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The effects of light, macronutrients, trace metals and CO_2 on the production of calcium carbonate and organic carbon in coccolithophores—A review

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Abstract

The ratio of calcium carbonate to organic carbon (C) production in the surface ocean is thought to be one of the key marine biotic climate variables, through its effect on ocean C cycling. This ratio is significantly affected by calcification and photosynthetic C fixation in coccolithophores. The abundance of coccolithophores and their rates of calcification and organic C fixation are in turn affected by climate-related changes in the ocean. However, there still exists disagreement on the strength of this feedback mechanism, which is due to the complexity of interactions of the factors regulating phytoplankton growth and ecosystem functioning. This review gives a qualitative overview on experimental and field data of coccolithophores, mainly *Emiliania huxleyi*, that are most relevant to actual oceanographic conditions and are likely to change in the foreseeable future under a changing climate. The focus is on the bottom-up control factors light, macronutrients, trace metals and carbon dioxide (CO_2), which can be of use in modelling studies.

Several trends have been identified that should be considered when attempting to simulate *E. huxleyi* growth. Light seems to be the central factor determining the occurrence of blooms. At low irradiance the calcite to organic C production ratio increases, but appears to decrease again when irradiance becomes severely limiting. Phosphate and nitrate limitation lead to an increase in the ratio of calcite to particulate organic carbon (POC), which is also shown for zinc but not for iron. This is mainly due to the fact that coccolith formation is generally less dependant on nutrient concentration than is cell replication. Finally, CO_2-related effects in *E. huxleyi* and the other bloom-forming coccolithophore species *Gephyrocapsa oceanica* have been observed. Under high light conditions, calcification decreases with increasing CO_2 concentration. Depending on the nutrient status of the cells, the production of POC strongly increases, or decreases under elevated CO_2 concentrations. In contrast, under low light conditions no sensitivity of calcification to CO_2 was observed, whereas POC production always strongly increases with CO_2 under nutrient-replete conditions. How different growth conditions taken together finally affect coccolithophore calcification and organic C production is discussed for some factors, but needs further investigation.

Keywords: Coccolithophores; Light; Nutrients (mineral); Metals; Carbon dioxide

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1. Introduction

The production of calcareous shells in the surface ocean by coccolithophores, foraminifera and pteropods results in a reduction of seawater alkalinity and hence in a shift towards a higher carbon dioxide (CO_2) partial pressure

$$Ca^{2+} + 2\ HCO_3^- \rightarrow CaCO_3 + CO_2 + H_2O. \quad (1)$$

Contrary, the production of organic carbon (C) by phytoplankton causes a reduction in the CO_2 concentration of the surface ocean (Volk and Hoffert, 1985; Bates et al., 1996; Zeebe and Wolf-Gladrow, 2001)

$$6\ CO_2 + 12\ H_2O \rightarrow C_6H_{12}O_6 + 6\ O_2 + 6\ H_2O. \quad (2)$$

This is the reason why marine biogeochemists widely agree that one of the key marine biotic climate variables is the ratio of calcite to organic C production in the surface ocean. Disagreement exists on the potential magnitude of effects, changes in this ratio may have on atmospheric CO_2, as well as to what extent it may vary. The calcite to organic C production ratio varies either due to a change on the organism level or a shift in ecosystem composition.

In spite of the biogeochemical and ecological importance of coccolithophores and the large amount of data from laboratory, mesocosm and field observations, especially for the species *Emiliania huxleyi*, relatively little is known about the factors initiating their blooms. The main reason is probably the complexity of interactions of the factors regulating growth under natural conditions. An algorithm accurately simulating trends in the production of calcite and particulate organic carbon (POC) by coccolithophores in ocean biogeochemistry models would be needed in order to better predict ocean C cycling.

