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Effects of elevated CO_2 partial pressure and temperature on the coccolithophore *Syracosphaera pulchra*

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ABSTRACT: The effects of elevated partial pressure of CO_2 (pCO_2) and temperature on the coccolithophore *Syracosphaera pulchra* were investigated in isolation and in combination. Both the diploid and the haploid life stages were studied. Batch cultures were grown under 4 conditions: 400 µatm and 19°C; 400 µatm and 22°C; 740 µatm and 19°C; and 740 µatm and 22°C. The growth rate (µ) significantly increased under elevated pCO_2 only in the haploid stage and showed a different pattern with respect to temperature: it was higher at an elevated temperature in the haploid stage at 400 µatm whereas it decreased in the diploid stage at 740 µatm. Increasing both parameters together increased the growth rate by 11% in the haploid stage only. Elevated pCO_2 had a negative impact on the content of particulate organic carbon (POC), production and cell size in both life stages at 19°C, while no significant effect was observed at 22°C. Increasing temperature significantly increased the content of POC and production in the diploid stage at 740 µatm, while at 400 µatm it significantly decreased both the content of POC and production in the haploid stage. A simultaneous increase in pCO_2 and temperature had a negative effect on the content of POC and production in the haploid stage only. Neither the rate of calcification (production of particulate inorganic carbon, PIC) nor the PIC:POC ratio were significantly affected by elevated pCO_2, temperature or their interaction. These results showed a strong interactive effect between pCO_2 and temperature in affecting the physiology of *S. pulchra*, an effect that was often more pronounced in the haploid life stage. Elevated pCO_2 had a stronger effect than temperature.

KEY WORDS: Coccolithophores · Ocean acidification · Global warming · Carbon dioxide · Temperature · Calcification · Primary production · PIC:POC ratio

INTRODUCTION

The concentration of atmospheric carbon dioxide (CO_2) has risen from 280 parts per million (ppm) before the industrial era to a current value of 387 ppm. It is currently rising at a rate of ~3.3% yr^{-1} and will reach more than 700 ppm by the end of this century (Solomon et al. 2007). Doubling atmospheric CO_2, and the concomitant increase in other atmospheric greenhouse gases, will have important consequences for the Earth's climate, leading to an average warming of 1.5 to >5°C of global air temperature at the Earth's surface by 2100 (Solomon et al. 2007). The global climate change is expected to increase the average sea-surface temperature (SST) by ~3°C (Solomon et al. 2007) and to increase the partial pressure of CO_2 (pCO_2) at the ocean's surface due to the oceanic uptake of atmospheric CO_2. Over the course of the present century, the concentration of CO_2 in seawater is projected to increase by about 25 µmol kg^{-1} with a corresponding decrease in the pH of seawater to ~7.8, roughly 0.3 units lower than today's value (Gattuso & Lavigne 2009).

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Such acidification of surface waters could affect marine organisms, in particular those having carbonate skeletons and shells (e.g. Gazeau et al. 2007, Cohen et al. 2009), and it will have major effects on marine phytoplankton communities in terms of both the physiology of individual species and the composition of the community (e.g. Tortell et al. 2002, Kim et al. 2006). It has been shown that CO_2 enrichment significantly influences growth and the elemental composition of important taxonomic groups of phytoplankton—such as marine diatoms, dinoflagellates (e.g. Burkhardt et al. 1999), cyanobacteria (e.g. Hutchins et al. 2007) and coccolithophores (e.g. Riebesell et al. 2000b). Moreover, some field studies have suggested that an increase in CO_2 might stimulate phytoplankton primary production in the open ocean (e.g. Hein & Sand-Jensen 1997) and that elevated concentrations of CO_2 could affect competition among groups (Tortell et al. 2002). A rising SST will also accelerate metabolic activity among phytoplankton, and it could shift the ratio of particulate organic carbon (POC) to dissolved organic carbon (DOC) toward an enhanced accumulation of dissolved compounds in the surface layer, eventually reducing the sinking of organic carbon and weakening the ocean's biological pump (Wohlers et al. 2009).

Although increasing attention has been paid to the response of phytoplankton to elevated pCO_2 or temperature, much less is known about the combined effect of these 2 environmental parameters. Laboratory studies have suggested that a simultaneous increase in temperature and pCO_2 may have interactive influences on natural assemblages, cyanobacteria and coccolithophores (e.g. Fu et al. 2007, Hare et al. 2007, Feng et al. 2008, De Bodt et al. 2010). However, the information available at present is limited to few species and selected functional groups of phytoplankton.

Coccolithophores (Prymnesiophyceae) are one of the major planktonic autotrophs in today's oceans, together with diatoms and dinoflagellates. They are considered to be the most productive calcifying organisms on the planet, and they play a crucial role in the marine carbon cycle through calcification and photosynthetic carbon production (Rost & Riebesell 2004). Owing to their abundance, they contribute significantly to the flux of organic matter from the sea surface to deep waters and sediments (Klaas & Archer 2002), and they are the most important pelagic calcifiers, responsible for about half of the global surface ocean calcification; next in importance are the foraminifera and pteropods (Broecker & Clark 2009). Moreover, it is suggested that the coccoliths (the body scales of calcium carbonate surrounding the coccolithophore cell) act as 'ballast minerals' in the process of aggregation of organic particles, enhancing the fraction of surface primary production that reaches the seabed (POC flux) by protecting the organic matter from oxidation and increasing the density, and thus the sinking rate, of organic aggregates (Ziveri et al. 2007). Thus, the predicted decrease in their capacity for calcification (e.g. Riebesell et al. 2000a) may lead to a significant decrease in the ratio of particulate inorganic carbon (PIC) to POC (the PIC:POC ratio) and may reduce the capacity of the ocean as a reservoir of dissolved inorganic carbon (DIC) (Iglesias-Rodriguez et al. 2002).

There have been many studies in the last decade examining the potential effects of global environmental change on the physiology of coccolithophores, mostly focusing on the influence of CO_2 enrichment on calcifying strains of 5 species: *Emiliania huxleyi*, *Gephyrocapsa oceanica*, *Calcidiscus leptoporous*, *Coccolithus pelagicus* and *Pleurochrysis carterae* (e.g. Riebesell et al. 2000a, Iglesias-Rodriguez et al. 2008, Casareto et al. 2009). These studies have reported quite diverse results, suggesting species-specific and even strain-specific responses (Langer et al. 2006, 2009).

