The response of *Emiliania huxleyi* (Lohmann) W. W. Hay et H. Mohler, *Calcisdiscus leptoporus* (G. Murray et V. H. Blackman) J. Schiller, and *Syracosphaera pulchra* Lohmann to elevated partial pressure of carbon dioxide (pCO$_2$) was investigated in batch cultures. We reported on the response of both haploid and diploid life stages of these three species. Growth rate, cell size, particulate inorganic carbon (PIC), and particulate organic carbon (POC) of both life stages were measured at two different pCO$_2$ (400 and 760 parts per million [ppm]), and their organic and inorganic carbon production were calculated. The two life stages within the same species generally exhibited a similar response to elevated pCO$_2$, the response of the haploid stage being often more pronounced than that of the diploid stage. The growth rate was consistently higher at elevated pCO$_2$, but the response of other processes varied among species. Calcification rate of *C. leptoporus* and of *S. pulchra* did not change at elevated pCO$_2$, whereas it increased in *E. huxleyi*. POC production and cell size of both life stages of *S. pulchra* and of the haploid stage of *E. huxleyi* markedly decreased at elevated pCO$_2$. It remained unaltered in the diploid stage of *E. huxleyi* and *C. leptoporus* and increased in the haploid stage of the latter. The PIC:POC ratio increased in *E. huxleyi* and was constant in *C. leptoporus* and *S. pulchra*. Elevated pCO$_2$ has a significant effect on these three coccolithophore species, the haploid stage being more sensitive. This effect must be taken into account when predicting the fate of coccolithophores in the future ocean.

*Key index words:* calcification; carbon dioxide; climate change; coccolithophores; haploid and diploid life stage; ocean acidification; Prymnesiophyceae

*Abbreviations:* DIC, dissolved inorganic carbon; μ, growth rate; pCO$_2$, carbon dioxide partial pressure; PIC, particulate inorganic carbon; POC, particulate organic carbon; TA, total alkalinity

Atmospheric CO$_2$ concentration has increased by about a third over preindustrial levels, with a continuing increment of ~0.4% per year. By the year 2100, the atmospheric pCO$_2$ is projected to be ~760 ppm (Solomon et al. 2007) against the present value of ~390 ppm. The invasion of atmospheric CO$_2$ in the surface ocean will dramatically affect the carbonate system. Surface-ocean pH will decrease three times more than the decrease that occurred during the transition from glacial to interglacial periods (Wolf-Gladrow et al. 1999), reducing the supersaturation of calcite. CO$_2$ enrichment will have a significant effect on growth, calcification, photosynthesis, and elemental composition of the main calcifying groups including foraminifera (e.g., Bijma et al. 2002), pteropods (e.g., Comeau et al. 2009), mollusks (e.g., Gazeau et al. 2007), and sea
urchin larvae (e.g., Kurihara and Shirayama 2004). It will also influence phytoplankton species composition and succession (e.g., Tortell et al. 2002) affecting the main groups such as diatoms (e.g., Trimborn et al. 2008), dinoflagellates (e.g., Rost et al. 2006), the diazotrophic cyanobacterium Trichodesmium (e.g., Hutchins et al. 2007), and coccolithophores (e.g., Riebesell et al. 2000). The latter are significant components of the earth’s biogeochemical cycles, owing to their great abundance, fast turnover rates, and capability to carry out photosynthesis and calcification (Baumann et al. 2004). Coccolithophores are one of the three taxa (with diatoms and dinoflagellates) in the modern ocean that contribute most to the export flux of organic matter to seabed and sediments (Falkowski et al. 2009), and also the most important pelagic calcifiers, responsible for about half of the global CaCO3 export (Broecker and Clark 2009). During calcification, they increase surface pCO2 producing by about one molecule of CO2 for each molecule of calcium carbonate (CaCO3) fixed, and they alter the so-called Corganic:Ccarbonate “rain ratio,” which is an important factor in determining the fate of the CO2 in seawater. Therefore, the predicted decrease in their calcification capacity in the future may potentially lead to a significant decrease in the PIC:POC ratio having a major effect on the capacity of the ocean as a reservoir of dissolved inorganic carbon (DIC; Iglesias-Rodriguez et al. 2002).