The different approaches to modelling production of organic C and calcite by coccolithophores that have been published to this date can be classified into three groups. The first group (e.g., Six and Maier-Reimer, 1996; Heinze, 2004) models changes in the ratio between net community calcification and primary production driven by changes in silicate concentration (Six and Maier-Reimer, 1996), or pCO_2 (Heinze, 2004). They do not distinguish between a change in this ratio caused by a shift in ecosystem structure (e.g., from coccolithophores to diatoms) or by a change in coccolithophore physiology (e.g., changes in the production per cell). While this approach is comparatively simple, it may describe the main effects of calcification on the global ocean biogeochemistry quite well.

The second group (Aksnes and Egge, 1991; Aksnes et al., 1994; Buitenhuis et al., 2001; Gregg et al., 2003; Le Quéré et al., 2005) attempts to describe the ecological success of coccolithophores relative to other phytoplankton groups as a function of environmental factors. The models describe the wax and wane of *E. huxleyi* blooms in terms of biomass, where calcite production is assumed to be in a fixed proportion to the coccolithophores primary production and can thus not be regulated separately. There are considerable differences in terms of the processes controlling coccolithophore growth between these models. For example, the study by Aksnes and Egge (1991) and Aksnes et al. (1994) identifies the high affinity of *E. huxleyi* for phosphate as a main controlling factor for the occurrence of blooms, while the model by Gregg et al. (2003) does not contain phosphate (and therefore no limitation by it). Some consensus seems to have evolved that the high light requirement by *E. huxleyi* is a major controlling factor. Regional studies (Aksnes and Egge, 1991; Aksnes et al., 1994; Buitenhuis et al., 2001; also Tyrrell and Taylor, 1996 and Merico et al., 2004, discussed further below) have achieved reasonable agreement with data, while the distribution of coccolithophores modelled by the global ecosystem models (Gregg et al., 2003; Le Quéré et al., 2005) still shows disagreement with observations (Anderson, 2005).

Similar to the models in the second group, the third group (Tyrrell and Taylor, 1996; Merico et al., 2004; Pasquer et al., 2005) has a separate component for the organic biomass of *E. huxleyi*, but in addition a calcite component. This allows a separate description of the dependency of calcification and organic C fixation on different environmental conditions. So far, these models reflect a decoupling of the regulation of calcification from organic C production in two aspects: Firstly, it is assumed that the production of calcite is not limited by the availability of nitrogen (N) or phosphorus (P), in contrast to the production of organic C. Secondly, calcification is made less dependant on light than organic C synthesis. Theoretically, there should be no difficulty to implement further dependencies, for example on CO_2 or trace metals, into this modelling approach.

To improve biogeochemical models in terms of ecophysiology, data on the general effects of growth

factors on phytoplankton production are needed, rather than their effects on cellular physiological functions. However, for modellers often coming from a completely different field, it may be a hard task to understand the observations published by marine biologists and use them for their ocean biogeochemistry models. It is for example of importance to appreciate the relevance of experimental conditions and procedures to be able to interpret the data. Are the experimental conditions relevant to the field situation? What units are used (for example cell numbers or biomass)? From what perspective are the data looked at (for example the changes in calcification and POC production or just changes in their ratio)? Are the observations in different experiments indeed contradictory, or has this something to do with different growth conditions or measuring techniques? These and other questions should be addressed, before experimentally derived trends on calcium carbonate and organic C production can be used in biogeochemistry models.

The purpose of this study is to give a qualitative overview on relevant observational data on general effects of the growth factors light, nitrate and phosphate, trace metals, and CO_2 on coccolithophores, as well as their interactions. The intention is not to give insight in the physiological mechanisms of this phytoplankton group. For data on the effect of temperature on growth rate and the ratio of calcification and organic C production in *E. huxleyi* the reader is referred to the review by Paasche (2002). Of the circa 200 coccolithophore species living today (Winter and Siesser, 1994), only one species, *E. huxleyi*, was studied intensively in laboratory experiments and in the field. This is mainly due to the fact that this species is easy to cultivate, its blooms can easily be observed by remote sensing techniques because coccoliths intensely backscatter light (Brown and Yoder, 1994; Voss et al., 1998; Tyrrell et al., 1999; Balch et al., 2001), and it is thought to be a good model organism (Westbroek et al., 1993). Therefore, this study inevitably focuses on this species.