At present, the combined effects of elevated pCO_2 and temperature increase have been investigated on only 2 calcifying (diploid) strains of *Emiliania huxleyi* (Feng et al. 2008, De Bodt et al. 2010). Both studies reported a decrease in the production of inorganic carbon (calcification) and in the PIC:POC ratio at elevated pCO_2, while a significant temperature effect was observed in only 1 of the 2 strains (De Bodt et al. 2010). Both studies found no significant effect of either temperature or pCO_2 on POC production. However, the growth rate was affected differently by elevated temperature and pCO_2 in these 2 strains: it was stimulated in *E. huxleyi* strain CCMP 371 (Feng et al. 2008), but in strain AC481 it showed no response, or was even depressed, under these conditions (De Bodt et al. 2010).

Although *Emiliania huxleyi* is the most widespread coccolithophore in the ocean, distributed from high to low latitudes, and extensively studied for its capacity to form blooms (Brown & Yoder 1994), it is also considered to be an atypical species. Due to its high genetic, morphological and physiological variability (Young et al. 2003, Iglesias-Rodriguez et al. 2006), it is regarded as a 'species complex' that is a diverse assemblage of genotypes with distinct calcification characteristics and ecological adaptations (Ridgwell et al. 2009). This could explain why a wide range of responses to changes in carbonate chemistry has been reported (Langer et al. 2009).

More than 100 species of the genus *Syracosphera* have been described, significantly contributing to the high diversity of extant coccolithophores (Cros & Fortuño 2002). *Syracosphaera pulchra* (Lohmann, 1902) is the best known species, possibly due to its relatively

large size (15 to 25 µm; Cros & Fortuño 2002). It shows a heteromorphic life cycle with 2 different ploidy phases. In the diploid life stage, the cells are surrounded by calcium carbonate platelets (heterococcoliths) which are composed of crystal units that have different shapes and sizes; these are produced within the cell (in coccolith vescicles) (Brownlee & Taylor 2004). The haploid cells are covered by holococcoliths formed from minute (<0.1 µm) crystallite units and are biomineralized on the cell membrane (Klaveness 1973). *S. pulchra* is a very common and representative coccolithophore (Inouye & Pienaar 1988): it has a wide geographical distribution, having been reported in the Atlantic, Indian, Pacific and Southern Oceans, the Mediterranean Sea and the Red Sea (e.g. Findlay & Giraudeau 2000, Balestra et al. 2004), and it is adapted to a wide range of temperatures. The highest abundances are typically observed in oligotrophic central gyres (e.g. Ziveri et al. 2004).

In the present study, the effects of the pCO_2 and temperature levels projected for the end of this century are examined on growth, photosynthesis, calcification and cell size of both life stages of *Syracosphaera pulchra* (strain AC418) to investigate the response of this species to future conditions of ocean warming and acidification.

MATERIALS AND METHODS

Strains and culture conditions. The diploid, heterococcolith-bearing stage and the haploid, holococcolith-bearing stage belonged to the same strain (unialgal but not axenic) of *Syracosphaera pulchra* AC418 (Western Mediterranean, Spain) and were provided by the Algobank-Caen culture collection at the University of Caen Basse-Normandie, France (http://www.unicaen.fr/ufr/ibfa/algobank/). All cultures were analysed by optical microscopy to verify the type of coccolith; the ploidy level was then determined by flow cytometry as already described by Houdan et al. (2003). Cultures were grown in sterile filtered (0.22 µm) seawater enriched with 160 µmol l^{-1} nitrate, 10 µmol l^{-1} orthophosphate, trace metals and vitamins according to K/5 receipe (Houdan et al. 2006) without Tris and Si. The medium was also enriched with a soil extract (1.5 ml l^{-1}) according to Houdan et al. (2006). The solutions used for the culture medium of both stock and batch cultures were prepared 2 mo before the beginning of the experiments. The solutions of major nutrients and trace metals were stored at 4°C, while the solution of vitamins was stored at −20°C and thawed 1 h before use.

The seawater used to prepare the culture medium (salinity 33) was collected in September 2008 from the surface of the North Sea, ~3 km off the Eastern Scheldt, pre-filtered (0.45 µm), and stored outdoors in the dark for 3 mo before use. The nutrient concentration and total alkalinity of the medium were measured before the acclimation and the experimental periods (see below).

Experimental design. Previous results obtained on batch cultures of *Syracosphaera pulchra* grown at 19°C and at 400 and 730 µatm (Fiorini et al. 2011) were complemented with new results on the same strain grown at 22°C and at 400 and 750 µatm. The second experiment was performed 3 d after the first one. Four experimental conditions were used for both life stages: (1) 400 µatm and 19°C; (2) 400 µatm and 22°C; (3) 740 µatm and 19°C; (4) 740 µatm and 22°C. The cultures were maintained in a Sanyo Gallenkamp incubator at an irradiance of 160 µmol photons m^{-2} s^{-1} provided by cool white fluorescent tubes (Philips Master TL-D, 18 W/840), under a photoperiod of 12:12 h light:dark.

The cultures were pre-adapted to the experimental conditions for ~8 generations (Riebesell et al. 2000a) (data not shown) and subsequently grown in triplicate batch cultures. Both the acclimation and the experiment started at low cell concentration (~40 cells ml^{-1}). The experiment was ended after 7 generations at a final average cell density of about 7000 ml^{-1}. This setup was chosen in order to keep the cell density low, ensuring well-controlled experimental conditions while having enough biomass to perform biological measurements. Cells were always harvested in the middle of the dark period, and cultures were still in exponential growth. The cells consumed ~1.4% of the DIC in the diploid, heterococcolith-bearing strain, and ~3.6% in the haploid, holococcolith-bearing strain, leading to a mean shift in pH of 0.05 and 0.17 units, respectively.

