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Growth of the coccolithophore *Emiliania huxleyi* in light- and nutrient-limited batch reactors: relevance for the BIOSOPE deep ecological niche of coccolithophores

Laura Perrin¹, Ian Probert², Gerald Langer³, and Giovanni Aloisi⁴

¹Sorbonne Universités, UPMC Univ. Paris 06-CNRS-IRD-MNHN, LOCEAN-IPSL, 75252 Paris, France
 ²CNRS-UPMC Université, Paris 06 FR2424, Roscoff Culture Collection, Station Biologique de Roscoff, 29680 Roscoff, France
 ³The Marine Biological Association of the United Kingdom, The Laboratory, Citadel Hill, Plymouth, Devon, PL1 2PB, UK
 ⁴LOCEAN, UMR 7159, CNRS-UPMC-IRD-MNHN, 75252 Paris, France

Correspondence to: Laura Perrin (lpelod@locean-ipsl.upmc.fr)

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Abstract. Coccolithophores are unicellular calcifying marine algae that play an important role in the oceanic carbon cycle via their cellular processes of photosynthesis (a CO₂ sink) and calcification (a CO2 source). In contrast to the wellstudied, surface-water coccolithophore blooms visible from satellites, the lower photic zone is a poorly known but potentially important ecological niche for coccolithophores in terms of primary production and carbon export to the deep ocean. In this study, the physiological responses of an Emiliania huxleyi strain to conditions simulating the deep niche in the oligotrophic gyres along the BIOSOPE transect in the South Pacific Gyre were investigated. We carried out batch culture experiments with an E. huxleyi strain isolated from the BIOSOPE transect, reproducing the in situ conditions of light and nutrient (nitrate and phosphate) limitation. By simulating coccolithophore growth using an internal stores (Droop) model, we were able to constrain fundamental physiological parameters for this E. huxleyi strain. We show that simple batch experiments, in conjunction with physiological modelling, can provide reliable estimates of fundamental physiological parameters for E. huxleyi that are usually obtained experimentally in more time-consuming and costly chemostat experiments. The combination of culture experiments, physiological modelling and in situ data from the BIOSOPE cruise show that E. huxleyi growth in the deep BIOSOPE niche is limited by availability of light and nitrate.

This study contributes more widely to the understanding of *E. huxleyi* physiology and behaviour in a low-light and oligotrophic environment of the ocean.

1 Introduction

Coccolithophores are unicellular, photosynthetic and calcifying algae that are very abundant in the marine environment and play key roles in the global carbon cycle (Paasche, 2002; Roth, 1994). Through photosynthesis they contribute to the upper ocean carbon pump (CO_2 sink), while via calcification they contribute to the carbonate counter-pump (CO₂ source) (Paasche, 2002; Westbroek et al., 1993). The relative importance of calcification and photosynthesis is one of the factors that dictates the effect of coccolithophores on oceanatmosphere CO₂ fluxes (Shutler et al., 2013). Environmental conditions such as temperature, irradiance, nutrient concentrations and pCO_2 exert a primary control on the calcification/photosynthesis ratio in coccolithophores and also affect cellular growth rates, which, together with grazing, mortality, sinking of cells and oceanic transport, define the biogeography of coccolithophores.

Despite the fact that certain coccolithophores have been fairly extensively studied in the laboratory (e.g. Daniels et al., 2014; Iglesias-Rodriguez et al., 2008; Krug et al., 2011; Langer et al., 2012; Rouco et al., 2013), the factors controlling their biogeography in the global ocean are poorly understood (Boyd et al., 2010). In controlled laboratory conditions, coccolithophore growth is monitored as given environmental parameters are varied (e.g. Buitenhuis et al., 2008; Feng et al., 2008; Fritz, 1999; Langer et al., 2006; Leonardos and Geider, 2005; Paasche, 1999; Trimborn et al., 2007). In the ocean, geographical surveys of coccolithophore abundance and concomitant measurements of environmental variables contribute to defining coccolithophore biogeography in relation to the environment (Claustre et al., 2008; Henderiks et al., 2012). Although extrapolation of results from laboratory experiments to field distributions might not be straightforward, this approach has been widely used and continues to yield important insights into coccolithophore ecology and its reaction to a rapidly changing environment.

In this respect, one of the least well-understood but possibly globally relevant niches where coccolithophores can be relatively abundant is that occurring at the deep pycnocline of oceanic gyres, probably the best studied example of which was observed during the BIOSOPE cruise in the South Pacific Gyre (Beaufort et al., 2008; Claustre et al., 2008). This deep coccolithophore niche occurred at about 200 m depth, at a very low irradiance level (< 20 μ mol photons m⁻² s⁻¹) and at a depth corresponding to the nitrate and phosphate nutricline with dissolved nitrate (NO₃) and phosphate (PO₄) concentrations of about 1 and 0.2 µM respectively. The niche was dominated by coccolithophore species belonging to the family Noëlaerhabdaceae, i.e. Emiliania huxleyi and species of Gephyrocapsa and Reticulofenestra (Beaufort et al., 2008). Deep-dwelling coccolithophores have also been observed in other geographic regions. Okada and McIntyre (1979) observed coccolithophores in the North Atlantic Ocean down to a depth of 100 m, where Florisphaera profunda dominated assemblages in summer and E. huxleyi dominated assemblages for the rest of the year. Deep coccolithophore populations dominated by F. profunda in the lower photic zone (LPZ > 100 m) of subtropical gyres were observed by Cortés et al. (2001) in the central North Pacific Gyre (station ALOHA) and by Haidar and Thierstein (2001) in the Sargasso Sea (North Atlantic Ocean). Jordan and Winter (2000) reported assemblages of coccolithophores dominated by F. profunda in the LPZ in the north-eastern Caribbean with a high abundance and co-dominance of E. huxleyi and G. oceanica through the water column down to the top of the LPZ. These deep-dwelling coccolithophores are not recorded by satellite-based remote sensing methods (Henderiks et al., 2012; Winter et al., 2014), which detect back-scattered light from coccoliths from a layer only a few tens of metres thick at the surface of the ocean (Holligan et al., 1993; Loisel et al., 2006).

Understanding the development of deep coccolithophore populations in low-nutrient, low-irradiance environments would contribute to building a global picture of coccolithophore ecology and biogeography. Laboratory culture experiments with coccolithophores that combine both nutrient and light limitation, however, are scarce. One reason is that investigating phytoplankton growth under nutrient limitation in laboratory experiments is complicated. In batch cultures the instantaneous growth rate decreases as nutrients become limited, making it hard to extract the dependence of growth rate on nutrient concentrations (Langer et al., 2013). This can be avoided by employing chemostat cultures, in which growth rates and nutrient concentrations are kept constant under nutrient-limited conditions (Engel et al., 2014; Leonardos and Geider, 2005; Müller et al., 2012). Physiological parameters obtained in chemostat experiments have been used in biogeochemical models to investigate environmental controls on phytoplankton biogeography (Follows and Dutkiewicz, 2011; Gregg and Casey, 2007). Despite their relevance to nutrient-limited growth, chemostat cultures are relatively rarely used because they are more expensive, timeconsuming and complicated to set up and run than batch cultures (LaRoche et al., 2010).