To understand how coccolithophores respond to elevated pCO2, laboratory and mesocosm experiments were initially carried out on the diploid stage of two prominent modern species, E. huxleyi and Gephyrocapsa oceanica, and on natural phytoplankton assemblages (Riebesell et al. 2000, Zondervan et al. 2002, Sciandra et al. 2003, Delille et al. 2005, Engel et al. 2005). These laboratory and mesocosm experiments showed a clear overall decrease in carbonate production with decreasing pH, together with the occurrence of malformed coccoliths. Subsequent research targeted other widespread species (Coccolithus braarudii and C. leptoporus) and revealed a wider range of responses, suggesting marked species-specific sensitivity to elevated pCO2 (Langer et al. 2006). Finally, recent laboratory experiments carried out on E. huxleyi (Iglesias-Rodriguez et al. 2008a, Langer et al. 2009, Shi et al. 2009) reported increased calcification at higher ambient CO2 and lower pH.

The interpretation of some of these results has been challenged (Riebesell et al. 2008), and progress has been made to unveil the specific mechanisms underlying coccolithophores’ physiological response to variations in DIC concentration (e.g., Rickaby et al. 2010), but it is clear that their response to ocean acidification is not as simple as it first appeared. Ridgwell et al. (2009) highlighted that such a difference in magnitude and even sign of the response raises challenges to model future changes in marine biogeochemical cycling and feedback to the atmosphere.

The difficulties of interpreting such variable results are due to the poor knowledge of calcification mechanisms in coccolithophores and to the limited number of model species investigated (only 5 vs. ~200 existing in the modern ocean) from selected morphological types and only one life stage. The life cycle of Prymnesiophyceae is distinct from other main groups of microalgae. In dinoflagellates, vegetative cells are haploid; diploid cells are restricted to zygotes. In diatoms, vegetative cells are diploid, and only the gametes are haploid, whereas it has been demonstrated that coccolithophores alternate asexual reproduction by binary fission to sexual reproduction by meiosis and syngamy (Billard and Inouye 2004). Concerning sexual reproduction, Billard and Inouye (2004) assumed that probably all the coccolithophores have a heteromorph life cycle alternating haploid (N) and diploid (2N) generations with each ploidy phase showing a different body scale morphology. The diploid cells are surrounded by calcium carbonate platelets (heterococcoliths) composed of crystal units with different shape and size having an intracellular origin (coccoliths vesicles) (e.g., Pienaar 1994, Brownlee and Taylor 2004), whereas the haploid cells are usually covered by holococcoliths formed by minute crystallites units (<0.1 μm) and biomineralized on the cell membrane (De Jong et al. 1976, Young et al. 2003). However, in some species, such as E. huxleyi, one of the most widespread extant coccolithophores, the haploid stage is covered by minute organic scales (no coccoliths), and it is usually referred to as scaly (Paasche 2002).

The haploid stage is historically less known, and the evidence of its autonomous existence is relatively recent (Cros et al. 2000, Geisen et al. 2002). At present, few studies have investigated the effects of some important environmental parameters such as irradiance and nutrients (Houdan et al. 2004, 2005, 2006, Noël et al. 2004) on both the life stages, highlighting that the haploid stage has a key role in the ecology of Prymnesiophyceae and is likely to contribute to the survival of this phytoplankton class in response to environmental stress and seasonal variations. Noël et al. (2004) stressed the advantages of the haploid-diploid alternation of generations, which are likely to broaden the ecological range of species and which may be a part of a survival strategy in response to an environmental change as caused by, for example, ocean circulation pattern changes. Houdan et al. (2006) asserted that each morphological stage within coccolithophores corresponds to a different ecological niche to maximize exploitation of resources such as nutrients or light.

At present, the effects of elevated atmospheric carbon dioxide on primary production by the haploid life stage, that is, free from the complications
in inorganic carbon chemistry associated with calcification, have been reported only twice (Leonardos and Geider 2005, Fiorini et al. 2010). The latter study also examined fatty acids and carbon isotopic composition relative to variations in DIC concentration.

The present study aims at deepening the knowledge on the effects of ocean acidification on coccolithophores and to provide an insight into how the future pCO$_2$ increase expected for the end of the 21st century may influence both life stages within the Prymnesiophyceae. To reach this goal, we compared the effect of two different pCO$_2$ in seawater (400 and 760 ppm) on the growth rate, calcification, production of organic carbon, and cell size of two species already investigated in previous studies (E. huxleyi and C. leptoporus) as well as in S. pulchra, a widespread and ecologically relevant species (Geisen et al. 2004, Ziveri et al. 2004). Both life stages—the diploid, heterococcolith bearing and the haploid, holococcolith bearing (C. leptoporus and S. pulchra) or scaly (E. huxleyi)—were studied to investigate possible differences in the response.