The carbonate chemistry was controlled by bubbling the culture medium in a sterile 20 l tank (Nalgene) at the experimental temperature with a gas mixture of CO_2-free air and pure CO_2, until the target pCO_2 was reached. Triplicate 2.4 l borosilicate bottles were then filled with the medium, with no headspace, and the medium was inoculated to reach a density of ~40 cells ml^{-1}; the bottles were sealed with Teflon-lined screw caps to avoid gas exchange between the medium and the atmosphere (Langer et al. 2006). The bottles were then maintained at the experimental temperature (±0.5°C) in a Sanyo Gallenkamp incubator and gently inverted manually 3 times a day to avoid settling of the cells.

Measurement of the carbonate system. The pH was measured at the experimental temperature (19 or 22°C, total scale pH [pH_T]) at the beginning (in the 20 l tank, immediately before filling the bottles) and at the end of both acclimation and experiment. To measure the pH we used a glass electrode (Metrohm LL Uni-

trode), calibrated on the total scale, using Tris/hydrochloric acid and 2-aminopyridine/hydrochloric acid buffer solutions with a salinity of 33, prepared according to Dickson et al. (2007). Samples for total alkalinity (TA) and salinity were taken at the beginning and at the end of both acclimation and experiment, filtered (0.22 µm), poisoned with mercuric chloride (Dickson et al. 2007), and stored in the dark at 4°C pending analysis. Total alkalinity was determined on triplicate 50 ml subsamples using a computer-controlled Gran titration technique with a Metrohm 713 pH meter and a 665 Metrohm Dosimat. Titrations of a total alkalinity standard, provided by A.G. Dickson (batch 83) (2334.31 ± 5.03 µmol kg^{-1}), were within 0.71 µmol kg^{-1} of the nominal value (2335.02 ± 0.91 µmol kg^{-1}; n = 3; mean ± 95% confidence limits).

pCO_2 and the other parameters of carbonate chemistry were calculated using the R package 'seacarb' (Gattuso & Lavigne 2009) from measured values of pH_T, TA, temperature and salinity. Silicate, phosphate and ammonia concentrations were not taken into account in TA calculations.

Growth rate. Samples (0.5 ml), used for monitoring cell density during the acclimation and the experiment, were taken from the triplicate bottles at the beginning of the experimental period (t_0) and at several times (3 or 4) during the experimental period at the same hour (10:00 h) until cells were harvested (t_f). The same volume of culture medium was added in order to maintain zero headspace. The bottles were left open for no more than 30 s during sampling. The maximum volume sampled in each bottle was 3 ml, causing a variation in the initial carbonate values smaller than 1.5% — which was considered to be negligible. The samples were fixed with a 37% formaldehyde solution buffered with sodium tetraborate (1%) and stored at 4°C before counting; the analysis was performed within 24 h under the microscope using a Lemaur haemocytometer. The growth rate (µ) was calculated by exponential regression. The goodness of fit of each curve was estimated by the coefficient of determination ($0.966 \leq R^2 \leq 1$).

Particulate organic and inorganic carbon. Samples (400 ml) for determination of total particulate carbon (TPC) and POC were taken at the end (t_f) of each experiment, filtered onto pre-combusted GF/F filters (4 h, 400°C), dried at 60°C overnight, and subsequently stored in a dry environment. Inorganic carbon was removed prior to measurement of POC by the addition of hydrochloric acid (25%) (Nieuwenhuize et al. 1994). TPC and POC were subsequently measured on a Thermo Electron Flash EA 1112 Analyzer according to Nieuwenhuize et al. (1994). The detection limit for carbon was about 2 µg, giving a precision (SD) of between 0.023 and 0.028%. PIC (pg C $cell^{-1}$) was calculated as a difference between TPC and POC. The production of inorganic and organic carbon (PIC and POC production, pg C $cell^{-1}$ d^{-1}) was calculated according to: P = µ × cellular carbon content.

Nutrients. Nutrient concentration in the cultures was measured at the beginning and at the end of the acclimation and the experimental period, in order to verify that cells had not experienced nutrient limitation. Samples (20 ml) for nutrient determination were filtered (0.22 µm) and stored at −20°C pending analysis. Measurements were carried out colorimetrically within 3 wk of sampling, according to Merks & Sinke (1981) using a Skalar SA 4000 analyzer.

Cell size. Cell diameter was measured from 1 ml samples at the end of each experiment with a Beckman Multisizer 3 Coulter Counter. The mean diameter (long axis) of each sample was considered and the average value of triplicates calculated.

Statistical analysis. Values are reported as mean ± standard deviation (SD). Significant differences between the treatments as well as interactive effects of pCO_2 and temperature were statistically analyzed by 2-way analysis of variance (2-way ANOVA) at confidence levels of 0.05, 0.01 and 0.001. The source of the main effect determined by ANOVA was assessed by a Tukey post hoc test. Pair-wise tests were conducted with 1-way ANOVA. Statistical analysis was performed using the software package STATISTICA 6.0 (StatSoft).

RESULTS

In the following section we present the results for the experimental period of the 4 pCO_2/temperature conditions tested. Each data point shown in the table and figures is the mean value of triplicate culture experiments. Data relating to the carbonate chemistry and nutrient concentrations in the medium, and to the cells' growth during the period of acclimation in each experiment, are not presented.

Carbonate chemistry and general considerations

The experiments began (t_0) with a mean (±SD, n = 4) pH_T of 8.05 ± 0.02 in the low pCO_2 treatment and 7.82 ± 0.01 in the high pCO_2 treatment. The average initial TA for the 8 experiments was 2227 ± 17 µmol kg^{-1} (Table 1). Calculated values of pCO_2, DIC and calcite saturation state (Ω_c) at the experimental temperatures (19 and 22°C) were, respectively, 398 ± 26 µatm, 1957 ± 33 µmol kg^{-1} and 4.6 ± 0.4 in the low pCO_2 treatments and 742 ± 11 µatm, 2077 ± 12 µmol kg^{-1} and 3.1 ± 0.2 in the high pCO_2 treatments.