In this study, we investigated growth of the coccolithophore E. huxleyi under light and nutrient co-limitation and applied the results of this culture study to investigate the conditions controlling growth in the deep niche of the South Pacific Gyre. Using an E. huxleyi strain isolated during the BIOSOPE cruise, we carried out batch culture experiments that reproduced the low- in situ light and nutrient conditions of the deep ecological niche. We monitored the nitrogen and phosphorus content of particulate organic matter, as well as cell, coccosphere and coccolith sizes, because these parameters are known to vary with nutrient limitation (Fritz, 1999; Kaffes, 2010; Rouco et al., 2013). To overcome the conceptual limitations inherent in nutrient-limited batch experiments (Langer et al., 2013), we modelled the transient growth conditions in the batch reactor assuming that assimilation of nutrients and growth are either coupled (Monod, 1949) or decoupled (Droop, 1968) processes in the coccolithophore E. huxleyi. An independent check of our modelling approach was obtained by also modelling the E. huxleyi batch culture data of Langer et al. (2013). The range of physiological parameters that can be directly assessed in batch culture experiments is limited (Eppley et al., 1969; Marañón et al., 2013). We show that batch cultures, if coupled to simple physiological modelling, may provide valuable estimates of fundamental physiological parameters that are more widely obtained in more time-consuming and costly chemostat experiments (Eppley and Renger, 1974; Terry, 1982; Riegman et al., 2000; Müller et al., 2012). Our joint culture and modelling approach also provides information on the conditions that control the growth of E. huxleyi in the deep ecological niche of the South Pacific Gyre.

2 Materials and methods

2.1 Experimental

2.1.1 Growth medium and culture conditions

Natural seawater collected near the Roscoff Biological Station (Brittany, France) was sterile-filtered and enhanced to K (-Si, -Tris, +Ni, -Cu) medium according to Keller et al. (1987), with only nitrate (no ammonium) as a nitrogen source. Emiliania huxleyi strain RCC911, isolated in summer 2004 from a water sample collected at 10 m depth near the Marquesas Islands during the BIOSOPE cruise (November to December 2004), was grown in batch cultures. Experiments were conducted in triplicate in 2.7 L polycarbonate bottles (Nalgene) with no head space. Experimental conditions were chosen to reproduce those prevalent in surface waters and at the nitricline of the oligotrophic gyre in the South Pacific Ocean (Morel et al., 2007). Cultures were grown under a 12:12 h light:dark (L:D) cycle (taken from a calculation of L:D cycle at the GYR station at the date of the sampling), at a temperature of 20 °C and at a salinity of 34.7. Cultures were grown at two irradiance levels: high light (ca. 140 µmol photons $m^{-2} s^{-1}$) and low light (ca. 30 µmol photons $m^{-2} s^{-1}$). The latter corresponds to the upper end of the irradiance range of the deep BIOSOPE coccolithophore niche (10- $30 \,\mu\text{mol photons m}^{-2} \,\text{s}^{-1}$). We chose not to run experiments at irradiance levels lower than $30 \,\mu\text{mol}$ photons m⁻² s⁻¹ in order to avoid very long experimental runs. Nutrient concentrations at the beginning of batch experiments were 100 and 2.5-5.1 µM for nitrate and 6.25 and 0.45-0.55 µM for phosphate in nutrient-replete and nutrient-limited conditions respectively. For each irradiance level, three experiments were carried out (in triplicate): control (nutrient-replete), phosphate limited (P-limited) and nitrate limited (N-limited) conditions. Cells were acclimated to light, temperature and carbon chemistry conditions for at least three growth cycles prior to experiments.

2.1.2 Cell enumeration and growth rate

The growth of batch cultures was followed by conducting cell counts every day or every other day using a BDFacs Canto II flow cytometer. Experiments were stopped before the cell density reached ca. 1.5×10^5 cells mL⁻¹ in order to minimise shifts in the dissolved inorganic carbon (DIC) system. Cultures remained in the exponential growth phase throughout the duration of the control (nutrient-replete) experiments. In these control cultures, the growth rate (μ) was obtained by conducting a linear regression of the cell density data on the logarithmic scale. Nutrient-limited experiments were allowed to run until growth stopped. The growth rate in nutrient-limited conditions decreases in time as nutrients are depleted and it is therefore not possible to calculate growth rate by means of regression analysis (Langer et al., 2013). The dependence of growth rate on nutrient concentration in nutrient-limited conditions was investigated with the numerical model introduced in Sect. 2.2 below.

2.1.3 Cell and coccosphere diameter and coccolith length

Samples were taken at the end of the experiments at roughly the same point in the L:D cycle (between noon and 16:00 UTC) to acquire images of cells using an optical microscope (×100, oil immersion, Olympus BX51 microscope). The internal cell diameter of 100 cells was measured for each experimental culture using the ImageJ software (http: //rsbweb.nih.gov/ij/). Images of coccospheres and coccoliths were obtained with scanning electron microscopy (SEM). For SEM observations, samples were filtered onto 0.8 µm polycarbonate filters (Millipore), rinsed with a basic solution (180 µL of 25 % ammonia solution in 1 L of MilliQ water) and dried at 55 °C for 1 h. After mounting on an aluminum stub, they were coated with gold-palladium and images were taken with a Phenom G2 pro desktop scanning electron microscope. For each experimental culture 100 coccospheres were measured using ImageJ. Three hundred coccoliths per sample were measured using a script (Young et al., 2014) that is compatible with ImageJ in order to measure the distal shield length (DSL) of coccoliths.

2.1.4 Dissolved inorganic carbon (DIC) and nutrient analyses

Subsamples for pH_T (pH on the total scale), DIC and nutrient analyses were taken from culture media at the beginning and at the end of each experiment. The pH was measured with a pH meter-potentiometer pHenomenal pH 1000 L with a Ross ultra combination pH electrode on the total scale (precision ± 0.02 pH units) and was calibrated with a TRIS buffer. Samples for the determination of DIC were filtered through pre-combusted (4 h at 450 °C) glass fibre filters (Whatman GF/F) into acid-washed glass bottles and poisoned with mercuric chloride. Bottles were stored at 4 °C prior to analysis. A LICOR7000 CO2 / H2O gas analyser was used for DIC analysis (precision $\pm 2 \,\mu\text{mol}\,\text{kg}^{-1}$). A culture aliquot (100 mL) was filtered onto pre-combusted (4 h at 450 °C) glass fibre filters (Whatman GF/F) and stored at -20 °C in a polyethylene flask until nutrient analysis. Nitrate and phosphate concentrations were measured using an auto-analyser Seal Analytical AA3 (detection limits were 0.003 µM for PO₄ and 0.01 µM for NO₃).

2.1.5 POC, PON, PIC, POP

For particulate organic carbon (POC), particulate organic nitrogen (PON) and particulate organic phosphorus (POP) analyses, samples (200 or 250 mL) were filtered onto precombusted (4 h at 450 °C) glass fibre filters (Whatman GF/F) and preserved at -20 °C. POC and PON were measured on

the same filter that was dried overnight at 50 °C after being placed in a fuming hydrochloric acid dessicator for 2 h to remove coccolith calcite. POC and PON were analysed using a NC Analyzer Flash EA 1112. Particulate inorganic carbon (PIC) was obtained by using a 7500cx Agilent ICP-MS to analyse the calcium concentration in samples filtered onto 0.8 µm polycarbonate filters (Millipore) and extracted by a 0.4 M solution of nitric acid. PIC was obtained considering a 1:1 stoichiometry between Ca^{2+} and PIC, i.e. all of the calcium on the filters was considered to have come from calcium carbonate (Fagerbakke et al., 1994). POP was determined as the difference between the total particulate phosphorus and particulate inorganic phosphorus, analysed using an auto-analyser Seal Analytical AA3, after the filters were placed in a solution of hydrochloric acid, according to the method of Labry et al. (2013).

2.2 Modelling

2.2.1 Monod and Droop model

Growth of *E. huxleyi* in the batch reactors was simulated using Monod and Droop models of cellular growth.