**MATERIALS AND METHODS**

*Strains and culture conditions.* The original strains (unialgal but not axenic) of the species studied, namely, E. huxleyi AC472 (1998 South Pacific, Western New Zealand), C. leptoporus AC370 (2000 South Atlantic, South Africa), and S. pulchra AC418 (Western Mediterranean, Spain), were selected because they represent some of the most abundant species of coccolithophores, widespread from high to low latitudes (Young et al. 2003). The diploid, heterococcolith-bearing stage and the haploid, holococcolith-bearing (C. leptoporus and S. pulchra) or naked (E. huxleyi) stage of each species belonged to the same strain 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 analyzed using optical microscopy to verify coccolith type, and then, the ploidy level was determined using flow cytometry as already described by Houdan et al. (2004) (I. Probert, pers. comm.).

Cultures were grown in sterile filtered (0.22 μm) seawater enriched with 160 μmol·L$^{-1}$ nitrate, 10 μmol·L$^{-1}$ orthophosphate, and trace metals and vitamins according to K/5 (-TRIS, -Si); the medium used for S. pulchra (I. Probert, pers. comm.). The medium and the atmosphere (Langer et al. 2006). The carbonate chemistry was controlled by bubbling the culture medium in a sterile 20 L tank (Nalgene, Thermo Fisher Scientific, Roskilde, Denmark) with a gas mixture of CO$_2$-free air or pure CO$_2$, until the required pCO$_2$ was reached (Gattuso et al. 2010). Triplicate 2.4 L borosilicate bottles were then filled up with the medium without headspace, the cultures were inoculated to reach a density of ~40 cells·mL$^{-1}$, and the bottles were sealed with Teflon-lined screw caps (Fisher Scientific, Illkirch, France) to avoid gas exchange between the medium and the atmosphere (Langer et al. 2006).

Measuring the carbonate system. pH (total scale) was measured at the experimental temperature (pH$_{19}$) with a glass electrode (Metrohm LL Unitrode, Metrohm, Herisau, Switzerland) calibrated on the total scale using Tris/hydrochloric acid and 2-aminoipiridyne/hydrochloric acid buffer solutions with a salinity of 33, prepared according to Dickson et al. (2007). Samples for TA were filtered (0.22 μm), poisoned with mercuric chloride (HgCl$_2$) (Dickson et al. 2007), and stored in the dark at 4°C pending analysis. TA 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 (Metrohm, Herisau, Switzerland). Titrations of a TA standard provided by A. G. Dickson (batch 83), (2,334.31 ± 0.03) were within 0.71 μmol·kg$^{-1}$ of the nominal value (2,335.02 ± 0.91 μmol·kg$^{-1}$); (n = 5; mean ± 95% confidence limits).

The partial pressure of the CO$_2$ as well as the other parameters of the carbonate chemistry were calculated using the R package “seacarb” (http://cran.r-project.org/web/packages/seacarb/index.html, Gattuso and Lavigne 2009) from measured values of pH, alkalinity, temperature, and salinity, as reported by Riebesell et al. (2010).

**Growth rate.** Samples (0.5 mL) to monitor cell density were taken daily or every other day at 10:00 h from the triplicate bottles, which were then refilled with the same volume of culture medium to have no headspace. Each time, the bottles were open for not more than 30 s. The maximum volume sampled in each bottle was 3 mL, causing a variation of the initial carbonate values not higher than 1.5%, which can be considered 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 by manual counting using a Lemaux hemocytometer (Fisher Scientific). The growth rate (μ) was calculated by means of exponential regression.

**Particulate organic and inorganic carbon.** Samples (400 mL) for determination of total particulate carbon (TPC, pg C·cell$^{-1}$) and POC (pg C·cell$^{-1}$) were filtered onto
precombusted GF/F filters (Whatman, Maidstone, UK) (4 h, 400°C), dried in the oven at 60°C overnight, and stored in a dry environment. For the POC measurement, inorganic carbon was completely removed before the analysis from the filters by in situ hydrochloric acid (25%) addition (Nieuwenhuize et al. 1994). TPC and POC were subsequently measured on a Thermo Electron Flash EA 1112 Analyzer (Thermo Scientific, Breda, the Netherlands) according to Nieuwenhuize et al. (1994). This method has a carbon blank of ~2 pg. PIC (pg C · cell−1) was calculated as a difference between TPC and POC. Production of inorganic and organic carbon (PIC and POC production, pg C · cell−1 · d−1) was calculated according to: 

\[ P = \mu \times c \]  

where \( P \) is production (pg C · cell−1 · d−1) and \( \mu \) is the growth rate (cell−1 · d−1). 

