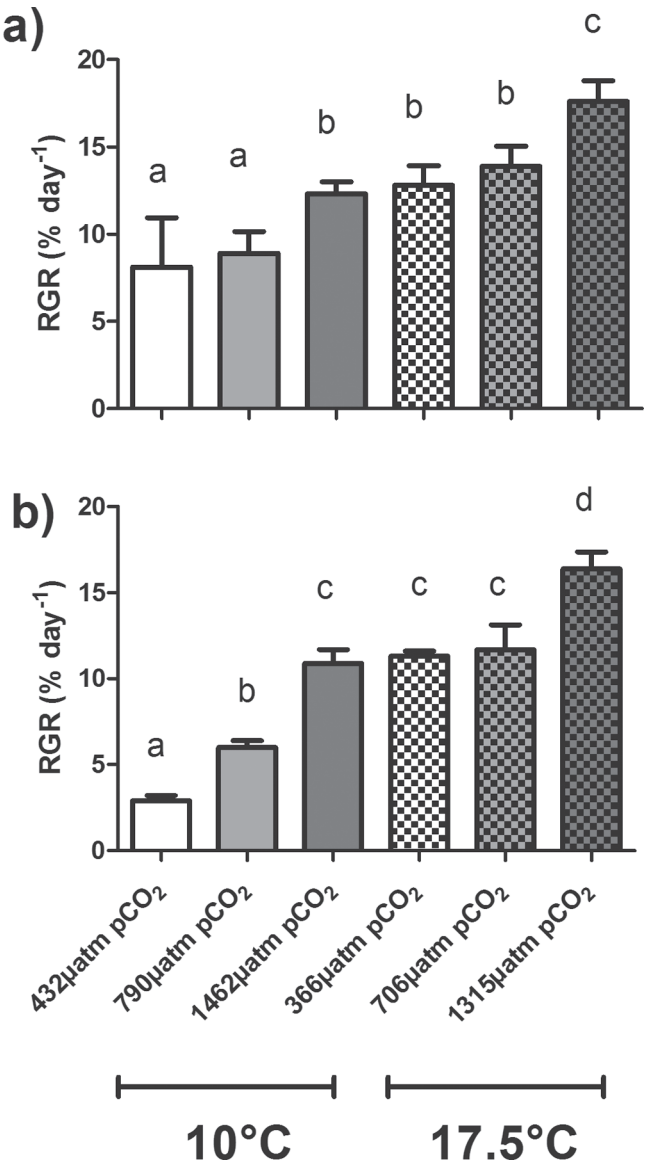

Fig. 5. RGRs (means+SD, *n*=6) of *N. harveyi* during (a) 14 days of cultivation under indicated pCO_2 and temperatures (*n*=6) and (b) between day 17 and 24 of cultivation (*n*=6 at 17.5 °C and *n*=3 at 10 °C) under the indicated pCO_2 and temperatures. Squared pattern indicates the 17.5 °C treatment. Different letters indicate significant differences revealed by *post hoc* comparision with Fisher's LSD test.

in the last week of the experiment ($P<0.05$, Fisher's LSD test). Regarding the comparison of RGRs at the beginning and the end of the experimental period, there was a reduction in RGR at intermediate and low CO_2 at 10 °C, whereas the rest remained at similar values, indicating that both CO_2 and temperature were responsible for long-lasting, actively growing thalli.

Discussion

It was shown that elevated pCO_2 supports the photosynthesis ($\Delta F/F'_m$, α, E_k, net-PS) and growth of *N. harveyi* alone and/or interactively with temperature. The beneficial effects of elevated pCO_2 were more pronounced at low temperatures. At high pCO_2 no temperature effect was evident in the photosynthetic characteristics. The pH-drift experiments indicate

the presence of a CCM and show that the importance of intCA for the functioning of photosynthesis decreases following cultivation at high pCO_2. Furthermore, the inhibitor studies revealed that the contribution to carbon acquisition for the photosynthetic carbon supply by both exCA and DBL acidification was higher at 17.5 °C, but was independent of the cultivation pCO_2.