In the Monod model (Monod, 1949), the growth rate depends on the external nutrient concentration and is calculated as

$$\mu = \mu_{\max} \times \frac{[R]}{[R] + K_R},\tag{1}$$

where μ_{max} (in days⁻¹) is the maximum growth rate in nutrient-replete conditions, K_R (in μ mol L⁻¹) is the (Monod) half-saturation constant for growth and [R] (in μ mol L⁻¹) is the concentration of nutrient R in the batch reactor. Both μ_{max} and K_R were obtained by fitting the model to the data, while [R] is the nutrient concentration in the culture experiments calculated as detailed below.

Two differential equations keep track of the total cell abundance in the batch reactor (Cells) and the limiting nutrient concentration in the reactor:

$$\frac{d \text{ Cells}}{dt} = \mu \times \text{Cells}$$
(2)

$$\frac{\mathrm{d}\left[R\right]}{\mathrm{d}t} = \frac{-R_{\mathrm{UP}} \times \mathrm{Cells}}{V},\tag{3}$$

where V (in L) is the volume of the batch reactor, Cells (in cells mL⁻¹) is the cell density measured during the experiments and $R_{\rm UP}$ is the cell-specific R uptake rate (in μmol_R cell⁻¹ d⁻¹) given by

$$R_{\rm UP} = \mu \times Q_R,\tag{4}$$

where Q_R , the (constant) cellular quota of nutrient R (in $\mu mol_R \text{ cell}^{-1}$), is the value of the quota R at the end of the control experiment.

In the Droop model (Droop, 1968) nutrient uptake and cellular growth are decoupled and cellular growth depends on the internal store of the limiting nutrient. The time-dependent rate of nutrient uptake, R_{up} (in μmol_R cell⁻¹ d⁻¹), is simulated using Michaelis–Menten uptake kinetics:

$$R_{\rm UP} = S_{\rm Cell} \times V_{\rm maxR} \times \frac{[R]}{[R] + K_R},\tag{5}$$

where S_{Cell} (in μ m³) is the surface area of the cell, $V_{\max R}$ (in μ mol_R μ m⁻² d⁻¹) is the maximum surface-normalised nutrient uptake rate (obtained by fitting the model to the data) and K_R (in μ mol L⁻¹) is the (Michaelis–Menten) half-saturation constant for uptake of nutrient *R*. The volume and surface of cells (S_{cell}) was obtained either by measurements of cells (both in the control culture and at the end of the nutrient-limited cultures) for the RCC911 strain experiments, or was estimated from Q_C , the cellular organic carbon quota (in pmol_C cell⁻¹), and the density of carbon in coccolithophore biomass (approximately equal to 0.015 pmol_C μ m⁻³; Aloisi, 2015) for the batch experiments of Langer et al. (2013), for which cell measurements were not made.

The phytoplankton growth rate μ (in d⁻¹) was calculated based on the normalised ^{*n*}Quota equation reported in Flynn (2008):

$$\mu = \mu_{\max} \frac{(1 + KQ_R) \times \left(Q - Q_R^{\min}\right)}{\left(Q - Q_R^{\min}\right) + KQ_R \times \left(Q_R^{\max} - Q_R^{\min}\right)},\tag{6}$$

where μ_{max} (in d⁻¹) is the maximum growth rate attained at the maximum nutrient cell quota Q_R^{max} (in µmol cell⁻¹), Q_R^{min} (in µmol cell⁻¹) is the minimum (subsistence) cellular quota of nutrient *R* below which growth stops and KQ_R is a dimensionless parameter that can be readily compared between nutrient types and typically has different values for NO₃ and PO₄ (Flynn, 2008). While Q_R^{max} was obtained from the analysis of the nutrient quota (N or P) at the end of the control experiments, Q_R^{min} was estimated by calculation described in the Sect. 2.2.2 below and KQ_R was obtained from fitting the model to the experimental data. Thus, in the Droop model the growth rate depends on the internal cellular quota of nutrient *R* rather than on the external nutrient concentration like in the Monod model of phytoplankton growth.

Three differential equations keep track of the total cell abundance in the batch reactor (Cells), the nutrient concentration in the reactor ([*R*], in μ mol L⁻¹) and the internal cellular quota of nutrient (*Q_R*, in μ mol cell⁻¹):

$$\frac{d \text{ Cells}}{dt} = \mu \times \text{Cells}$$
(7)

$$\frac{\mathrm{d}[R]}{\mathrm{d}t} = \frac{-R_{\mathrm{UP}} \times \mathrm{Cells}}{V} \tag{8}$$

$$\frac{\mathrm{d}Q_R}{\mathrm{d}t} = R_{\mathrm{UP}} - \mu \times Q_R. \tag{9}$$

These three differential equations are integrated forward in time starting from initial conditions chosen based on experimental values of the number of cells, nutrient concentration at the beginning of the experiment and the cellular nutrient quota determined during growth in nutrient-replete conditions.

The dependence of the maximum growth rate on irradiance was determined independently by fitting the growth rate determined in the exponential growth phase in our experiments and in the experiment of Langer et al. (2013) to the following equation from MacIntyre et al. (2002):

$$\mu = \mu_{\max} \left(1 - e^{\left(\frac{-\ln r}{K_{\ln r}}\right)} \right), \tag{10}$$

where K_{Irr} is the light-saturation parameter of growth in µmol photons m⁻² s⁻¹ (MacIntyre et al., 2002; Fig. S1 in the Supplement) and was determined by this equation.

2.2.2 Modelling strategy

The Droop model presented here does not take into account the variation of size of coccolithophore cells between the different experiments. This model has eight parameters. Four are considered to be known and constant for a given experiment: batch volume V, cell volume (and surface area S_{Cell}) and minimum and maximum cellular quota of nutrient respectively Q_{min} and Q_{max} . The unknown parameters (the physiological parameters of interest) are: the (Michaelis– Menten) half-saturation constant for nutrient uptake K_R , the maximum surface-normalised nutrient uptake rate V_{maxR} , the maximum growth rate µmax and the dimensionless parameter $K Q_R$. The Monod model has fewer known parameters: batch volume V and cellular quota of nutrient Q_R . Unknown parameters are: maximum growth rate μ_{max} and the (Monod) half-saturation constant for growth K_R .

Concerning Q_R^{\min} , the measured minimum PON value $(5.71 \text{ fmol cell}^{-1})$ for the N-limited experiment of Langer et al. (2013) is very low compared with the PON quota in other N-limited E. huxleyi experiments reported in the literature $(38.9-39.3 \text{ fmol cell}^{-1} \text{ in Sciandra et al., 2003; and}$ 51.4 fmol cell⁻¹ in Rouco et al., 2013). When the $Q_{\rm N}^{\rm min}$ value of Langer et al. (2013) was used in the model, the model fit to the experimental data degraded considerably (data not shown). Consequently, we decided to recalculate Q_N^{\min} using the initial concentration of dissolved N and the final cell density in the reactor (column "Calculation" in Table 3). This calculated value of $Q_{\rm N}^{\rm min}$, that in all cases except for the Nlimited experiments of Langer et al. (2013) was very similar to the measured minimum PON quota, was comparable to values reported in the literature for E. huxleyi and resulted in a very good fit of the model to the experimental data. To be coherent, we applied this approach to all values of $Q_{\rm N}^{\rm min}$ and $Q_{\rm P}^{\rm min}$ used in the modelling exercise.

A point to note concerning the Q_P^{max} used for the P-limited experiment of Langer et al. (2013) is that the initial C : P ratio for the control experiment was 214, which is much higher than the Redfield ratio of 106 (Redfield, 1963). It is not possible to reproduce the experimental data when imposing such a high C: P ratio on the model. Thus, the Q_P^{max} value had to be increased in order to reproduce the data and thus estimate additional physiological parameters for this experiment. For this reason, the modelling results for this particular experiment should be taken with caution.