Table 1. Initial and final carbonate chemistry of the culture medium in the 4 experiments performed. pCO_2 = µatm; CO_2 = µmol kg^{-1}; HCO_3^- = µmol kg^{-1}; CO_3^{2-} = µmol kg^{-1}; Ω_c = calcite saturation state; pH_T = pH (total scale); TA = total alkalinity (µmol kg^{-1}); DIC = dissolved inorganic carbon (µmol kg^{-1}); NO_3^- = µmol l^{-1}; PO_4^{3-} = µmol l^{-1}. Values are given at the experimental temperatures (19 and 22°C). Initial values are those calculated from values of pH_T and TA (means, SD in parentheses; n = 3) measured in the 20 l tank. Final values are reported as means (SD in parentheses) of the values measured in the replicate bottles (n = 3)

	400 µatm and 19°C		740 µatm and 19°C		400 µatm and 22°C		740 µatm and 22°C	
	Diploid	Haploid	Diploid	Haploid	Diploid	Haploid	Diploid	Haploid
Initial medium chemistry								
pCO_2	416	416	730	730	379	379	751	745
CO_2	13.8	13.8	24.1	24.1	11.8	11.8	22.5	22.7
HCO_3^-	1789	1789	1944	1944	1720	1720	1922	1912
CO_3^{2-}	178	178	119	119	198	198	133	129
Ω_c	4.3	4.3	2.9	2.9	4.8	4.8	3.2	3.1
pH_T	8.03 (0.008)	8.03 (0.008)	7.82 (0.002)	7.82 (0.002)	8.06 (0.004)	8.05 (0.004)	7.81 (0.006)	7.80 (0.003)
TA	2222 (5)	2222 (5)	2235 (12)	2235 (12)	2205 (2)	2205 (2)	2222 (3)	2234 (3)
DIC	1980	1980	2088	2088	1933	1933	2078	2064
NO_3^-	156 (6)	156 (6)	165 (13)	165 (13)	157 (10)	157 (10)	157 (6)	153 (15)
PO_4^{3-}	10 (0.1)	10 (0.1)	10 (0.3)	10 (0.3)	9 (0.4)	9 (0.4)	9 (0.7)	10 (0.5)
Final medium chemistry								
pCO_2	344 (9)	341 (3)	619 (15)	457 (27)	359 (8)	200 (17)	665 (10)	460 (16)
CO_2	11.4 (0.03)	11.3 (0.01)	20.1 (0.05)	15.4 (0.09)	11.2 (0.02)	6.2 (0.05)	20.3 (0.03)	14.1 (0.05)
HCO_3^-	1756 (0.1)	1772 (0.8)	1921 (0.1)	1870 (0.2)	1730 (0.2)	1528 (0.3)	1926 (0.1)	1832 (0.1)
CO_3^{2-}	183 (0.3)	188 (0.2)	125 (0.2)	158 (0.6)	189 (0.2)	270 (0.1)	130 (0.3)	169 (0.3)
Ω_c	4.4 (0.07)	4.5 (0.05)	3.0 (0.04)	3.8 (0.02)	4.6 (0.07)	6.5 (0.03)	3.1 (0.07)	4.1 (0.08)
pH_T	8.09 (0.01)	8.1 (0.002)	7.88 (0.01)	7.99 (0.02)	8.07 (0.01)	8.27 (0.03)	7.86 (0.01)	7.99 (0.01)
TA	2210 (6)	2237 (8)	2230 (7)	2260 (7)	2196 (5)	2199 (2)	2245 (2)	2249 (5)
DIC	1933 (8)	1956 (8)	2057 (9)	2030 (15)	1913 (3)	1788 (17)	2066 (16)	2007 (13)
NO_3^-	155 (11)	154 (9)	146 (18)	148 (29)	135 (15)	141 (13)	157 (8)	132 (5)
PO_4^{3-}	9 (0.3)	9 (0.4)	10 (0.5)	9 (0.9)	8 (0.6)	7 (0.3)	10 (0.6)	9 (0.1)

At the end of each experiment (t_f) the shifts in pH and DIC were always below 0.05 ± 0.03 units and 1.4 ± 0.8%, respectively, in the diploid (calcifying) cultures and below 0.17 ± 0.06 and 3.6 ± 2.7%, respectively, in the haploid (non-calcifying) cultures (Fig. 1). This led to an average change in pCO_2 of 24 ± 15% (n = 8), which was higher in the haploid cultures compared to the diploid cultures, as reported by Shi et al. (2009). TA decreased by 65 ± 55 µmol kg^{-1} in the calcifying cultures and increased by 14 ± 14 µmol kg^{-1} in the non-calcifying ones (n = 4). Initial and final values of the carbonate chemistry of each experiment are reported in Table 1.

Growth

The growth rates ranged between 0.53 and 0.66 d^{-1} (Fig. 2). The growth rate of the haploid stage was significantly higher at 740 µatm (+15%) when cells were incubated at 19°C (2-way ANOVA, $p < 0.01$; Tukey post hoc, $p < 0.001$), while no significant effect of elevated pCO_2 was observed at 22°C (Tukey post hoc, $p > 0.05$) nor on the growth rate of the diploid life stage (2-way ANOVA, $p > 0.05$).

Increasing temperature alone, or in conjunction with variation in pCO_2, had a significant effect on the growth rate of both life stages (2-way ANOVA, $p < 0.05$ for diploid stage and $p < 0.01$ for haploid stage). Elevated temperature significantly increased the growth rate of the haploid stage at ambient pCO_2 (Tukey post hoc, $p < 0.01$) and significantly decreased the growth rate of the diploid stage at elevated pCO_2 (Tukey post hoc, $p < 0.05$). A simultaneous increase in temperature and pCO_2 increased the growth rate of the haploid stage by 11% (Tukey post hoc, $p < 0.01$) but had no significant effect on the diploid stage.

Particulate organic and inorganic carbon

The cellular quota of PIC measured in the haploid stage was not significantly different from zero (1-way ANOVA, $p > 0.05$). Elevated pCO_2 and temperature, separately or combined, had no significant effect on the content of PIC, the PIC production or the PIC:POC ratio in either the diploid or haploid stage (2-way ANOVA, $p > 0.05$; Fig. 3A,C).

The effect of elevated temperature, pCO_2, and their interaction, was significant on the organic carbon content of the cell in both life stages; it was more marked in the haploid stage (2-way ANOVA; $p < 0.05$, $p < 0.01$ and $p < 0.001$, respectively).