The $\Delta F/F'_m$ benefitted from high pCO_2 but not from elevated temperature and the interaction of temperature with pCO_2 (Fig. 1a). Hence, thalli grown at high pCO_2 can use light more efficiently. The missing effect of temperature on $\Delta F/F'_m$ is explainable by the insensitivity of light absorption, excitation, and energy transfer with respect to temperature (Raven and Geider, 1988). The absence of interactions between temperature and future pCO_2 on $\Delta F/F'_m$ has been reported previously for the diatom *N. directa* (Torstensson *et al.*, 2012).

In contrast, α shows a temperature-dependent response (Fig. 1b). A temperature effect on α was demonstrated for *E. huxleyi* cultivated at low light (Feng *et al.*, 2008) (obtained with P-E curves related per chlorophyll *a*), for the cyanobacterium *Synechococcus* sp. (Fu *et al.*, 2007), the raphidophyte *Heterosigma akashiwo*, and the dinoflagellate *P. minimum* (Fu *et al.*, 2008). However, whether α is sensitive to temperature might be dependent on further environmental constraints. The α of low-light-adapted *E. huxleyi* is temperature-dependent, whereas at saturating light there was no temperature dependence detectable (Feng *et al.*, 2008). Accordingly, temperature sensitivity of α can be dependent on the prevailing irradiance. Moreover, it could be demonstrated that carbon availability is an additional factor that can influence α (Fig. 1b).

The data of the present study demonstrate that the effects of pCO_2 on α are dependent on temperature (Fig. 1b). That pCO_2 effects on α values were influenced by temperature was previously shown for *E. huxleyi* cultivated at low light, for *Synechococcus* sp., and for *H. akashiwo* (Fu *et al.*, 2007, 2008; Feng *et al.*, 2008). Clearly, α is not affected by pCO_2 at 17.5 °C, whereas pCO_2 has a strong effect at lower sub-optimal temperatures. The conclusion is that elevated pCO_2 has no effect on α if a species is cultivated within its optimal temperature range and that elevated pCO_2 can compensate for sub-optimal temperature effects.

An unchanged α in response to OA as presented here at 17.5 °C (Fig. 1b) is in agreement with findings obtained with the diatom *Phaeodactylum tricornutum* Bohlin and the red alga *Hypnea spinella* (C.Agardh) Kützing (Wu *et al.*, 2010; Suárez-Àlvarez *et al.*, 2012). In general, α reflects the energetic costs of photosynthesis (Wu *et al.*, 2010). Accordingly, the findings suggest that future pCO_2 does not influence the cost for photosynthesis at 17.5 °C treatment, whereas at low temperatures elevated pCO_2 decreases the costs of photosynthesis. Furthermore, Fu *et al.* (2007) postulated that an increased α at elevated pCO_2 is attributed to a lower energy demand of the carbon acquisition and more efficient light use. The presented findings support the conclusions of Fu *et al.* (2007) since a decreased contribution of intCA is indicated by the pH-drift experiments (Fig. 3b). However, the external carbon acquisition by DBL acidification and exCA was not affected by pCO_2, irrespective of temperature (Fig. 4).