The time-dependent cell density, limiting nutrient concentration and cellular particulate organic nitrogen and phosphorus calculated by the models were fitted to the same quantities measured in the experiments. For our experiments there were only two nutrient cellular quota data points, one at the beginning and one at the end of the experiments. We artificially inserted a third nutrient-quota data point at the end of the exponential growth phase, setting it equal to the nutrient quota at the beginning of the experiment. In this way the model is forced to keep the nutrient quota unchanged during the exponential growth phase. This is a reasonable assumption, as cellular nutrient quotas should start to be affected only when nutrient conditions become limiting.

The quality of the model fit to the experimental data was evaluated with a cost function. For a given model run, the total cost function was calculated as follows:

$$TotCost = \sum_{i=1}^{n} (\Delta x_i)^2,$$
(11)

where *n* is the number of data points available and Δx_i is the difference between the data. The model for the *i*th data point is as follows:

$$\Delta x_i = \text{Data}(x_i) - \text{Model}(x_i), \qquad (12)$$

where x_i is the data or model value for the considered variable (cell density, limiting nutrient concentration or cellular limiting nutrient quota). The lower the cost function is, the better the quality of the model fit to the data. For a given experiment, the best-fit of the model to the data was obtained by running the model and repeatedly imposing a high number of combinations of input parameters (typically 500 000 model runs for every experiment) and selecting the parameter setting that yielded the lowest cost.

3 Results

3.1 Laboratory experiments with *E. huxleyi* strain RCC911

Growth curves for all experiments with *E. huxleyi* strain RCC911 are shown in Fig. 1. Experiments run in high-light conditions attained target cell densities (in nutrient-replete, control experiments) or nutrient limitation (in nutrient-limited experiments) in a shorter time compared to experiments run in low-light conditions. Growth in nutrient-replete cultures in both light conditions followed an exponential growth curve (growth rates in the control nutrient-replete experiments were 0.91 ± 0.03 and $0.28 \pm 0.01 \text{ d}^{-1}$



Figure 1. The evolution of cell density with time in culture experiments with *E. huxleyi* strain RCC911 in (a) high irradiance, (b) low irradiance and cell density on a logarithmic scale for nutrient-replete cultures in (c) high irradiance and (d) low irradiance.

for the high-light and low-light experiments respectively; Table 1) whereas in nutrient-limited experiments growth evolved from an exponential to a stationary phase at the end of the experiment, except the P-limited culture at low light where the stationary phase was not attained (growth rate of $0.13 \pm 0.01 \, d^{-1}$).

In the high-light experiment, NO₃ concentration decreased to $0.18 \pm 0.03 \,\mu\text{M}$ in N-limited cultures and PO₄ concentration decreased to $0.011 \pm 0.004 \,\mu\text{M}$ in P-limited cultures at the end of the experiments, and in low-light conditions the final NO₃ and PO₄ concentrations were 0.13 ± 0.02 and $0.008 \pm 0.006 \,\mu\text{M}$ respectively (Table 1). Thus, nutrients where nearly completely exhausted at the end of our nutrient-limited experiments. Seawater carbonate chemistry was quasi-constant over the course of the experiments in all treatments, with, as reported by Langer et al. (2013), the Plimited cultures undergoing the largest change in DIC (12– 13 %; Table 1).

Compared to the control experiments, cellular POC, PIC and PON quotas increased in the P-limited cultures at both light levels, while cellular POP quota decreased (Table 2; Fig. 2d). In the N-limited cultures, cellular PIC and POC quotas (Fig. 2a and b) increased, with the exception of POC at low light, which remained nearly unchanged, while cellular PON and POP quotas (Fig. 2c and d) decreased at both light levels. N-limiting conditions resulted in an increase of the POC : PON ratio in both light regimes (Fig. 3a, Table 2). POC : POP (Fig. 3b) was higher in P-limited experiments compared to nutrient-replete experiments. The PIC : POC ratio increased with both N and P limitation (Fig. 3c) at both light regimes. For the high-light experiment, the PIC : POC ratio was highest in the P-limited culture (0.52 ± 0.14), while in the low-light conditions, the highest ratio was recorded in the N-limited culture (0.33 ± 0.02) (Fig. 3c).

Light limitation led almost invariably to a decrease in POC and PIC, with the exception of POC in nutrient-replete conditions (Table 2, Fig. 2). In P-limited cultures POP and PON decreased with light limitation, whereas in N-limited cultures POP and PON increased with light limitation (Fig. 2). With the exception of the POC : POP ratio in P-limiting conditions, which was not affected by the change in light regime, both POC : PON and POC : POP ratios decreased with light limitation. Finally, the PIC : POC ratio decreased with light limitation in all three nutrient conditions.

Cell size varied with both nutrient and light limitation (Table S1 in the Supplement). Compared to the control culture in high-light conditions, the cell volume was higher for the P-limited culture $(77.2 \pm 19.9 \,\mu\text{m}^3)$ and was similar for the N-limited culture $(47.33 \pm 11.13 \,\mu\text{m}^3)$. The same pattern was observed in low-light conditions. In both light regimes P limitation resulted in higher coccosphere volume and higher DSL than the other nutrient conditions (Table S1).

Table 1. Growth rate, nutrient concentration, pH, DIC at the end of the experiments and shift in DIC compared with the initial DIC (averages from triplicate, n = 3).

Sample	Growth rate* (d^{-1})	SD	NO_3 (µmol L ⁻¹)	SD	$\begin{array}{c} PO_4 \\ (\mu mol L^{-1}) \end{array}$	SD	pН	SD	$\begin{array}{c} \text{DIC} \\ (\mu mol kg^{-1}) \end{array}$	SD	DIC shift (%)
High light											
Control	0.91	0.03	67.92	1.98	3.95	0.12	8.13	0.01	2177	19.14	2.1
PO ₄ lim	0.00		80.88	0.35	0.01	0.00	8.21	0.01	1894	21.01	12.1
NO3 lim	0.00		0.18	0.03	5.74	0.00	8.14	0.00	2060	3.61	4.7
Low light											
Control	0.28	0.01	79.10	1.15	4.90	0.04	8.13	0.02	2161	7.55	4.1
PO ₄ lim	0.13	0.01	75.25	1.24	0.01	0.01	8.30	0.01	1956	8.33	13.2
NO ₃ lim	0.00		0.13	0.02	5.83	0.02	8.09	0.00	2139	4.16	39

* Cells are in exponential growth phase at the end of control experiments.



Figure 2. Cellular PIC (a), POC (b), PON (c), POP (d) quotas.

For example, the coccosphere volume in high light was $260 \pm 88 \,\mu\text{m}^3$ for the P-limited experiment, whereas it was $109 \pm 23 \,\mu\text{m}^3$ for the control experiment and $139 \pm 41 \,\mu\text{m}^3$ for the N-limited experiment. There was no measurement of coccosphere volume and DSL in the low-light control culture because of a lack of visible cells on the filters. However, the coccosphere volume for the P-limited treatment followed the same trend as the cell size, i.e. a decrease with lower light. Figure 4a shows the correlation between POC content and cell volume ($R^2 = 0.85$, p < 0.05, n = 6) and Fig. 4b shows the correlation between cell and coccosphere volume ($R^2 = 0.92$, p < 0.03, n = 5). Relationships between

DSL and coccosphere size ($R^2 = 0.68$, p < 0.3, n = 5) and between DSL and cell size ($R^2 = 0.86$, p < 0.06, n = 5) are illustrated in Fig. 4c. These parameters were not significantly correlated, but the sample size was rather low. The thickness of the coccolith layer, calculated by subtracting the cell diameter from the coccosphere diameter and dividing by two, was higher for P-limited cultures in both light conditions: $1.294 \pm 0.099 \,\mu\text{m}$ for high light and $1.02 \pm 0.043 \,\mu\text{m}$ for low light compared with the other cultures, which were between 0.66 and $1 \,\mu\text{m}$. These observations are consistent with the high PIC quota and relatively large size of coccospheres and coccoliths of *E. huxleyi* under P limitation.