Specifically, elevated pCO_2 dramatically decreased the cell content of POC at 19°C (–32% and –53% in the

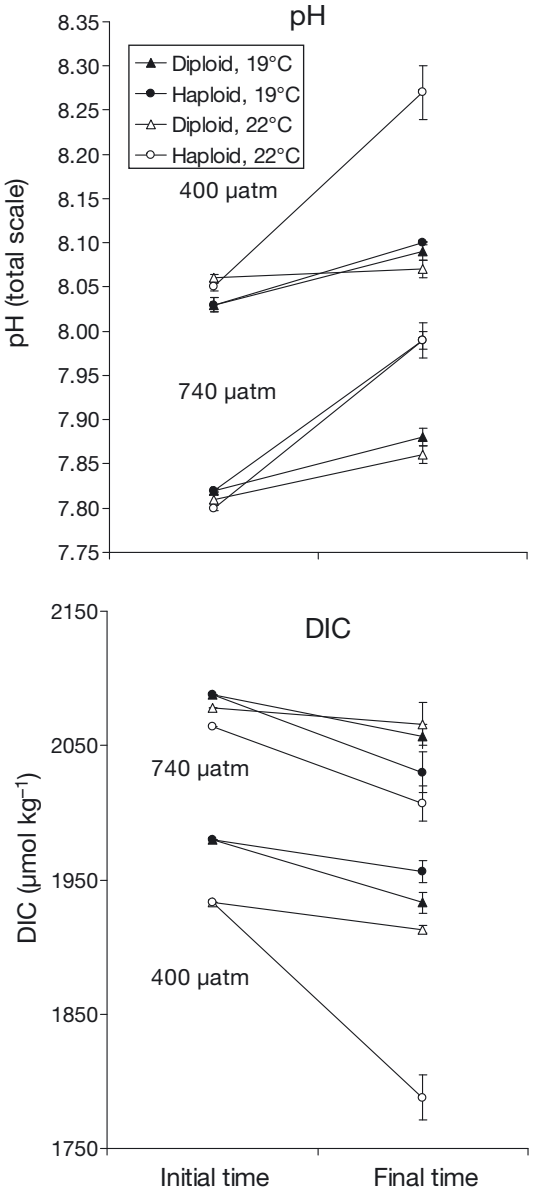

Fig. 1. Shift in pH and dissolved inorganic carbon (DIC) in the culture medium during the experiments. Data are reported as mean ± SD (n = 3)

diploid and haploid stage, respectively) (Tukey post hoc, diploid stage $p < 0.05$; haploid stage $p < 0.001$) but had no significant effect at 22°C (Fig. 3B). Elevated temperature increased the POC content of the diploid life stage (+40%) only when cells were grown at 740 µatm (Tukey post hoc, $p < 0.05$,), while at 400 µatm the organic carbon content of the cell was significantly decreased (–37%) in the haploid stage (Tukey post hoc, $p < 0.001$). Elevated pCO_2 together with increased temperature also had a negative effect on the POC content of the haploid life stage (–46%; Tukey post hoc, $p < 0.001$).

The POC production followed the same pattern as the cellular POC content in the 4 treatments (2-way ANOVA, $p < 0.05$, $p < 0.001$; Fig. 4B) but with smaller changes in general.

Elevated pCO_2 decreased the organic carbon production by 25 and 45% in the diploid and haploid stages, respectively (Tukey post hoc, diploid stage $p < 0.01$; haploid stage $p < 0.01$), when cells were grown at 19°C but had no significant effect at 22°C. Increased temperature alone stimulated the production of organic carbon in the diploid stage by 21% at 740 µatm (Tukey post hoc, $p < 0.05$) whereas it induced a significant decline in the haploid stage (–28%) at 400 µatm (Tukey post hoc, $p < 0.001$). The combined increase in temperature and pCO_2 significantly decreased POC production (Tukey post hoc, $p < 0.001$) in the haploid stage. The combined effect of pCO_2 and temperature on POC production was greater in the haploid stage than in the diploid stage (2-way ANOVA, $p < 0.05$ and $p < 0.001$ for the diploid and haploid stage, respectively).

Cell size

The sizes of the cells (cell diameter) in the 2 life stages of *Syracosphaera pulchra* were not significantly different (10.7 ± 0.2 µm and 11.1 ± 0.2 µm in the diploid and haploid stage, respectively) at lower values of pCO_2 and temperature (19°C and 400 µatm). The cell size was significantly affected only by elevated pCO_2 (2-way ANOVA, $p < 0.05$) or by elevated temperature alone (2-way ANOVA, $p < 0.05$), while the simultaneous increase in pCO_2 and temperature had no significant effect (2-way ANOVA, $p > 0.05$) (Fig. 5). Moreover, although a negative pCO_2 effect (–9 and –5%, diploid and haploid stage, respectively) was observed on both life stages at ambient temperature (Tukey post hoc, both life stages $p < 0.05$), it was not significant at 22°C, and elevated temperature significantly increased the cell size of both life stages only at 740 µatm (Tukey post hoc, both life stages $p < 0.05$) (Fig. 5). The statistical analysis also revealed a significant interaction between pCO_2 and temperature on the cell size of the diploid life stage (2-way ANOVA, $p < 0.05$) (Fig. 5).

DISCUSSION AND CONCLUSIONS

The environmental changes expected in the near future will impact marine autotrophs, but knowledge of the response of phytoplankton to the combined effect of a rising concentration of CO_2 and a rising temperature is presently limited to a few functional