Nutrients. Samples (20 mL) were taken for nutrient determination at the beginning and end of each experiment, filtered (0.22 μm), and stored at 4°C pending analysis. Measurements were carried out colorimetrically using a Skalar SA 4000 analyzer (Skalar, Breda, the Netherlands).

Cell size. Cell diameter as well as cell volume were measured on 1 mL samples at the end of each experiment using a Beckman Multisizer 3 Coulter Counter (Beckman Coulter, Woerden, the Netherlands). The mean diameter and the mean volume of each sample were considered, and the average value between replicates was calculated (n = 3 ± SD).

Statistical analysis. Values are reported as mean ± SD. Statistical analysis was conducted using Microsoft Excel (11.0) and the software package Tanagra (1.4.30). Homoscedasticity was checked using F-test (F2,2) before choosing which statistical test to apply. When data obeyed normality and homoscedasticity criteria, significant differences between the treatments were assessed using one-way analysis of variance (ANOVA) at confidence level of 0.05 (*), 0.01 (**), and 0.001 (***) and, and degree of freedom (n−1) of 5; otherwise, the less powerful Mann–Whitney U-test was used with P = 0.05 (*). In addition, post hoc power analysis was performed (PHP) for each test. PHP values >0.8 are considered to indicate reliable significant differences, but this type of power analysis has its limitations (Hoenig and Heisey 2001).

RESULTS

The data presented on carbonate chemistry and growth results have already been reported by Fiorini et al. (2010).

Carbonate chemistry and general considerations. The experiments were started (t0) with a mean (±SD) pH of 8.04 ± 0.01 in the low-pCO2 treatment and 7.81 ± 0.01 in the high-pCO2 treatment. The average initial TA for the six experiments was 2,235 ± 22 μmol · kg−1 (Table 1). Calculated values of pCO2, DIC, and calcite saturation state (Ωcalcite) were 403 ± 13 ppm, 1,977 ± 9 μmol · kg−1, and 4.4 ± 0.1, respectively, in the low-pCO2 treatment, and 759 ± 25 ppm, 2107 ± 23 μmol · kg−1, and 2.8 ± 0.1, in the high-pCO2 treatment. For the sake of convenience, the low- and high-pCO2 conditions will be referred to as 400 and 760 ppm, respectively.

At the end of each experiment (t), the shifts in pH and DIC were never higher than 0.07 ± 0.04 and 1.7 ± 1.3%, respectively, leading to an average change in pCO2 of 17 ± 9.5% (n = 12). TA decreased by 34 ± 52 μmol · kg−1 in the calcifying cultures and increased by 48 ± 39 μmol · kg−1 in the noncalcifying ones.

Growth. The lowest and highest growth rates were measured in the diploid stages of C. leptoporus (0.33 d−1) and E. huxleyi (0.99 d−1), respectively (Table 2). The growth rates of the three species were significantly higher (U-test, P < 0.05; ANOVA, P < 0.05. C. leptoporus diploid stage PHP = 1; S. pulchra diploid stage PHP = 0.59; E. huxleyi PHP = 0.77 and 0.99 for the diploid and the haploid stages, respectively) at 760 than at 400 ppm (Fig. 1). The increase was more pronounced in the haploid stage of S. pulchra (ANOVA, P < 0.01, PHP = 0.86) and C. leptoporus (ANOVA, P < 0.001, PHP = 1). In experiments 3, 4 some pseudocolonies of C. leptoporus (Houdan et al. 2006) were observed in the haploid cultures, but each cell was counted.