Sample	PIC (pg cell ⁻¹)	SD	POC (pg cell ⁻¹)	SD	PON (pg cell ⁻¹)	SD	POP (pg cell ⁻¹)	SD	PIC: POC	SD	POC: PON	SD	POC: POP	SD
High light														
Control	3.46	0.36	10.8	1.38	1.45	0.21	0.16	0.03	0.32	0.05	8.72	1.45	173	14.0
PO ₄ lim	14.16	3.19	27.49	1.53	2.66	0.10	0.11	0.01	0.52	0.12	12.05	0.70	661	24.3
NO3 lim	7.06	0.55	15.77	0.95	0.4	0.04	0.07	0.00	0.45	0.04	45.59	4.12	600	16.7
Low light														
Control	0.89	0.10	10.98	0.41	1.98	0.07	0.18	0.00	0.08	0.01	6.46	0.28	158	2.51
PO ₄ lim	3.53	0.25	16.25	0.56	2.08	0.08	0.06	0.00	0.22	0.017	9.11	0.41	693	13.4
NO3 lim	3.15	0.13	9.67	0.21	0.79	0.02	0.11	0.00	0.33	0.015	14.35	0.37	226	3.38

Table 2. Cellular carbon, nitrogen and phosphorus quotas (averages from triplicate, n = 6 for cellular quotas measurements).

Table 3. Values of Q_R^{\min} (which correspond to the cellular PON (POP) at the end of the experiment: values measured and calculated) and the parameters obtained with the best-fit indicated for N- and P-limited experiment (high light = HL and low light = LL).

		Limitation	$Q_{\rm I}^{\rm I}$	nin R	Best-fit				
Strain	Light		Analysis (fmol cell ⁻¹)	Calculation (fmol cell ^{-1})	$V_{\max R}$ (µmol cell ⁻¹ d ⁻¹)	$K_{\rm R}$ (µmol L ⁻¹)	$_{(d^{-1})}^{\mu_{max}}$	KQR	
PML B92/11		NO ₃	5.71	27.7	$1.46.10^{-7}$	0.35	1.3	0.39	
PML B92/11		PO ₄	0.645	2.04	$1.36.10^{-8}$	0.051	1.57	0.98	
RCC911	HL	NO ₃	28.57	31.28	$1.05.10^{-7}$	0.205	1.01	0.25	
RCC911	HL	PO ₄	3.464	5.931	$1.47.10^{-8}$	0.35	1.2	0.9	
RCC911	LL	NO ₃	56.14	78.99	$3.34.10^{-8}$	0.09	0.2	0.3	
RCC911	LL	PO ₄	1.968	2.875	$5.74.10^{-10}$	0.275	0.52	0.47	

3.2 Modelling results

We applied the modelling approach to both the data from our batch culture experiments with strain RCC911 and to the batch culture data of Langer et al. (2013), who tested Nand P-limited growth of *E. huxleyi* strain PML B92/11 cultured in high-light conditions (400 µmol photons $m^{-2} s^{-1}$), optimal temperature (15 °C) and quasi-constant carbon system conditions. Measurements of cell density, nutrient concentrations and cellular particulate matter from both sets of experiments were used for the present modelling study.

The Droop model was able to accurately reproduce both experimental data sets (Figs. 5, 6 and 8; Figs. S2, S3 and S4), whereas the Monod model was not able to reproduce the rise in cell number after the limiting nutrient had been exhausted (Fig. 5). The modelling approach allows evaluation of the evolution of experimental variables that are complicated to determine analytically, i.e. (1) the nutrient-uptake rate, which follows the same trend as the nutrient concentration in the reactor, (2) the C / limited-nutrient ratio, that starts at a minimum value, stays constant during the duration of the external nutrient, reaching a maximum as the culture attains the stationary phase and (3) the instantaneous growth rate, which follows the trend of the limiting nutrient ratio, reaching zero when the culture attains the stationary phase.

The values for the physiological parameters of the best-fit obtained by applying the Droop model to our experiments with E. huxleyi strain RCC911 and to the experiments of Langer et al. (2013) are presented in Table 3. Overall, the best-fit values for the two strains in high-light conditions were very similar, suggesting that the modelling approach is sound. Values for the half-saturation constant for nitrate uptake $K_{\rm N}$ determined in our experiments in high-light conditions and in those of Langer et al. (2013) were comparable. However, for K_P the value was consistent between our high- and low-light experiments, but considerably lower for the Langer et al. (2013) experiment, which, as noted above, is a result that should be taken with caution. The maximum surface nutrient-uptake rates V_{maxR} were similar between our high-light experiment and that of Langer et al. (2013). The dimensionless parameters $KQ_{\rm N}$ and $KQ_{\rm P}$ were also comparable between the two studies for high-light conditions and in both cases KQ_P was higher than KQ_N . Maximum growth rates in high-light conditions were similar for both N-limited and P-limited experiments. As expected, maximum growth rates for our low-light cultures were considerably lower (Table 3).

To test the reliability of the model to obtain estimates of the physiological parameters, we forced the model to run with a range of values for a given parameter, while letting the other three parameters vary over a wide range. These tests



(a) 25 POC quota (pg cell⁻¹) 20 15 HL-Control HL-PO₄ lim 10 HL-NO3 lim LL-Control 5 LL-PO4 lim LL-NO3 lim 0 20 60 80 100 40 Cell volume (µm³) 100 (b) 80 Cell volume (µm³) 60 40 20 0 100 300 0 200 400 Coccosphere volume (µm³) (c) 3.4 3.2 (ml) 3 DSL (µm) 2.6 2.4 2.2 5 6 7 8 3 4 g Cell/coccosphere size (µm)

30

Figure 3. Cellular POC : PON (a), POC : POP (b) and PIC : POC (c) ratios.

give us plots of the value of the cost function (Eq. 11) as a function of the value of the imposed parameter. The process was repeated separately for the four unknown parameters and Fig. S5 shows the results for the N-limited culture of Langer et al. (2013). For all of the parameters except for K_R , this exercise yielded a U shaped curve with a minimum of the cost function corresponding to the best-fit parameter values presented in Table 3. This shows that the model is well suited to find a best-fit value for these parameters. Three minima of the cost function were found for K_R (Fig. S5), of which only the lowest was consistent with values reported in the literature (e.g. Riegman et al., 2000). This value was chosen to obtain the best-fit of the model to the experimental data.

Figure 4. (a) POC quota vs. cell volume, (b) Cell volume vs. coccosphere volume in high-light (HL) and low-light (LL) conditions, (c) Distal shield length (DSL) vs. coccosphere and cell diameter. Solid symbols are cell size and open symbols are coccosphere size. Dotted line is the linear regression.