2. Light

2.1. Field situation

Many blooms of *E. huxleyi* have been studied in the past 50 years and although they are very different in size, timing and location, there seems to be a general pattern with respect to hydrographical conditions in these blooms. Whether they occur in the south-eastern Bering Sea (Stabeno et al., 2001; Stockwell et al., 2001; Hunt and Stabeno, 2002; Shin et al., 2002; Tyrrell and Merico, 2004), in the Gulf of Maine (Balch et al., 1991, 1992; Townsend et al., 1994), in the North Sea (Wal et al., 1995; Buitenhuis et al., 1996; Marañón and Gonzalez, 1997; Head et al., 1998; Rees et al., 2002), on the Celtic and Armorican Shelf (Holligan et al., 1983), in the northeast Atlantic Ocean south of Iceland (Fernández et al., 1993; Holligan et al., 1993; Robertson et al., 1994; Tyrrell and Taylor, 1996), in the western English Channel (Garcia-Soto et al., 1995), in a Norwegian Fjord (Berge, 1962; Kristiansen et al., 1994; Veldhuis et al., 1994; Fernández et al., 1996), off Bermuda (Haidar and Thierstein, 2001) or in the Northeast Subarctic Pacific (Putland et al., 2004), they all appear to be confined to the top 10–20 m and to seldom more than 30 m of the surface ocean in stable, stratified waters. Monthly observations of satellite data by Iglesias-Rodríguez et al. (2002) suggest that *E. huxleyi* blooms primarily in temperate, and high-latitude oceans with relatively high irradiance. Nanninga and Tyrrell (1996) and Tyrrell and Taylor (1996) suggest that intense light is an essential, although not a sufficient, requirement for bloom formation by *E. huxleyi* (see also Nanninga and Tyrrell, 1996, for overview and literature on this topic).

Maximum downwelling irradiance at the sea surface can reach up to 2000 μmol photons $m^{-2} s^{-1}$ in the photosynthetically active range at noon under clear skies (Kirk, 1983). Average irradiances within the surface mixed layer vary strongly with mixed-layer depth and light attenuation. Values of more than 500 μmol photons $m^{-2} s^{-1}$ have been found during *E. huxleyi* blooms in shallow mixed layers (Nanninga and Tyrrell, 1996 and references therein). However, also values of less than 10% of the surface irradiance have been observed, caused either by deep mixing (about 100 m) or high concentrations of chlorophyll or suspended particulate matter (e.g. 10 mg Chl m^{-3} at 20 m mixed-layer depth).

2.2. Growth rate

In spite of the abundant evidence that *E. huxleyi* requires high light conditions to form blooms, this species appears to grow well under low irradiance (Paasche, 1999). Bleijswijk et al. (1994b) showed that *E. huxleyi* growth was light dependant between

5.9 and 70 μmol photons $m^{-2} s^{-1}$, with no growth below an irradiance level of 5.9 μmol photons $m^{-2} s^{-1}$ at 10 °C. At 15 °C, Zondervan et al. (2002) reported growth rates of 0.5 d^{-1} at 15 μmol photons $m^{-2} s^{-1}$, increasing to a maximum growth rate of 1.1 d^{-1} at 150 μmol photons $m^{-2} s^{-1}$, consistent with Nielsen (1997), who found light saturation at 200 μmol photons $m^{-2} s^{-1}$. In a non-calcifying strain grown at 15 °C, growth rates increase from 0.38 to 0.83 d^{-1} over a photon flux density range of 50–800 μmol photons $m^{-2} s^{-1}$ (Harris et al., 2005). At a temperature of 20–24 °C growth becomes fully light saturated at circa 300 μmol photons $m^{-2} s^{-1}$ (Paasche, 1967; Brand and Guillard, 1981; Paasche, 1999; see overview by Paasche, 2002). Thus, saturating light photon flux densities for growth appear to depend on the temperature, increasing at higher temperatures. Note that the reported irradiance saturation levels for *E. huxleyi* are higher than those typical for diatoms and dinoflagellates (Richardson et al., 1983).