The hypothesis that increasing pCO_2 compensates for temperature effects is further supported by the E_k values (Fig. 1c). These were significantly lower with increasing pCO_2 at 10 °C only. At the light saturation point (E_k value) the temperature-independent photosynthetic energy capture and the temperature-dependent capacity of the photosynthetic system to process this energy are balanced (Falkowski and Raven, 1997). According to Raven and Geider (1988) algae tend to keep the ratio between temperature-independent light absorption and temperature-sensitive processes constant, thus avoiding photoinhibition. The E_k values obtained at 10 °C and high pCO_2 are comparable to those obtained at 17.5 °C, irrespective of pCO_2 treatment (Fig. 1c). Again, this pattern mirrors the physiological response reported for *Synechococcus* sp. (Fu *et al.*, 2007) and *E. huxleyi* grown under low light conditions (Feng *et al.*, 2008). However, *E. huxleyi* responded also with decreasing E_k values to future pCO_2 at higher temperatures (Feng *et al.*, 2008). On the other hand, increasing E_k values as response to OA were reported for *H. spinella* and were explained by a stimulation of the maximal photosynthetic capacity (Suárez-Àlvarez *et al.*, 2012), which was not recorded here (Fig. 1d). The temperature effect on rETR(max) of PSII is significant but not very pronounced and the pCO_2 effect on rETR(max) was insignificant (Fig. 1d). This indicates that a slightly lower maximal amount of light could be processed at low temperatures. An unchanged ETR(max) as a response to increased pCO_2 was previously reported in *Phaeodactylum tricornutum* (Wu *et al.*, 2010).

The overall conclusion from chlorophyll fluorescence data is that temperature and pCO_2 are of minor importance for the maximal photosynthetic capacity in algae cultivated at saturating irradiance for growth (Fig. 1d) but that high pCO_2 can enhance the effectiveness of light harvesting and processing at sub-optimal temperatures (Fig. 1a, b), since at high pCO_2 fewer photons are needed to reach E_k (Fig. 1c). This indicates that fewer electrons have to be spent in side reactions of the photosynthesis involving PSII, such as pseudocyclic phosphorylation. Remarkably, the acclimation to elevated CO_2 at low temperatures revealed a similar pattern to the low-light adaptation of the *Cyclotella* type [increased α, similar rETR(max); Sommer, 2005]. Hence, photosynthetic characteristics might be regulated under sub-optimal conditions by the carbon availability in a comparable manner to that known from the light-harvesting side.

The pCO_2-compensation effect of temperature on processes at PSII was also reflected in the net-PS. C_3 plants respond to increased pCO_2 if the net-PS is controlled by the regeneration capacity of ribulose 1,5-bisphosphate, i.e. under conditions when photorespiration occurs (Sage *et al.*, 2007). In such cases the sensitivity of the net-PS to pCO_2 reflects the competition of O_2 and CO_2 for ribulose 1,5-bisphosphate due to the competing oxygenase and carboxylase activity of Rubisco (Sage *et al.*, 2007). Hence, photorespiration decreases with increasing pCO_2 but increases with rising temperature (Sage *et al.*, 2007). Accordingly, higher photorespiration at 17.5 °C and present pCO_2 would be expected. However, the rate of diffusion through water adjacent to the photosynthetic thalli is several orders of magnitude lower than in air (Maberly, 1990).