Cell size. The size of the cells was different among the species and between the two life stages. The cell diameter was significantly larger in the diploid stage of C. leptoporus (11.9 ± 0.2 μm) and E. huxleyi (4.8 ± 0.1 μm) than in their haploid stages.
Table 2. Biological parameters sampled at the end of each experiment (means ± SD; n = 3; POC content (µg C cell⁻¹), PIC production (µg C cell⁻¹), growth rate (µm d⁻¹), PIC:POC (µg C µg⁻¹), cell number (cells mL⁻¹), cell volume (µm³), nitrate consumption (D.L. = detection limit of the method used for the analysis). PIC, particulate inorganic carbon; POC, particulate organic carbon. ANOVA, analysis of variance. P values are given in parentheses. Significant differences between treatments (U-test, P < 0.05) are shown by asterisks (* U-test, ** ANOVA). Emiliania huxleyi, Calcidiscus leptoporus, Syracosphaera pulchra.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Emiliania huxleyi</th>
<th>Calcidiscus leptoporus</th>
<th>Syracosphaera pulchra</th>
</tr>
</thead>
<tbody>
<tr>
<td>Life stage</td>
<td>POC content</td>
<td>PIC content</td>
<td>PIC production</td>
</tr>
<tr>
<td>Low pCO₂</td>
<td>16 (±1.1)</td>
<td>10 (±0.2)</td>
<td>8 (±0.3)</td>
</tr>
<tr>
<td>Diploid</td>
<td>15 (±1.1)</td>
<td>10 (±0.2)</td>
<td>8 (±0.3)</td>
</tr>
<tr>
<td>Haploid</td>
<td>15 (±1.1)</td>
<td>10 (±0.2)</td>
<td>8 (±0.3)</td>
</tr>
<tr>
<td>High pCO₂</td>
<td>22 (±2.3)</td>
<td>15 (±1.1)</td>
<td>10 (±0.2)</td>
</tr>
<tr>
<td>Diploid</td>
<td>16 (±1.1)</td>
<td>10 (±0.2)</td>
<td>8 (±0.3)</td>
</tr>
<tr>
<td>Haploid</td>
<td>15 (±1.1)</td>
<td>10 (±0.2)</td>
<td>8 (±0.3)</td>
</tr>
</tbody>
</table>

Particulate organic and inorganic carbon. Elevated pCO₂ had a significant effect on the organic carbon content per cell in S. pulchra, which decreased by 32% and 53% in the diploid and haploid stages, respectively (Fig. 3) (U-test, P < 0.05, PHP = 0.96 and 1.00 for the diploid and the haploid stages, respectively). A smaller but significant effect on the cellular quota of organic carbon was observed in the haploid stage of E. huxleyi (23% lower in cells grown under high pCO₂) (ANOVA, P < 0.05, PHP = 0.68), whereas neither its diploid stage nor C. leptoporus were significantly altered.

The cellular quota of inorganic carbon measured in the haploid stages was below the detection limit of the method applied for the analysis (see above) and therefore was not taken into account. The PIC content per cell in the diploid stage of E. huxleyi slightly increased at 760 ppm compared with 400 ppm (from 9 ± 0.2 to 10 ± 0.2 pg C cell⁻¹) (ANOVA, P < 0.001, PHP = 0.99); it was not significantly affected in C. leptoporus nor in S. pulchra (Fig. 3). The PIC:POC ratio of E. huxleyi was higher at elevated pCO₂ than in the control (U-test, P < 0.05, PHP = 1.00), whereas, it did not exhibit significant changes in C. leptoporus and S. pulchra (Fig. 3).

Fig. 1. Growth rates (µ) of the three species in the two pCO₂ treatments. ANOVA, analysis of variance.
POC production followed the same pattern as the cellular POC content for the three species, although with weaker variations due to increase in the growth rate (Fig. 4). It decreased at elevated pCO₂ by 25% and 45% in the diploid and haploid stages of *S. pulchra* (*U*-test, *P* < 0.05, PHP = 0.66 and 1.00 for the diploid and the haploid stages, respectively) and by 20% in the haploid stage of *E. huxleyi* (ANOVA, *P* < 0.05, PHP = 0.56). pCO₂ had no significant effect on the POC production of the diploid stage of *E. huxleyi* and of *C. leptoporus*. In the haploid stage of the latter, the POC production significantly increased (*U*-test, *P* < 0.05, PHP = 0.40). Calcification rate (pg C cell⁻¹ d⁻¹) exhibited a response similar to one of the cellular PIC content: it did not change in *C. leptoporus* and *S. pulchra* and increased by 25% in *E. huxleyi* (ANOVA, *P* < 0.001, PHP = 0.95) (Fig. 4).

**DISCUSSION**

Although it is widely accepted that the haploid life stage is critically important in the ecology of coccolithophores to optimize resource exploitation (Young 1994, HouDan et al. 2006), and that it plays a key role in genetic structuring of wild populations (Paasche 2002), this life stage has received little attention, and its response to carbonate chemistry variations is almost unknown. Moreover, the present knowledge of coccolithophores’ sensitivity to elevated pCO₂ is based on experiments testing only the diploid stage of a few species (*E. huxleyi*, *Gephyrocapsa oceanica*, *Coccolithus braarudii*, *C. leptoporus*, and *Pleurochrysis carterae*), and the results reported by previous studies showed a large variability of response, painting a highly incoherent view.