2.3. Photosynthetic C fixation and calcification

In addition to cellular growth rate, the volume of *E. huxleyi* cells increases with increasing irradiance (Paasche, 1967; Bleijswijk et al., 1994a; Muggli and Harrison, 1996; Paasche, 1999; Sunda and Huntsman, 2000). Since *E. huxleyi* has no rigid cell wall or a large vacuole, the cell volume is probably closely proportional to the amount of the cellular organic C content (Paasche, 1968). Bleijswijk et al. (1994b) observed an increase in POC content of 40–140% (depending on the time of sampling) with increasing photon flux density from circa 6 to more than 70 μmol photons $m^{-2} s^{-1}$. Similar results were obtained in a later study by Zondervan et al. (2002), where cellular POC content increased by 56% when photon flux density increased from 15 to 150 μmol photons $m^{-2} s^{-1}$.

In experiments by Nimer and Merrett (1993) photosynthetic C fixation in *E. huxleyi* was light-limited at 50 μmol photons $m^{-2} s^{-1}$, increasing rapidly with increasing photon flux density up to 200 μmol photons $m^{-2} s^{-1}$ with a 2-fold increase to a maximum rate at 300 μmol photons $m^{-2} s^{-1}$. Bleijswijk et al. (1994b) and Zondervan et al. (2002) reported that photosynthesis by *E. huxleyi* was saturated already at 140–150 μmol photons $m^{-2} s^{-1}$, whereas Balch et al. (1992) and Nanninga and Tyrrell (1996) reported maximum rates of photosynthesis at irradiances above 500 μmol photons $m^{-2} s^{-1}$ (see also Nanninga and Tyrrell, 1996; Paasche, 2002 for summary of experimentally derived *P–I* curves of *E. huxleyi*). Thus, photosynthetic C fixation in *E. huxleyi* is highly sensitive to irradiance, increasing strongly with increasing photon flux density in laboratory experiments (Paasche, 1964; Balch et al., 1992; Fernández et al., 1993; Nimer and Merrett, 1993; Paasche and Brubak, 1994; Bleijswijk et al., 1994b; Paasche, 1999; Zondervan et al., 2002).

In contrast to other phytoplankton species (Kirk, 1983), *E. huxleyi* shows no photoinhibition even at irradiances of up to 1700–2500 μmol photons $m^{-2} s^{-1}$ (Balch et al., 1992; Nielsen, 1995; Nanninga and Tyrrell, 1996). These findings, as well as the timing of the blooms, often in mid-summer (Balch et al., 1991; Fernández et al., 1993) when surface irradiances are high, suggests that these algae preferably grow at high irradiance (Baumann et al., 2000; Ziveri and Thunell, 2000).

Because calcification in *E. huxleyi* also requires energy (Anning et al., 1996), it is a light-dependant process (see Paasche, 2002, for a review). This was shown in several studies, where calcification strongly depends on the irradiance. Bleijswijk et al. (1994b) showed that the calcium carbonate ($CaCO_3$) content per cell increased by 30–110% (depending on time of sampling) with increasing irradiance from 5.9 to more than 70 μmol photons $m^{-2} s^{-1}$. Similar results were obtained in laboratory experiments by Zondervan et al. (2002), where cellular $CaCO_3$ content increased by 32% over a photon flux density range of 15–150 μmol photons $m^{-2} s^{-1}$. Paasche (1999) reported a doubling in cellular calcium content with irradiance increasing from 8 to 330 μmol photons $m^{-2} s^{-1}$. Nimer and Merrett (1993) observed a doubling in calcification rates when the irradiance was raised from 50 to 300 μmol photons $m^{-2} s^{-1}$. This is consistent with findings in the field, where calcification rates were frequently high in the surface, decreasing steeply to subsurface layers (Fernández et al., 1993; Wal et al., 1995).