Therefore, in algae operating a CCM the mitigation of photorespiration at low temperatures might be complicated by the temperature requirements of the CCM enzymes. It can be demonstrated that the contribution of external carbon acquisition to photosynthesis clearly decreases at low temperature (Fig. 4b). This is in accordance with previous findings by Shiraiwa and Miyachi (1985) and Wu *et al.* (2011), who showed that in the cyanobacterium *Microcystis* and eukaryotic microalga *Chlorella vulgaris* (Beyerinck) carbonic anhydrase activity and the affinity for dissolved inorganic carbon increased at higher temperatures. Furthermore, in *Macrocystis pyrifera* (Linnaeus) C.Agardh carbonic anhydrase activity increased in response to above-ambient temperatures at two out of three stations along a latitudinal gradient in Chile (Rothäusler *et al.*, 2011). Accordingly, in *N. harveyi* low temperatures could have caused greater photorespiration due to inhibition of the CCM. This might be reflected in the lower net-PS measured at present pCO_2 and low temperatures (Fig. 2). Elevated pCO_2 could have overcome this effect by favouring the diffusive entry of CO_2 into the chloroplast, resulting in a higher steady-state CO_2 concentration around Rubisco, as would have been reached by the CCM under optimal temperature conditions (Hepburn *et al.*, 2011). On the other hand, Raven *et al.* (2002) predicted that low temperatures decrease the need to operate a CCM due to a higher concentration of dissolved pCO_2 in sea water, which favours faster passive diffusion of CO_2 into the chloroplast. Gordillo *et al.* (2006) measured extraordinarily high exCA activity in a number of polar macroalgae and concluded that in arctic algae high levels of exCA are part of the evolutionary strategy to cope with low arctic temperatures. Our findings support Raven *et al.* (2002) and Gordillo *et al.* (2006). The presented results prove a reduced contribution of the CCM to photosynthetic carbon demand at low temperatures (Fig. 4). This finding supports Raven *et al.* (2002). However, the enrichment of pCO_2 increases the photosynthetic performance strongly (Fig. 2), indicating carbon limitation and/or high photorespiration. It should be considered that *N. harveyi* grows faster under warm conditions (Olischläger and Wiencke, unpublished data) and hence is a species adapted to warm temperatures. Therefore, it might not be well prepared to cope with low temperatures. Accordingly, it is reasonable to assume that *N. harveyi* is not able to express exCA in the quantities needed to cope with low temperatures, as expected by Gordillo *et al.* (2006), and therefore benefits from elevated pCO_2 at low temperatures.

Acclimation to low temperature is energetically costly (Raven and Geider, 1988). This is reflected in the lower RGRs of *N. harveyi* at 10 °C (Fig. 5). The RGRs of *N. harveyi* reflect the pattern described for the photosynthetic response. However, the photosynthetic response measured after 14 days is more reflected in the growth rate measured between day 17 and 24. In this week the RGRs decreased compared to the RGRs measured between day 1 and 14. Potentially, in the experiment the impact of low temperature was not fully pronounced at the first part of the experiment but became more severe with time.

Physiologically, the beneficial effect of pCO_2 at 10 °C on growth can be explained by a reduction of photorespiration (Hepburn *et al.*, 2011), potentially combined with a lower expression of intCA (Fig. 3b). Both ways could have saved energy in a synergistic manner and be responsible for the maintenance of higher RGRs at high pCO_2 and low temperature. The energy to drive the CCM is suggested to be obtained from mitochondrial ATP (Klenell *et al.*, 2004), and cyclic or pseudocyclic photophosphorylation (Sültemeyer *et al.*, 1993; Giordano *et al.*, 2005). The lower E_k indicates that *N. harveyi* needs fewer photons to reach its maximal photosynthetic level, which could indicate decreased pseudocyclic photophosphorylation. Furthermore, decreased pseudocyclic photophosphorylation and decreased photorespiration would lower the rate of reactive oxygen species (ROS) production (Lesser, 2006). Hence, in *N. harveyi* elevated pCO_2 could have prevented the formation of ROS, as has been shown for the limnic microalga *Peridinium gatunense* (Vardi *et al.* (1999). ROS are harmful for photosynthetic organisms; they are detoxified by antioxidants and/or ROS-detoxifying enzymes (Lesser, 2006). The increased availability of carbon can be responsible for a decreased need of the ROS-detoxifying enzymes (Sültemeyer *et al.*, 1993), the activity of which is known to be temperature-dependent (Choo *et al.*, 2004). The conclusion is that damage generated by ROS could have been more severe under carbon limitation and low temperatures.