Together with our companion study (Fiorini et al. 2010), which focused on coccolithophores’ fatty acids and carbon isotopic composition related to variations in seawater carbonate chemistry, the present work is the first to investigate both life stages of three ecologically relevant species: *E. huxleyi*, *C. leptoporus*, and *S. pulchra*. It provides novel data on the haploid stage, useful to more completely understand coccolithophores’ response to carbonate chemistry variations and to better predict how Prymnesiophyceae will function and proliferate in future scenarios of ocean acidification.

The large variety of responses of the diploid life stage of coccolithophores to increasing pCO₂ and decreasing calcite saturation state has raised much attention and debate. Iglesias-Rodriguez et al. (2008a,b) and Riebesell et al. (2008) expressed opposing views about the contrasting results. The debate focused on experimental protocols, in particular, the need for an adequate cell acclimation and data processing. This debate also involved discussions regarding the appropriate approach to adjust pCO₂ levels (acid or base addition vs. bubbling with CO₂-enriched air) to reproduce, as realistically as possible, future changes of the carbonate chemistry (Schulz et al. 2009). This methodological aspect was resolved by Shi et al. (2009) who tested both pCO₂ adjustment methods on the same strain of *E. huxleyi* as used by Iglesias-Rodriguez et al. (2008a) and found similar responses when bubbling with CO₂-enriched air or acid addition was used. In our experiments, the carbonate chemistry was manipulated by bubbling with a mixture of CO₂-free air and pure CO₂ to reach the experimental conditions by manipulating DIC at constant TA. This mimics the natural mechanism of ocean acidification (Gattuso and Lavigne 2009, Hurd et al. 2009, Gattuso et al. 2010). The shift in carbonate parameters due to cell activity was negligible as dilute cultures were used, which also limited bacterial contamination and the stress caused by continuous bubbling (Shi et al. 2009). The culture medium in the
experiments was the same for the three species investigated, but the one used for *S. pulchra* was enriched with soil extract, which is required to successfully grow it (data not shown; I. Probert, pers. comm.). Except for this difference, the techniques used in the present study were identical for all the species and life stages investigated, and so, the responses observed can be attributed to physiological responses to changes in carbonate chemistry rather than to experimental procedures. It therefore seems that the method used to manipulate the carbonate chemistry is not an important aspect to explain the wide range of response of coccolithophores to ocean acidification. Ridgwell et al. (2009) proposed that the high variability in calcification observed across studies is due to the large intraspecific variability of *E. huxleyi*. That proposal is supported by the results of Langer et al. (2009) who reported very different responses in four strains of *E. huxleyi* with heterogeneous morphotypes and different geographic origin. In this work, we investigated one of the strains of *E. huxleyi* studied by Langer et al. (2009; strain AC472/RCC1216/TQ26), but we obtained a response of calcification (25% increase at 760 compared with 400 ppm) opposite to the one they reported (22% decrease at 729 compared to 422 ppm). The only significant difference in the methods used is the irradiance level were respectively, 160 μmol photons m$^{-2}$ s$^{-1}$ on a 12:12 L:D photoperiod in the present study and 400 μmol photons m$^{-2}$ s$^{-1}$ on a 16:8 h L:D photoperiod in Langer et al. (2009). Feng et al. (2008) reported a combined effect of CO2 and light on calcification by another strain of *E. huxleyi*, with a large decrease of the cellular PIC content and PIC:POC ratio at elevated irradiance, and a further decrease by increased pCO2 only at elevated irradiance. Calcification data reported on *E. huxleyi* at different pCO2 levels are compiled in Table 3. The pCO2 levels were distributed in three groups: (i) <405 ppm, (ii) between 405 and 700 ppm, and (iii) >700 ppm. For each experiment, the calcification rate measured in group (3) was divided by the calcification rate measured in group (1) to calculate the $C_{high/low}$ ratio. Ratios <1 indicate an inhibition of calcification at elevated pCO2, whereas ratios >1 indicate a
Table 3. Calcification (PIC production) of *Emiliania huxleyi* versus CO₂ environment and irradiance experiments available in literature.