4 Discussion

4.1 Batch culture experiments

The batch culture experiments presented here provide new insights into the physiology of the numerically dominant coccolithophore *E. huxleyi* under conditions of light and nutrient limitation. Leonardos and Geider (2005) carried out cultures in low-light and low-phosphate conditions with a non-calcifying *E. huxleyi* strain and thus did not report PIC : POC ratios. The culture study reported here is thus the first experiment where changes in the PIC : POC ratio due to light limitation are explored for nutrient-limited cultures. In our



Figure 5. Model fitted to the data of the nitrate-limited cultures of Langer et al. (2013) (Inst = instantaneous).

experiments, cultures were harvested at relatively low cell densities, i.e. a maximum of ca. 1.6×10^5 cells mL⁻¹ in the P-limited low-light experiment and $< 1.3 \times 10^5$ cells mL⁻¹ in all other treatments. The aim was to ensure that changes in the carbonate system were within a minimal range (<10% except for the P-limited experiments in which the DIC changes were 12 and 13%; Table 1) that is not expected to have a significant influence on measured physiological parameters (Langer et al., 2007; LaRoche et al., 2010). Hence, it can be stated that the observed phenomena stem from N or P limitation and/or light limitation (depending on the treatment) rather than from carbon limitation.

Comparison of the growth curves illustrated in Fig. 1 demonstrates that growth limitation was attained in both our low-nutrient and low-light treatments relative to control conditions. Consistent with previous experimental results (Langer et al., 2013; Leonardos and Geider, 2005; Müller et al., 2012; Oviedo et al., 2014; Rouco et al., 2013), the relatively low cellular PON or POP quotas (and high POC : PON and POC : POP ratios) at the end of the low-nutrient experiments relative to the control indicate that nutrient limitation

of growth occurred in our low-nutrient experiments. The stationary phase was not attained in the P-limited low-light culture, but it can be inferred that cells were P limited from (a) the POP quota, which was lower than that of the control, (b) the POC : POP ratio, which was higher than that of the control and (c) a deviation of the growth curve from exponential growth starting (at the latest) on day 16 of 19. While a decline in POP quota is an early sign of limitation, the decline in growth rate occurs later, indicating more severe limitation. The cessation of cell division (stationary phase) would be the last stage in the process of becoming fully P limited over the course of a batch culture.

In nutrient-replete conditions, low light had no effect on POC quota (Fig. 2b) and cell size (Fig. 4) within the limit of uncertainty of the measurements, whereas it caused a decrease in PIC quota (and therefore a decrease in PIC : POC ratio). Although PIC quota also decreased in low light for nutrient-limited conditions (Fig. 2a), the PIC quota for nutrient-replete conditions in low light was unexpectedly low indicating a potential anomaly in the calcification process for this experiment.



Figure 6. Model fitted to the data of the nitrate-limited cultures of strain RCC911 in high-light conditions.

In our experiments N limitation led to an increase in the PIC: POC ratio in both high- and low-light conditions, a result that is consistent with most previous N limitation studies with E. huxleyi (see review by Raven and Crawfurd, 2012), but the cause of this increase appears to vary. According to Müller et al. (2008) and Raven and Crawfurd (2012), Nlimited cells decrease in volume due to substrate limitation and lower assimilation of nitrogen in the G1 phase of the cell division cycle, but in our experiments N limitation did not cause an obvious decrease in cell volume or POC quota, but rather an increase in PIC quota relative to nutrient-replete cells in both high- and low-light conditions (Fig. 2a). Both Müller et al. (2008) and Fritz (1999) also reported an increase of the PIC content of E. huxleyi in N-limited conditions. The increase in PIC quota is difficult to explain in light of the observations that coccolith size was lower in N-limited cultures and coccosphere volume was broadly comparable (given the error margins) in control and N-limited cultures (Fig. 4).

P limitation had the greatest effect on cell size, cells being significantly larger under P limitation than in control conditions, for both high- and low-light regimes. The increase in cell volume was accompanied by increases in both POC and PIC quotas, again in both light conditions (Fig. 2). According to Müller et al. (2008), P limitation inhibits DNA replication while biomass continues to build up, leading to an increase in cell volume. This could explain the very high volume of P-limited cells in high-light conditions in our experiments, and the slightly increased cell volume in the Plimited, low-light experiment, compared to experiments not limited by PO₄. P limitation resulted in a considerably higher coccosphere volume than the other nutrient conditions, in line with the observations of Müller et al. (2008) and Oviedo et al. (2014). In high light, the PIC quota in P-limited cells was more than tripled relative to nutrient-replete conditions. This general effect of phosphate limitation was also reported by Raven and Crawfurd (2012) and is likely due to the occurrence of larger (as shown by high DSL values) and potentially more numerous coccoliths (Gibbs et al., 2013). In the P-limited experiment, PIC: POC ratios increased relative to nutrient-replete cultures, similar to the experiments of van Bleijswijk et al. (1994) and Berry et al. (2002), although Oviedo et al. (2014) reported that the response of the PIC : POC ratio to P limitation is strain specific in E. huxleyi. The increase in PIC: POC in E. huxleyi is often greater for P limitation than for N limitation (Zondervan, 2007), as in our high-light experiment. However, in low light the PIC: POC ratio was higher under N limitation, highlighting that colimitation can have unexpected physiological consequences.

In our experiments the PIC : POC ratio decreased with light limitation in nutrient-replete and nutrient-limited conditions (Fig. 3). Zondervan (2007) stated that the ratio of calcification to photosynthetic C fixation increases with decreasing light intensities due to the lower saturation irradiance for calcification than for photosynthesis in *E. huxleyi*. However, due to a more rapid decline of calcification relative to photosynthesis below saturation levels, this ratio decreases again under strong light-limiting conditions (below approximately 30 µmol photons m⁻² s⁻¹). Several culture studies us-



^a = model results; ^b = analysis results

Figure 7. (a) Maximum normalised surface uptake rate V_{maxN} for nitrate vs. the cell volume. Data from Marañón et al. (2013) in black, data from Litchman et al. (2007) in red and the Droop model output for the experiments presented in this work in blue and green depending on the strain. (b) Minimum cellular quota Q_{min} for nitrate vs. the cell volume. Data of Marañón et al. (2013) and the results from the model and analysis of the present study. (c) V_{maxN} vs. the half-saturation constant for nitrate uptake K_{N} . Data of Litchman et al. (2007) and results from the Droop model in nitrate-limited conditions.

ing different *E. huxleyi* strains have reported this trend. Using the same L:D cycle (12:12) as employed in our experiments, Feng et al. (2008) also reported a decreasing PIC : POC ratio between 400 and 50 µmol photons $m^{-2} s^{-1}$. Comparable observations have been reported in studies that used a 16:8 L:D cycle, with decreasing light from 300 down to a minimum of 30 µmol photons $m^{-2} s^{-1}$ (Trimborn et al., 2007; Rokitta and Rost, 2012). Again with a 16:8 L:D cycle, Rost et al. (2002) reported a decrease of the PIC : POC ratio between 80 and 150 µmol photons $m^{-2} s^{-1}$ (for a *p*CO₂ level comparable to that in our experiments), but an increase of the ratio to 80 µmol photons $m^{-2} s^{-1}$. Our results indicate that calcification was more severely limited than photosynthesis at 30 µmol photons $m^{-2} s^{-1}$ in strain RCC911.

The non-significant correlation between DSL and coccosphere size (Fig. 4c) is not consistent with the correlation reported by Gibbs et al. (2013) between coccolith and coccosphere size in fossil sediment samples, but the number of observations in our study was too low to draw a robust conclusion about the relationship. The significant correlation between cell and coccosphere volume (Fig. 4b) and observations of other studies (e.g. Aloisi, 2015; Gibbs et al., 2013) support the conclusion that coccosphere size in the water column and in sediments could be used as a proxy for cell size (and thus POC quota).