However, laboratory experiments and field observations have shown that calcification in *E. huxleyi* is considerably less light-dependant than photosynthesis, saturating at lower irradiance than photosynthesis (Paasche, 1964; Balch et al., 1992; Fernández et al., 1993; Bleijswijk et al., 1994b; Paasche and Brubak, 1994; Wal et al., 1994, 1995). Paasche (1964) reported saturation levels somewhat below 90 μmol photons $m^{-2} s^{-1}$, which was confirmed by his later study (Paasche, 1999), where light saturation

between 50 and 100 μmol photons $m^{-2} s^{-1}$ was reported. This is consistent with observations by Bleijswijk et al. (1994b), Paasche (1998), and Zondervan et al. (2002), where calcification in *E. huxleyi* saturated at levels of circa 70 and 80 μmol photons $m^{-2} s^{-1}$, respectively, although Nimer and Merrett (1993) reported light saturation levels of 300 μmol photons $m^{-2} s^{-1}$. Balch et al. (1992), modelling the ratio of calcification to photosynthetic C fixation and assuming uniform mixing, suggested that due to the lower light-saturation of calcification compared to photosynthetic C fixation in *E. huxleyi*, the $CaCO_3$:POC production ratio should increase dramatically in the deeper reaches of the euphotic zone.

It should be noted, however, that the observations by Paasche (1964), Balch et al. (1992) and Nimer and Merrett (1993) were based on short-term incubations. In investigations by Bleijswijk et al. (1994b), Paasche (1999) and Zondervan et al. (2002), where *E. huxleyi* cultures were acclimated to growth conditions, a decrease in calcification relative to photosynthesis at irradiances below 30 μmol photons $m^{-2} s^{-1}$ was shown. This was mainly due to a more rapid decline in calcification under light limitation, which is probably the sum of two separate effects: less calcite is deposited in each coccolith, and (in some clones) fewer coccoliths are produced by each cell (Paasche, 1999). Due to the lower saturation irradiance for calcification, the ratio of calcification to photosynthetic C fixation increases with decreasing light intensities. However, due to a more rapid decline of calcification relative to photosynthesis this ratio decreases again under strongly light-limiting conditions. Consequently, the $CaCO_3$:POC ratio should at first increase with depth and then decrease again in deeper reaches.

3. Nitrate and phosphate

3.1. Field situation

Blooms of *E. huxleyi* almost always occur in areas where nitrate and/or phosphate concentrations are very low (Balch et al., 1991; Fernández et al., 1993; Holligan et al., 1993; Kristiansen et al., 1994; Townsend et al., 1994; Wal et al., 1995; Ziveri et al., 1995; Buitenhuis et al., 1996; Stockwell et al., 2001; Iglesias-Rodríguez et al., 2002; Olson and Strom, 2002; Rees et al., 2002; Tyrrell and Merico, 2004). From these findings it was argued that *E. huxleyi* is able to build up its massive blooms by outcompeting other phytoplankton species at levels of inorganic P down to the nM range (e.g. Riegman et al., 1992, 2000; Egge and Heimdal, 1994).

However, *E. huxleyi* appears to grow well under both high and low nutrient concentrations or high as well as low ratios of nitrate to phosphate. Wal et al. (1994) and Bleijswijk and Veldhuis (1995) found that different nutrient regimes in mesocosm enclosures had no influence on the gross growth rate of the *E. huxleyi* population. Results from Egge and Aksnes (1992) and Bratbak et al. (1993) illustrate that *E. huxleyi* reaches high cell densities both at high and low N:P ratios with high and low nutrient concentrations. Egge and Heimdal (1994) showed that *E. huxleyi* blooms at phosphate concentrations ranging from 0.4 to 1 μmol l^{-1}.