At optimal temperatures the situation might be different. Accelerated metabolic activity, photosynthesis, and growth are common responses in marine phytoplankton to moderately enhanced temperatures (Falkowski and Raven, 1997). At 17.5 °C the stimulatory effect of increasing pCO_2 decreases, and only high pCO_2 caused significantly elevated photosynthesis and growth. One explanation for the reduced effect of elevated pCO_2 on *N. harveyi* at 17.5 °C might be that the CCM increases the CO_2 concentration around Rubisco to a level at which photorespiration is almost completely avoided. The further enhancement of growth at high pCO_2 might have been caused by reduced operation costs of the CCM and/or by a relief of carbon limitation. As mentioned previously, down-regulation of the CCM is indicated by lower sensitivity to ethoxyzolamide recorded for thalli grown at high pCO_2, which increased the pH of the medium faster than the thalli cultivated at present pCO_2. The faster pH increase of the medium persisted after exCA and intCA inhibition. This indicates that the improved photosynthetic capacity is at least partly due to reorganization of the algal pigmentation, which is reflected in the higher $\Delta F/F'_m$ value and the tendency towards higher chlorophyll *a* content of thalli cultivated at high pCO_2 (Figs S1 and S2). Fu *et al.* (2007) reported similar results for *Synechococcus* and additionally showed higher phycocyanin and phycoerythrin contents. In conclusion, in *N. harveyi* grown at high pCO_2 a higher fraction of the incident photons was absorbed and this in turn might explain the faster pH increase.

The finding that elevated pCO_2 mitigates low-temperature effects on *N. harveyi* is ecologically relevant. Temperature is believed to be one of the crucial factors for the biogeographical distribution of seaweeds (Lüning, 1990). Hence, in an acidified ocean this invasive species could potentially prolong its growth period and expand its biogeographical distribution

towards areas that are currently outside its thermal range. But this also depends on other factors, such as successful sexual reproduction, resistance to grazing, and high competitiveness.

Supplementary material

Supplementary material is available at *JXB* online.

The effects of pCO_2 and temperature on chlorophyll *a* content and the FW/chlorophyll *a* ratio of the thalli are available online as supplementary material (Figs S1 and S2).

Acknowledgements

We wish to thank Francisco Gordillo for very helpful comments on an earlier version of the manuscript and Andreas Wagner and Claudia Daniel for assistance with lab work. This work was supported by the German Federal Ministry for Science and Education (BMBF; Förderkennzeichen 03F0608B) as part of the BIOACID-program (subproject 4.1.1).

References

Choo K-s, Snoeijs P, Pedersén M. 2004. Oxidative stress tolerance in the filamentous green algae *Cladophora glomerata* and *Enteromorpha ahlneriana*. *Journal of Experimental Marine Biology and Ecology* **298,** 111–123.

Connell SD, Russell BD. 2010. The direct effects of increasing CO_2 and temperature on non-calcifying organisms: increasing the potential for phase shifts in kelp forests. *Proceedings of the Royal Society B Biological Sciences* **277,** 1409–1415.

Davison IR. 1991. Environmental-effects on algal photosynthesis - temperature. *Journal of Phycology* **27,** 2–8.

Doney SC, Fabry VJ, Feely RA, Kleypas JA. 2009. Ocean acidification: the other CO_2 problem. *Annual Review of Marine Science* **1,** 169–192.

Falkowski PG, Raven JA. 1997. *Aquatic Photosynthesis* . Oxford: Blackwell Science.

Feng Y, Warner ME, Zhang Y, Sun J, Fu FX, Rose JM, Hutchins DA. 2008. Interactive effects of increased pCO_2, temperature and irradiance on the marine coccolithophore *Emiliania huxleyi* (Prymnesiophyceae). *European Journal of Phycology* **43,** 87–98.

Fu FX, Warner ME, Zhang YH, Feng YY, Hutchins DA. 2007. Effects of increased temperature and CO_2 on photosynthesis, growth, and elemental ratios in marine *Synechococcus* and *Prochlorococcus* (Cyanobacteria). *Journal of Phycology* **43,** 485–496.

Fu FX, Zhang YH, Warner ME, Feng YY, Sun J, Hutchins DA. 2008. A comparison of future increased CO_2 and temperature effects on sympatric *Heterosigma akashiwo* and *Prorocentrum minimum*. *Harmful Algae* **7,** 76–90.

Giordano M, Beardall J, Raven JA. 2005. CO_2 concentrating mechanisms in algae: mechanisms, environmental modulation, and evolution. *Annual Review of Plant Biology* **56,** 99–131.