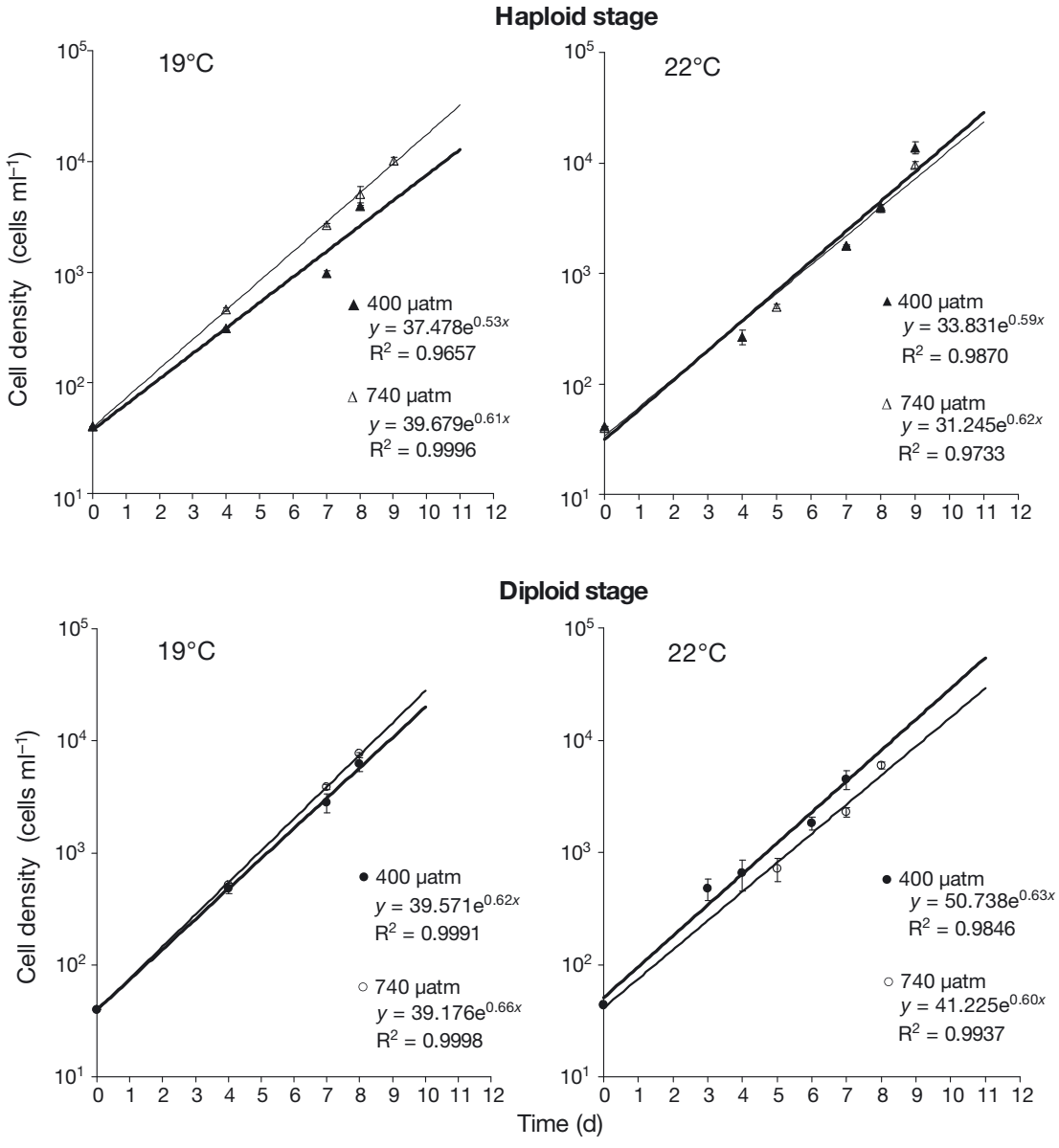

Fig. 2. *Syracosphaera pulchra*. Growth curves in the 4 different temperature and pCO_2 treatments (closed symbols: 400 µatm; open symbols: 740 µatm) calculated by exponential regression. Cell density (cells ml^{-1}) is represented in logarithmic scale. Data are reported as mean ± SD (error bars) of the values measured in the replicate bottles (n = 3). R^2 is the coefficient of determination of each curve

groups, species and assemblages (e.g. Hare et al. 2007, Hutchins et al. 2007, Feng et al. 2008, 2009). Our study is the first to investigate the effects of a rising seawater temperature and an increase in the partial pressure of CO_2 on growth, primary production, cell size and calcification of the widespread organism *Syracosphaera pulchra*; in this study we extended the analysis to both life stages (haploid and diploid) — which differ in coccolith cover, physiology, seasonal succession and ecology. The experimental setup of our study was designed following the recommendations of *Guide to best practices for ocean acidification research and data reporting* (Riebesell et al. 2010), after a careful analysis of the previous experimental approaches reported in the literature (e.g. Riebesell et al. 2000a, Zondervan et al. 2002, Sciandra et al. 2003, Langer et al. 2006, Feng et al. 2008, Iglesias-Rodriguez et al. 2008, Shi et al. 2009) in order to reproduce the experimental environment as close as possible to natural seawater, ensuring appropriate comparison of data between studies. The manipulation of the carbonate system was achieved by bubbling the culture medium with a mixture of CO_2-free air and pure CO_2 before inoculating the cells, and the experiment was consequently performed in sealed