In summary, apart from the phosphate-limited low-light experiment, nutrient limitation led to a cessation of cell division (entry into stationary phase) at the end of the experiment. Nutrient limitation decreased the particulate organic P or N quota for the limiting nutrient (POP for P limitation and PON for N limitation) and increased the PIC : POC ratio under both light conditions. Discerning the effect of nutrient limitation on morphological properties was complicated by the relatively large margins of error, but the overall trend was of an increase in cell/coccosphere size under P limitation and no obvious effect under N limitation. Light limitation decreased the PIC quota, the cell size and the PIC : POC ratio in every nutrient condition, whereas POC : PON and POC : POP decreased with light limitation. Further investigations need to be carried out to improve the understanding of the effect of light intensity on the PIC : POC ratio.

4.2 *E. huxleyi* physiological parameters obtained by modelling growth in a batch reactor

In contrast to the Monod model, the Droop model was able to accurately reproduce the experimental data obtained in experiments with *E. huxleyi* strain RCC911 as well as the experiments of Langer et al. (2013). The Droop model was notably able to reproduce the increase in cell number after the limiting nutrient had been exhausted. This indicates that, as for several other phytoplankton groups (Lomas and Glibert, 2000), *E. huxleyi* has the ability to store nutrients internally to continue growth to some extent when external nutrient lev-



Figure 8. Model fitted to the data of the nitrate-limited cultures on RCC911 strain in low light. The shaded area corresponds to the equivalent nitrate concentration in the BIOSOPE ecological niche of coccolithophores at the GYR station (between 150 and 200 m depth).

els become very low. In our experiments and those of Langer et al. (2013), cells grew on their internal nutrient reserves and managed two to three cell divisions in the absence of external nutrients. These observations are consistent with the explanation of both Monod and Droop models by Bernard (2011).

Numerous studies have estimated the maximum nutrient uptake rate V_{maxR} and the half-saturation constant for nutrient uptake K_R , especially for nitrate uptake, for a variety of phytoplankton species. The values obtained in our study for K_N for high-light *E. huxleyi* cultures (Table 3) are comparable to those reported in the literature. Using *E. huxleyi* in chemostat experiments, Riegman et al. (2000) found K_N values between 0.18 and 0.24 μ M and K_P between 0.10 and 0.47 μ M. In addition, they reported a V_{maxN} of 7.4.10⁻⁶ μ mol cell⁻¹ d⁻¹, which is similar to that found for RCC911 and PML B92/11 (Table 3).

When comparing physiological parameters between phytoplankton taxa, the scaling of physiological parameters with cell size has to be taken into account (Marañón et al., 2013). Marañón et al. (2013) plotted Q_{min} and μ_{max} against cell size (see Fig. 7b for Q_{min} vs. cell size) for different phytoplankton species. In these plots coccolithophores fall with the smallest diatoms. Figure 7a reports V_{maxN} vs. cell size for different groups of phytoplankton based on the results of Litchman et al. (2007) (using a compiled database) and of Marañón et al. (2013) (22 cultivated species) and the results obtained with the Droop model in this study. Despite the different procedures used to obtain V_{maxN} (simulated with a model or measured experimentally), all values for coccolithophores fall in the same range. Collos et al. (2005) and Litchman et al. (2007) found a linear correlation between the maximum uptake rate and the halfsaturation constant for nitrate uptake across several phytoplankton groups (Fig. 7c). This correlation defines a physiological relation between the capacity to assimilate nutrients efficiently (high V_{maxR}) and the capacity to assimilate nutrients in low-nutrient environments (low K_R), and thus thrive in oligotrophic conditions. This analysis shows that large phytoplankton like diatoms and dinoflagellates have high maximum nitrate uptake rates and high half-saturation constants for nitrate uptake. The half-saturation constant for nitrate uptake for *E. huxleyi* is consistently low compared to other groups of phytoplankton, which means that it will be competitive in low-nitrate waters (Litchman et al., 2007).

4.3 Controls on *E. huxleyi* growth in the deep BIOSOPE niche

The BIOSOPE cruise was carried out in 2004 along a transect across the South Pacific Gyre from the Marquesas Islands to the Peru–Chili upwelling zone. The aim of this expedition was to study the biological, biogeochemical and bio-optical properties (Claustre et al., 2008) of the most oligotrophic zone of the world ocean (Claustre and Maritorena, 2003). The deep ecological niche of coccolithophores along this transect occurred at the deep chlorophyll maximum (DCM; Beaufort et al., 2008). According to Claustre et al. (2008) and Raimbault et al. (2008), the nitrate concentration at the GYR station at the DCM (between 150 and 200 m depth) was be-



Figure 9. Left panel: In situ data (0 to 250 m) at the GYR station of the BIOSOPE transect (114.01° W, 26.06° S). Profiles of in situ measured chlorophyll *a*, PAR irradiance and nitrate concentration are shown. The dashed line represents an extrapolation of the irradiance between 117 m (last point measured) and 250 m considering a constant attenuation coefficient K_d ($K_d = 0.025 \text{ m}^{-1}$ from Claustre et al., 2008) and a simple light calculation taken from MacIntyre et al. (2002). The dotted black line is the depth at which the K_N (0.09 µM) is observed. This depth also corresponds to the lower limit of nitrate limitation. Light limitation starts above the DCM and intensifies with depth. The green shaded area corresponds to the location of the maximum of coccosphere abundance taken from Beaufort et al. (2008) between 120° and 107° W. The right panel shows the growth rate of *E. huxleyi* with depth at the GYR station (calculated using Appendix Eq. A8).

tween 0.01 and 1 μ M. In our nitrate-limited low-light culture experiment (Fig. 8), this concentration occurred between the end of the exponential growth phase and the beginning of the stationary phase (days 8 to 9), when nitrate limitation began to affect instantaneous growth rates. Claustre et al. (2008) reported a nitrate concentration < 3 nM (i.e. below the detection limit) in the 0–100 m water column, whereas phosphate concentration was always above 0.1 μ M in surface layers (Raimbault and Garcia, 2008). Moutin et al. (2008) concluded that phosphate was apparently not the limiting nutrient for phytoplankton along the BIOSOPE transect. A potential influence of organic nitrogen sources, which *E. huxleyi* is capable of using (Benner and Passow, 2010), cannot be excluded, but these would be expected to have been distributed vertically in a similar way to nitrate.

The picture that emerges from Fig. 9 is consistent with the model of Klausmeier and Litchman (2001), who predicted that growth in a DCM should be limited by both light and one nutrient, with the upper layer of the DCM being limited by nutrient supply and the deeper layer by light. The experiments and modelling work presented here allow us to confirm that growth of *E. huxleyi* in the deep niche at the GYR station of the BIOSOPE transect was clearly limited by light in the lower part of the DCM, and by nitrogen in the upper part of the DCM and upper water column. Ni-

trification and the vertical diffusivity of nitrate through the nitracline (Holligan et al., 1984) needs to be taken into account and could potentially be a source of dissolved nitrate in the deep niche of coccolithophores. The depth-distribution of the modelled E. huxleyi growth rate, and of dissolved nitrogen, light intensity, chlorophyll a concentration and coccolithophore abundance supports the inferred light-nitrate co-limitation (Fig. 9). We used the physiological parameters constrained in our experiments together with a steady state assumption for uptake and assimilation of nitrate (see appendix) to obtain the vertical profile of E. huxleyi growth rate at the GYR station (Fig. 9). This calculation, forced by the irradiance and nitrate data from the GYR station, shows that E. huxleyi growth rate was maximal at a depth corresponding to that of the measured maximum chlorophyll a concentration. The half-saturation constant for nitrate uptake $K_{\rm N}$ constrained with the Droop model (0.09 μ M) lies within the deep niche (Fig. 9). The maximum estimated growth rate at the GYR station (0.06 d^{-1} at 173 m depth) corresponds to an E. huxleyi generation time of 11.5 days, suggesting that division rate at the DCM was extremely slow (0.086 division per day), all the more so since this estimate does not consider grazing and vertical export of cells. Reports of the in situ growth rate of phytoplankton are not common, including for E. huxleyi, due to the inherent difficulties in measuring this parameter (Laws, 2013). Goldman et al. (1979) reported phytoplankton doubling times in the North Pacific around 0.36-0.89 per day which corresponds to a growth rate of approximately $0.25 d^{-1}$. Selph et al. (2011) estimated growth rates in the equatorial Pacific between 110 and 140° W to be below $0.3 d^{-1}$ for the phytoplankton community living at 1% of surface irradiance with net growth rates (considering mortality rates) around zero.