Gordillo FJL, Aguilera J, Jimenez C. 2006. The response of nutrient assimilation and biochemical composition of Arctic seaweeds to a nutrient input in summer. *Journal of Experimental Botany* **57,** 2661–2671.

Gordillo FJL, Niell FX, Figueroa FL. 2001. Non-photosynthetic enhancement of growth by high CO_2 level in the nitrophilic seaweed *Ulva rigida* C. Agardh (Chlorophyta). *Planta* **213,** 64–70.

Hepburn CD, Pritchard DW, Cornwall CE, McLeod RJ, Beardall J, Raven JA, Hurd CL. 2011. Diversity of carbon use strategies in a kelp forest community: implications for a high CO2 ocean. *Global Change Biology* **17,** 2488–2497.

Holbrook GP, Beer S, Spencer WE, Reiskind JB, Davis JS, Bowes G. 1988. Photosynthesis in marine macroalgae - evidence for carbon limitation. *Canadian Journal of Botany Revue Canadienne De Botanique* **66,** 577–582.

Israel A, Hophy M. 2002. Growth, photosynthetic properties and Rubisco activities and amounts of marine macroalgae grown under current and elevated seawater CO_2 concentrations. *Global Change Biology* **8,** 831–840.

Jassby AD, Platt T. 1976. Mathematical formulation of relationship between photosynthesis and light for phytoplankton. *Limnology and Oceanography* **21,** 540–547.

Klenell M, Snoeijs P, Pedersen M. 2004. Active carbon uptake in *Laminaria digitata* and *L. saccharina* (Phaeophyta) is driven by a proton pump in the plasma membrane. *Hydrobiologia* **514,** 41–53.

Lesser MP. 2006. Oxidative stress in marine environments: biochemistry and physiological ecology. *Annual Review of Physiology* **68,** 253–278.

Lüning K. 1990. *Seaweeds. Their Environment Biogeography and Ecophysiology*. Wiley-Interscience, New York, pp 360–364.

Maberly SC. 1990. Exogenous sources of inorganic carbon for photosynthesis by marine macroalgae. *Journal of Phycology* **26,** 439–449.

Mathieson AC, Pederson JR, Neefus CD, Dawes CJ, Bray TL. 2008. Multiple assessments of introduced seaweeds in the Northwest Atlantic. *Ices Journal of Marine Science* **65,** 730–741

Mercado JM, Andria JR, Perez-Llorens JL, Vergara JJ, Axelsson L. 2006. Evidence for a plasmalemma-based CO_2 concentrating mechanism in *Laminaria saccharina*. *Photosynthesis Research* **88,** 259–268.

Moulin P, Andria JR, Axelsson L, Mercado JM. 2011. Different mechanisms of inorganic carbon acquisition in red macroalgae (Rhodophyta) revealed by the use of TRIS buffer. *Aquatic Botany* **95,** 31–38.

Olischläger M, Bartsch I, Gutow L, Wiencke C. 2012. Effects of ocean acidification on different life-cycle stages of the kelp *Laminaria hyperborea* (Phaeophyceae). *Botanica Marina* **55,** 511–525.

Olischläger M, Bartsch I, Gutow L, Wiencke C. 2013. Effects of ocean acidification on growth and physiology of *Ulva lactuca* (Chlorophyta) in a rockpool-scenario. *Phycological Research* **61,** 180–190.

Orr JC, Caldeira K, Fabry V *et al.* 2009. Research priorities for understanding ocean acidification. Summary from the second

symposium on the Ocean in a High-CO_2 World. *Oceanography* **22,** 182–189.

Provasoli L. 1968. *Media and prospects for the cultivation of marine algae*. Cultures and collections of algae. Proceedings of the U.S.-Japan conference, Hakone 1966. Japanese Society for Plant Physiology, Tokyo, pp 63–75.