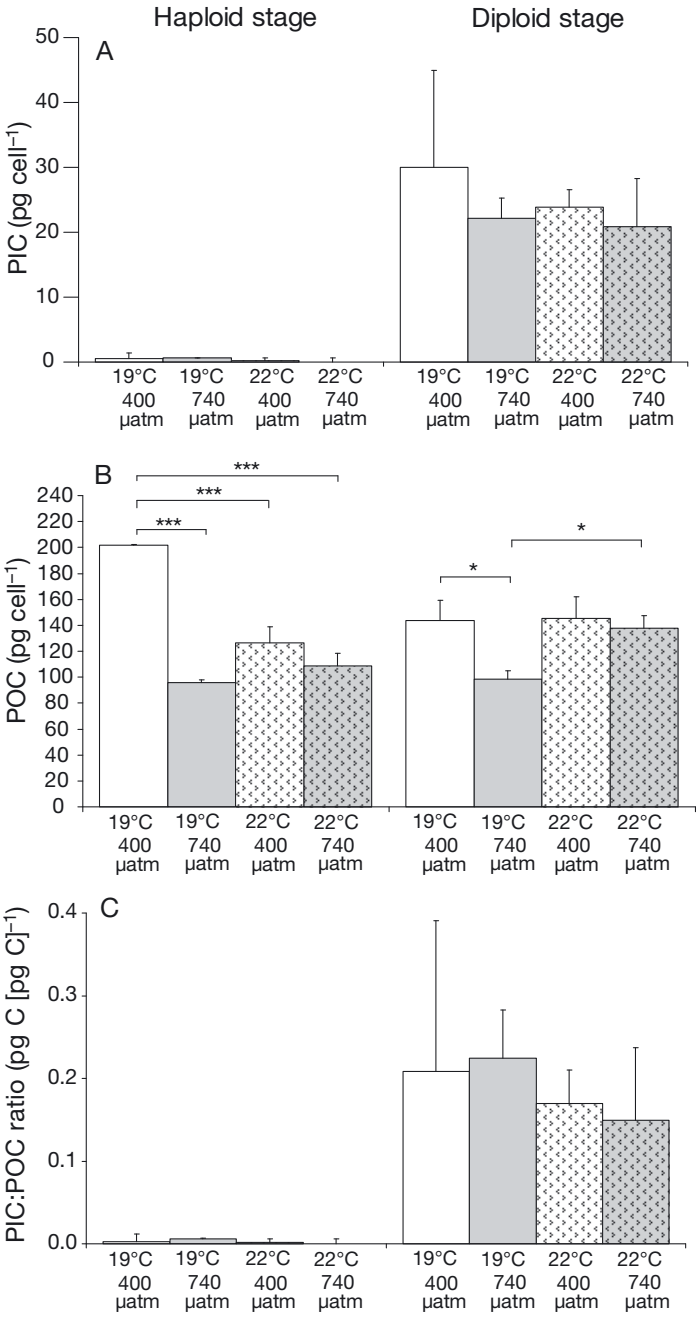

Fig. 3. *Syracosphaera pulchra*. The cells' content of (A) particulate inorganic carbon (PIC) and (B) particulate organic carbon (POC), and (C) the PIC:POC ratio in the 4 different treatments. Data are reported as mean ± SD (error bars) of the values measured in the replicate bottles (n = 3). Significance (2-way ANOVA): *p < 0.05; ***p < 0.001

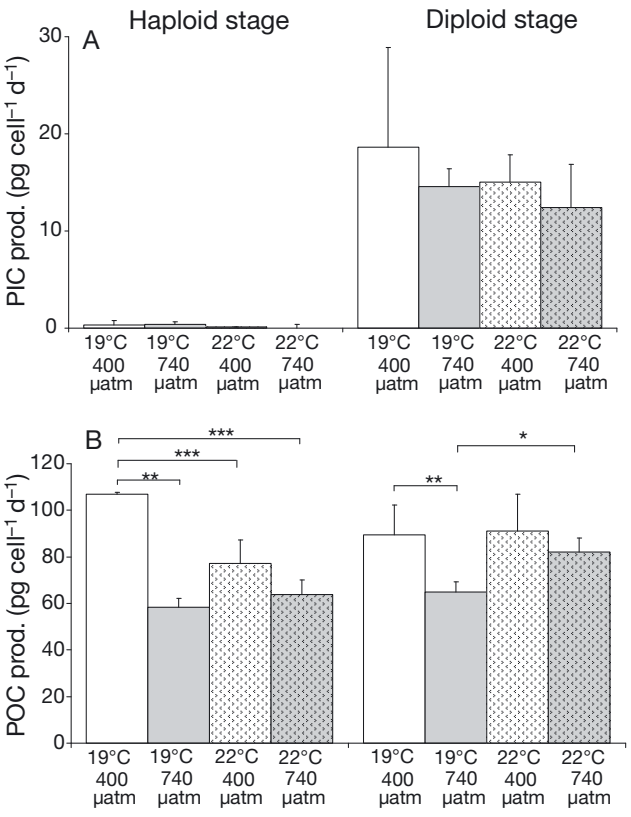

Fig. 4. *Syracosphaera pulchra*. The cells' production of (A) particulate inorganic carbon (PIC) and (B) particulate organic carbon (POC) across the 4 treatments. Data are reported as mean ± SD (error bars) of the values measured in the replicate bottles (n = 3). Significance (2-way ANOVA): *p < 0.05; **p < 0.01; ***p < 0.001

bottles, avoiding continuous bubbling of the medium and gas exchanges with the atmosphere (closed system) during the experimental period. This method involves changes in pCO_2, DIC and pH, while TA remains constant (Gattuso & Lavigne 2009), mimicking in the most realistic way the natural environment. As already described (e.g. Shi et al. 2009, Riebesell et al. 2010), fragile phytoplankton, such as some coccolithophore species, might be affected by the turbulence created by aeration/bubbling — which has an effect on the growth rate and general physiology of the organism. By avoiding continuous bubbling during the experiments we protected the cultures from the stress of aeration and thereby eliminated variability in the response to tested parameters; with such an approach, any change observed during the experiments can be attributed exclusively to physiological changes in response to the CO_2 perturbation (Fiorini 2010).

Our results show that the increase in temperature and pCO_2 expected by 2100 will have significant individual and interactive effects on growth rate, organic carbon production and the cell size of *Syracosphaera pulchra* but, in contrast to recent findings with *Emeliania huxleyi* (De Bodt et al. 2010), the calcification rate and PIC:POC ratio will not be significantly reduced. Although significant, the physiological variations ob-

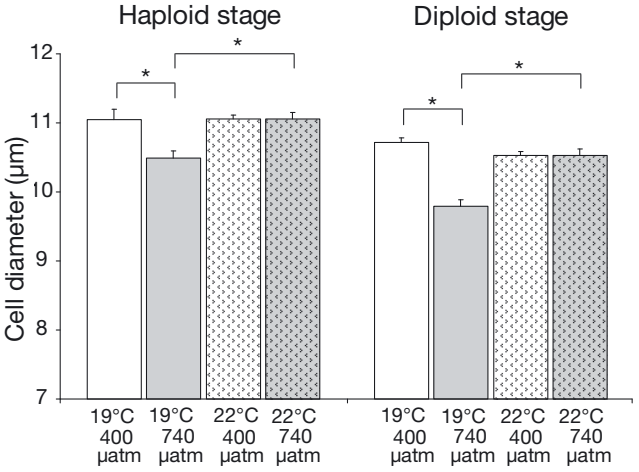

Fig. 5. *Syracosphaera pulchra*. Cell diameter of the 2 life stages (diploid and haploid) under the different pCO_2 and temperature conditions. Significance (2-way ANOVA): *$p < 0.05$