<table>
<thead>
<tr>
<th>Reference</th>
<th>Strain</th>
<th>Year of collection and location</th>
<th>Average pCO₂ (ppm)</th>
<th>Light/dark cycle (h:h)</th>
<th>Irradiance (µmol m⁻² s⁻¹)</th>
<th>Daily irradiance (mol m⁻² d⁻¹)</th>
<th>PIC production (pg cell⁻¹ d⁻¹)</th>
<th>C_high:low</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Ch 24-90</td>
<td>1991, North Sea</td>
<td>190</td>
<td>16:8</td>
<td>300</td>
<td>17.28</td>
<td>0.87</td>
<td>1.34</td>
</tr>
<tr>
<td>2</td>
<td>TW1</td>
<td>2001, W. Mediterranean Sea</td>
<td>400</td>
<td>24:0</td>
<td>570</td>
<td>49.25</td>
<td>2.38</td>
<td>0.85</td>
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<tr>
<td>3a</td>
<td>CCMP371</td>
<td>1987, Sargasso Sea</td>
<td>375</td>
<td>12:12</td>
<td>400</td>
<td>17.28</td>
<td>1.47</td>
<td>0.38</td>
</tr>
<tr>
<td>4</td>
<td>NZEH</td>
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<td>12:12</td>
<td>150</td>
<td>6.48</td>
<td>1.15</td>
<td>0.70</td>
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<tr>
<td>5</td>
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<td>24:0</td>
<td>150</td>
<td>12.96</td>
<td>0.64</td>
<td>1.56</td>
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<tr>
<td>6a</td>
<td>RCC1238</td>
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<td>400</td>
<td>23.04</td>
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<td>6b</td>
<td>RCC1216</td>
<td>1998, S. Pacific</td>
<td>218</td>
<td>16:8</td>
<td>400</td>
<td>23.04</td>
<td>13.48</td>
<td>1.01</td>
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<td>15</td>
<td>1.30</td>
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References: (1) Buitenhuis et al. (1999), (2) Sciandra et al. (2003), (3, a and b) Feng et al. (2008), (4) Iglesias-Rodriguez et al. (2008a), (5) Shi et al. (2009), (6, a–d) Langer et al. (2009), (7) present work, (8, a–d) Riebesell et al. (2000) and Zondervan et al. (2002). The data were obtained either from the articles, downloaded from the EPOCA database (http://www.epoca-project.eu/index.php/what-do-we-do/science/data.html), or interpolated from the figures using GraphClick 3.0 for Mac OS X. When pCO₂ was not reported, it was calculated from total alkalinity and [CO₂] using “seacarb” (Gattuso and Lavigne 2009). Chigh:low is the ratio of the mean calcification at pCO₂ above 700 ppm and calcification at pCO₂ below 405 ppm. PIC, particulate inorganic carbon.

Stimulation of calcification at elevated pCO₂. Daily irradiances (mol photons m⁻² d⁻¹) are used to compare experiments carried out using different photoperiods. The C_high:low ratio ranges between 0.4 and 1.8 at daily irradiances ranging between 1.3 and 49.3 mol photons m⁻² d⁻¹, but there is no consistent trend when all data are plotted (data not shown). However, a clearer pattern emerges when data are grouped according to strains. The C_high:low ratio decreases significantly at high daily irradiance in strains CCMP371 (0.7 vs. 0.4, respectively, at 2.2 and 17.3 mol photons m⁻² d⁻¹), AC472 (1.3 vs. 0.8, respectively, at 6.9 and 23.0 mol photons m⁻² d⁻¹), and NZEH (1.8 vs. 1.6, respectively, at 6.5 and 13.0 mol photons m⁻² d⁻¹). These strains therefore exhibit a significant interaction between light and pCO₂, confirming the conclusion of Feng et al. (2008). The different responses found for strain AC472 in the present study and by Langer et al. (2009) could thus be due to the different irradiance used in the experiments. In contrast, the response of strain PML B92/11A to elevated pCO₂ is not significantly affected by irradiance, and the C_high:low ratio varies between 0.7 and 0.9 (mean = 0.80 ± 0.13) for daily irradiances ranging from 1.3 to 13.0 mol photons m⁻² d⁻¹. This analysis of the combined effect of pCO₂ and irradiance confirms major physiological differences in the strains of *E. huxleyi* available in culture collections (Paasche 2002).

The calcification capacity of the other two species studied (*C. leptoporus* and *S. pulchra*) was not negatively affected by reduced pH in the experimental environment, as the calcification rate showed no significant change. Our results are in agreement with recent reports showing that pCO₂ and saturation state corresponding to projections for the end of this century do not negatively affect the precipitation of calcite in three species of coccolithophores (Iglesias-Rodriguez et al. 2008a, Shi et al. 2009). They confirm the wide range of responses found in coccolithophores (Langer et al. 2006, 2009).