Raven JA, Geider RJ. 1988. Temperature and algal growth. *New Phytologist* **110,** 441–461.

Raven JA, Giordano M, Beardall J, Maberly SC. 2011. Algal and aquatic plant carbon concentrating mechanisms in relation to environmental change. *Photosynthesis Research* **109,** 281–296.

Raven JA, Giordano M, Beardall J, Maberly SC. 2012. Algal evolution in relation to atmospheric CO_2: carboxylases, carbon-concentrating mechanisms and carbon oxidation cycles. *Philosophical Transactions of the Royal Society B Biological Sciences* **367,** 493–507.

Raven JA, Hurd CL. 2012. Ecophysiology of photosynthesis in macroalgae. *Photosynthesis Research* **113,** 105–125.

Raven JA, Johnston AM, Kubler JE *et al.* 2002. Seaweeds in cold seas: evolution and carbon acquisition. *Annals of Botany* **90,** 525–536.

Rothäusler E, Gomez I, Hinojosa IA, Karsten U, Tala F, Thiel M. 2011. Physiological performance of floating giant kelp *Macrocystis pyrifera* (Phaeophyceae): latitudinal variability in the effects of temperature and grazing. *Journal of Phycology* **47,** 269–281.

Sage RF, Kubien DS. 2007. The temperature response of C-3 and C-4 photosynthesis. *Plant Cell and Environment* **30,** 1086–1106.

Sarker MY, Bartsch I, Olischläger M, Gutow L, Wiencke C. 2013. Combined effects of CO_2, temperature, irradiance and time on the physiological performance of *Chondrus crispus* (Rhodophyta). *Botanica Marina* **56,** 63–74.

Shiraiwa Y, Miyachi S. 1985. Effects of temperature and CO_2 concentration in induction of carbonic-anhydrase and changes in the efficiency of photosynthesis in *Chlorella vulgaris* 11H. *Plant and Cell Physiology* **26,** 543–549.

Sokahl RR, Rohlf FJ. 1995. *Biometry, the Principles and Practice of Statistics in Biological Research* , 3rd edn. W.H. Freeman and Company, San Francisco, pp 419–422.

Sommer U. 2005. *Biologische Meereskunde 2. Auflage* . Springer-Verlag, Berlin, pp 68–71 (in German).

Staehr PA, Wernberg T. 2009. Physiological responses of *Ecklonia radiata* (Laminariales) to a latitudinal gradient in ocean temperature. *Journal of Phycology* **45,** 91–99.

Suárez-Àlvarez S, Gómez-Pinchetti JL, García-Reina G. 2012. Effects of increased CO_2 levels on growth, photosynthesis, ammonium uptake and cell composition in the macroalga *Hypnea spinella* (Gigartinales, Rhodophyta). *Journal of Applied Phycology* **24,** 815–823.

Sültemeyer D, Biehler K, Fock HP. 1993. Evidence for the contribution of pseudocyclic photophosphorylation to the energy requirement of the mechanism for concentrating inorganic carbon in Chlamydomonas. *Planta* **189,** 235–242.

Torstensson A, Chierici M, Wulff A. 2012. The influence of increased temperature and carbon dioxide levels on the benthic/sea ice diatom *Navicula directa*. *Polar Biology* **35,** 205–214.

Vardi A, Berman-Frank I, Rozenberg T, Hadas O, Kaplan A, Levine A. 1999. Programmed cell death of the dinoflagellate *Peridinium gatunense* is mediated by CO_2 limitation and oxidative stress. *Current Biology* **9,** 1061–1064.

Wu XH, Wu ZX, Song LR. 2011. Phenotype and temperature affect the affinity for dissolved inorganic carbon in a cyanobacterium *Microcystis*. *Hydrobiologia* **675,** 175–186.

Wu Y, Gao K, Riebesell U. 2010. CO_2-induced seawater acidification affects physiological performance of the marine diatom *Phaeodactylum tricornutum*. *Biogeosciences* **7,** 2915–2923.