served among treatments are small, and the results obtained are not easily interpreted. While the experimental planning (2 pCO_2 and 2 temperature experimental values) and the sampling strategy chosen for the present study (i.e. sampling at the beginning and at the end of the experimental period for most parameters, and several times during the experiment to follow the cultures' growth) allow an excellent control of the carbonate chemistry and of the physiological activity of the cultures, the data set has its limitations. It does not allow powerful statistical analysis which would require a larger number of sampling points and the testing of more experimental conditions. Hence, we would like to stress that the statistical results reported in the text are intended as a complement for a better understanding of the data. Overall, the increase in pCO_2 had an effect generally greater than the increase in temperature on *S. pulchra* physiology. The effect that was similar, in sign, in the 2 life stages, although sometimes more marked in the haploid life stage. Elevated pCO_2 stimulated the growth rate of this species while it had a negative effect on the organic carbon production and cell size of both life stages. Apart from being of a smaller magnitude, the effect of temperature depended highly on pCO_2 and was often opposite in the 2 life stages. Besides the statistical outcome, physiological and ecological reasons may explain the observed patterns. *S. pulchra* thrives in a wide range of temperatures (from 6 to 30°C) (Okada & McIntyre 1979, Ziveri et al. 2004), showing optimum growth in tropical–temperate waters (Findlay & Giraudeau 2000, Ziveri et al. 2004). This wide temperature range suggests a high degree of flexibility to different temperature conditions. According to some field studies (e.g. Beaufort & Heussner 2001, Malinverno et al. 2009), the haploid life stage is more abundant in summer (high sea-surface temperature, and stratification of the water column), while the diploid life stage shows maximum abundance peaks in spring (March to May) and autumn (October to November; lower temperature, and extensive mixing of the water column). The experimental temperatures tested in the present study fall in the optimal range for growth of both life stages of *S. pulchra* and this reasonably explains why the observed effect of this parameter on the physiology of this species is relatively minor and difficult to summarize. A significant temperature effect on *S. pulchra* would probably be observed >30°C (Okada & McIntyre 1979) and this would be an interesting hypothesis to test for a better understanding of the physiology of this species.

The effect of elevated pCO_2 was not significant on calcification in *Syracosphaera pulchra* but there was a clear effect on the other processes investigated. The stimulation of microalgal growth has already been observed for different classes of algae (e.g. Beardall & Raven 2004, Kim et al. 2006), but the decreased primary production at elevated pCO_2 observed in both life stages is uncommon. Furthermore, the haploid stage seemed more sensitive than the diploid stage to elevated pCO_2. The main differences between the 2 life stages lie in their different cell covering (heterococcoliths and holococcoliths for the diploid and the haploid life stage, respectively) and in their different cell processes of biomineralization (see 'Introduction'). It can be suggested that the process of calcification might be more vulnerable in the haploid life stage because calcite precipitation is separated from chemical reactions in the surrounding seawater by only one biomembrane (Klaveness 1973), whereas the coccolith vesicle in diploid cells (Brownlee & Taylor 2004) is presumably a highly controlled environment. Moreover, although the functional role of coccoliths is not completely clear, one of the hypotheses is that they might act as protective screens against the harmful effects of environmental factors (Young 1994). Globally, the haploid life stage would seem to be more vulnerable than the diploid life stage to external environmental factors. The more pronounced response of the haploid life stage to pCO_2 and temperature variations shown in this study seems to support this hypothesis. Moreover, it has already been highlighted that the 2 life stages have different environmental preferences (e.g. Noel et al. 2004). In the ocean, the abundance of the 2 life stages is related to their growth strategies and to different adaptations to environmental conditions — with their heteromorphic life cycle linked to their ecology, and with each morphological stage corresponding to a different ecologi-

cal niche (Houdan et al. 2006). In *S. pulchra* the 2 life stages proliferate under different conditions during the year: the haploid stage reaches maximum densities in highly stratified, oligotrophic surface waters while the diploid stage is well adapted to higher turbulence, mesotrophic conditions, lower salinity and irradiance (Beaufort & Heussner 2001, Malinverno et al. 2009). This intra-specific seasonal succession clearly shows the affinity of the 2 life stages for different environmental conditions, and it also explains some of the differences in the responses observed in the present study. Diversity at the physiological level, especially in the sensitivity of the enzymatic reactions of photosynthesis (Geider & Osborne 1992) and in the mechanisms of carbon concentration (Shiraiwa 2003, Feng et al. 2008), might also play a role, but at present the physiology of this species is too poorly known to go further with this hypothesis.

In conclusion, the aim of the present study was to investigate whether the conditions of global warming and ocean acidification expected by the end of this century will have a significant effect on the widespread coccolithophore *Syracosphaera pulchra*. Our results confirm that the expected 3°C increase in the present seawater temperature (Solomon et al. 2007) will not strongly affect the physiology of this eurythermal species. The effect of an elevated pCO_2 in seawater will not be significant on calcification or on the PIC:POC ratio in either life stage, but it will be marked in other processes. The observed decrease in POC production at 740 µatm and 19°C is an uncommon result with no simple interpretation. Instead, the higher sensitivity to variations in pCO_2 observed in the haploid life stage, compared to the diploid life stage, might be explained by the higher vulnerability of the haploid stage to environmental factors or by the different affinity of the 2 life stages for different environmental conditions. Differences in the mechanisms underlying photosynthesis, and in the utilization of inorganic carbon, could also play a role. Current knowledge of the physiology of *S. pulchra* and of the response of this species to variations in environmental factors — such as temperature and pCO_2 — is quite limited compared to our knowledge about other coccolithophore species (Buitenhuis et al. 2008, Fiorini et al. 2010, 2011). Further investigation of the mechanisms underlying the physiology of this species would help in the interpretation of some of the present results. Overall, as already reported for the common coccolithophore *Emiliania huxleyi* (Feng et al. 2008, De Bodt et al. 2010), the present study shows that the future increase in pCO_2 may have a greater adverse impact on *S. pulchra* than the increase in temperature alone or the interacting effects of temperature and pCO_2.

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