The growth rate of the three species tested, in particular, *C. leptoporus*, was stimulated by elevated pCO₂.
differently from other recent studies that showed the growth rate to be either insensitive to ocean acidification or to decrease at high pCO2 levels (Langer et al. 2006, 2009, Iglesias-Rodriguez et al. 2008a).

The production of organic carbon did not significantly respond to an increase of pCO2 in the diploid stage of E. huxleyi and C. leptoporus and decreased in both the life stages of S. pulchra. These results are not in agreement with previous reports of an increase in POC production coupled to the decrease in calcification rate (Zondervan et al. 2002). Variations in PIC and POC production rates indicate rapid changes (min) in calcification and primary production in response to variations of environmental parameters (e.g., pCO2 and irradiance) or to variations in cell physiology (e.g., exponential vs. stationary growth phases). In contrast, changes in the PIC or POC quota are the cumulative effect of short-term changes in the PIC or POC production over a longer period (days). Hence, the significant decrease in organic carbon quota observed in S. pulchra and in the haploid stage of E. huxleyi, as well as the increase of the inorganic carbon content in the diploid stage of the latter, confirm a significant effect of reduced pH on the two species, which could have biogeochemical implications. In the haploid stage of S. pulchra, the decrease of organic carbon production was even more marked than in the diploid stage (Fig. 4). While the POC production and cell size did not change significantly in the diploid stage of E. huxleyi and C. leptoporus, as previously stated, the haploid stage exhibited a significant response (Figs. 2 and 4). Along with the variation in the organic carbon production in S. pulchra and E. huxleyi, the growth rate increase was more pronounced in the haploid stages of C. leptoporus and S. pulchra compared with their diploid stages (Fig. 1). Our results indicate that the response of the haploid, noncalcifying stage to increased pCO2 is similar to that of the diploid stage, and it is often more pronounced.

The motile haploid stage has been shown to be the meiotic product of the diploid stage and to probably have the gamete function (Billard and Inouye 2004, Houdan et al. 2004). It is therefore important for the ability of coccolithophores to reproduce not only vegetatively (binary fission) but also sexually (syngamy). In species such as E. huxleyi or S. pulchra, in which the haploid stage is negatively affected by the increase in pCO2, as our data show, sexual reproduction could be limited, and asexual reproduction could be dominant. This would restrict both the genetic differentiation of the populations (Valero et al. 1992) and the genetic inheritance within the species with a possible reduction of diversity. In E. huxleyi, which is considered a “species complex” encompassing a wide range of genotypic variations (Ridgwell et al. 2009), the less sensitive strains could dominate, resulting in an intraspecific impoverishment. This hypothesis could stimulate future research on the evolution of coccolithophores.

On the basis of our findings and on the results reported by Fiorini et al. (2010), we can conclude that each of the three investigated species responds in a different way to carbonate chemistry variations. These results are consistent with those reported for C. leptoporus and C. braarudii by Langer et al. (2006) who showed that the response to pCO2 variation follows a pattern specific for each species. Our results suggest that the variability in the response to the CO2 partial pressure increase within coccolithophores also depend on the different life stages. The phenotypic and physiological differences of the two life stages allow each species to use two different niches to exploit a wider range of ecological conditions (Cros et al. 2000), to limit the competition in the utilization of resources (food, light) inside the species and to rapidly escape negative selection pressures exerted on one stage such as grazing, viral attack, parasitic infections (Frada et al. 2008), or abrupt environmental changes (Noël et al. 2004). In this way, the survival of a species is ensured by one life stage when the environmental conditions do not favor the development of the other life stage (Houdan et al. 2005). Therefore, the differential response of coccolithophore species, strains, and life stage to changes in the carbonate chemistry state predicted by the end of the century could lead to significant changes in the geographic distribution (Cubillos et al. 2007, Tyrrell et al. 2008) and in the community structure, by providing a competitive advantage to the types most tolerant or profiting from ocean acidification. The less competitive types would probably give in, and the ecological niche that they presently occupy in the seas would be taken by the fittest ecotypes or by other phytoplankton groups having similar ecological affinities. This has to be taken into account when predicting coccolithophores’ fate in the future scenario of climate change and in model predictions of future atmospheric CO2 levels and global carbon cycle.

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