With the above limitation pattern in mind, it is possible to predict the effect of nitrate and light variability on the vertical evolution of the E. huxleyi PIC : POC ratio in gyre conditions. According to our experimental results, the PIC: POC ratio increases slightly with nitrate limitation but the strongest effect on PIC : POC ratio seems to be in response to light intensity. As noted above (Sect. 4.1), several studies have shown that the PIC : POC ratio increases with decreasing irradiance down to an average of $55 \pm 25 \,\mu\text{mol}$ photons m⁻² s⁻¹, but that it decreases with light limitation below this value. At the BIOSOPE GYR station, the PIC: POC ratio of E. huxleyi would be expected to be intermediate in surface waters (nitrate-poor but high light intensity) and then to increase and attain a maximum value in lower subsurface waters down to the upper part of the deep niche (between 80 and 30 μ mol photons m⁻² s⁻¹; therefore between 110 m and 150 m depth). The PIC: POC ratio would then decrease in the lower part of the deep niche, and finally decrease drastically in deeper, relatively nitrate-rich but extremely lowirradiance waters. This prediction cannot be verified with the available published data from the BIOSOPE transect and PIC: POC ratio of coccolithophores are not only controlled by light and nitrate conditions, but a comparable pattern for the upper part of the ocean was observed through in situ measurements by Fernández et al. (1993). Our predictions need to be verified via in situ studies of DCM zones dominated by coccolithophores. Klaas and Archer (2002) reported that coccolithophores are responsible for the main part of calcium carbonate export to the deep sea and that the rain of organic carbon is mostly associated with calcium carbonate particles, because of their higher density than opal particles and higher abundance than terrigenous material. The gyre ecosystem is a good example of the fact that effects on the rain ratio, and therefore on the carbon pump and carbonate counter-pump, need to be integrated over the whole photic zone. A low PIC quota due to the majority of production occurring at low irradiance in the deep niche would limit the E. huxleyi-related calcium carbonate rain to the sediments, and potentially also limit the ballasting of organic carbon to the deep ocean.

5 Conclusions

We present one of the few laboratory culture experiments investigating the growth and PIC : POC ratio of the coccolithophore *E. huxleyi* in light- and nutrient-limited conditions, mimicking those of the deep ecological niche of coccolithophores in the South Pacific Gyre (Beaufort et al., 2008; Claustre et al., 2008). By combining batch culture experiments with a simple numerical model based on the internal stores (Droop) concept, we show that (1) E. huxleyi has the capacity to divide up to several times in the absence of external nutrients by using internal nutrient stores; (2) a simple batch culture experimental set-up combined with a Droop model, as opposed to the more time-consuming and expensive continuous culture approach, can be used to estimate fundamental physiological parameters that describe the response of phytoplankton growth to nutrient availability and (3) the position of the deep coccolithophore niche of the South Pacific Gyre coincides with the depth of maximum potential growth rate calculated by our physiological model for E. huxleyi; at shallower depths growth is strongly limited by dissolved nitrate availability, while at greater depths it is strongly limited by the paucity of light. These observations confirm the theoretical prediction of Klausmeier and Litchman (2001) with regard to the environmental controls of growth in the DCM. Our conclusions were based on experiments using E. huxleyi strain RCC911 that was isolated from surface waters of the BIOSOPE transect and it will be important to repeat this approach using deep-dwelling strains. There is potential for our approach to shed light on the functioning of other oligotrophic, low-light phytoplankton ecosystems live in cold, dark, nutrient-poor Arctic and Antarctic waters.

6 Data availability

Data from the BIOSOPE transect of attenuation coefficient, nitrate concentration and chlorophyll *a* were taken from the BIOSOPE database available on the website: http://www.obs-vlfr.fr/proof/php/x_datalist.php?xxop= biosope&xxcamp=biosope; and access have been provide by C. Schmechtig.

Appendix A

To obtain the growth rate through the vertical profile at the station GYR, we needed to express the cellular quota Q_N as a function of the nitrate concentration NO₃ [*N*]. To achieve this, we resolved the system of three equations from the Droop theory:

$$\frac{\mathrm{d}Q_{\mathrm{N}}}{\mathrm{d}t} = N_{\mathrm{UP}} - \mu \times Q_{\mathrm{N}} \tag{A1}$$

$$N_{\rm UP} = S_{\rm Cell} \times V_{\rm maxN} \times \frac{[N]}{[N] + K_{\rm N}}$$
(A2)

$$\mu = \mu_{\max} \times \frac{(1 + KQ_N) \times (Q - Q_N^{\min})}{(Q - Q_N^{\min}) + KQ_N \times (Q_N^{\max} - Q_N^{\min})}.$$
 (A3)

Considering a stationary state (uptake-assimilation steady state) and thus assuming the differential Eq. (A1) equal to zero, we resolved the system to express the cellular quota $Q_{\rm N}$ vs. the nitrate concentration (see Fig. A1):

$$A = \frac{1}{2 \times (1 + KQ_{\rm N}) \times \mu_{\rm max} \times (K_{\rm N} + [N])} \times \left(K_{\rm N} \times (1 + KQ_{\rm N}) \times \mu_{\rm max} \times Q_{\rm N}^{\rm min}\right)$$
(A4)

$$B = \left((1 + K Q_{\rm N}) \times \mu_{\rm max} \times [N] \times Q_{\rm N}^{\rm min} \right) + ([N] \times S_{\rm cell} \times V_{\rm maxN}).$$
(A5)

$$C = \frac{4(1+KQ_{N}) \times \mu_{\max} \times [N]}{\left(K_{N}+[N]\right) \times \left(KQ_{N} \times Q_{N}^{\max} - (1+KQ_{N}) \times Q_{N}^{\min}\right) \times S_{cell} \times V_{maxN}} + \left((1+KQ_{N}) \times \mu_{\max} \times (K_{N}+[N]) \times Q_{N}^{\min} + [N] \times S_{cell} \times V_{maxN}\right)^{2}}$$
(A6)

$$Q_{\rm N} = A \times (B+C) \tag{A7}$$



Figure A1. Cellular quota of nitrogen vs. the nitrate concentration using parameters of the best-fit results of the model ran for the low-light and nitrate-limited experiment with RCC911.

Thus, the growth rate can be expressed depending on the irradiance (and K_{Irr} ; see Sect. 2.2.1) and the cellular quota Q_N . Physiological parameters are known (output of the model for the experiment in high-light and low-nitrate conditions):

$$\mu = \mu_{\max} \times \frac{(1 + KQ_N) \times (Q - Q_N^{\min})}{(Q - Q_N^{\min}) + KQ_N \times (Q_N^{\max} - Q_N^{\min})} \times \left(1 - e^{\left(\frac{-\ln r}{K_{\ln r}}\right)}\right).$$
(A8)

The vertical profile of the growth rate of coccolithophores at the GYR station, calculated with this equation, is shown in Fig. 9.

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