Egge and Aksnes (1992) convincingly showed in mesocosm studies that diatoms dominate when the silicate concentration is above 2 μmol l^{-1}. This seemed to apply over a wide range of environmental conditions. It is well known that diatoms bloom even when the mixed layer is deep and that these blooms are followed by coccolithophore blooms when surface waters become stable with a shallow mixed layer (Margalef, 1978). It can be inferred that factors other than the nutrient concentrations or their ratio, or at least in addition to the nutrient concentration, are a causative factor of blooms of *E. huxleyi* (for example light and temperature) (see also Balch et al., 1991). This is confirmed by the study of Lessard et al. (2005), who showed that there is no strong association between high N:P ratios (P more limiting than N) and the occurrence of *E. huxleyi* blooms (see their Table 2 for overview on N and P concentrations in blooms). They suggest that it is the combined abilities of *E. huxleyi* to use non-nitrate N (Palenik and Henson, 1997) in addition to its exceptional P acquisition capacity (Riegman et al., 2000) that provide it a competitive edge in nutrient-depleted waters with shallow mixed layers and high light. They believe that ecosystem modelling including *E. huxleyi* should not contain a dependency on high N:P ratios as a trigger for blooms of this species (see also Merico et al., 2004).

3.2. Photosynthetic C fixation and calcification

Although nutrient concentrations or their ratio do probably not determine whether *E. huxleyi* blooms in nature, phosphate and nitrate appear to have a pronounced effect on the regulation of

cellular calcification and organic C production (see for review on physiology Berry et al., 2002; Paasche, 2002). Riegman et al. (2000) reported from laboratory experiments an exceptional P assimilation capability by *E. huxleyi*, expressing the highest affinity for inorganic P ever recorded for a phytoplankton species, with a maximum phosphate uptake rate of $9\,h^{-1}$ and an affinity for P uptake of $20\,L\,\mu mol^{-1}\,h^{-1}$ (their Table 4). With respect to N-limited growth, *E. huxleyi* showed a rather low uptake rate, with a maximum N uptake rate of $0.07\,h^{-1}$ and an affinity for N uptake of $0.37\,L\,\mu mol^{-1}\,h^{-1}$. In comparison with other algal species, *E. huxleyi* therefore is a poor competitor for nitrate under N-limitation (Riegman et al., 2000, their Table 4).

It is of importance to realize that many analyses of *E. huxleyi* cultures in steady-state growth indicate that although calcification roughly equals photosynthesis in terms of the amount of C transported, the ratio of the two processes seldom reaches unity (see review by Paasche, 2002, his Table 2, for summary on the $CaCO_3$:POC ratio in *E. huxleyi*). Studies of calcification in relation to nutrient availability in culture experiments indicate that reduced levels of both P and N increase the number of coccoliths produced per cell of *E. huxleyi* (Paasche, 1998). Riegman et al. (2000) observed at lower growth rates due to P-limitation that *E. huxleyi* cells contained more organic and inorganic C. These cells also expressed the highest coccolith coverage of the cell surface and increased $CaCO_3$:POC ratios. Similar results were obtained by Linschooten et al. (1991) in batch cultures, although in this study it was not clear whether *E. huxleyi* was limited by P or N. Chemical data reported by Paasche and Brubak (1994) and Bleijswijk et al. (1994b) indicate that if *E. huxleyi* cells are starved of P, the ratio of $CaCO_3$ to C incorporated by photosynthesis may climb from just below unity at nutrient sufficiency to circa 2.2 under P-limitation. The proportion of cells with two or more coccolith layers increased markedly in the chemostats (Paasche and Brubak, 1994). Fritz (1999) observed in N-limited cyclostat experiments an increase in the $CaCO_3$ content and detached coccolith number with decreasing growth rate. In batch or semi-continuous cultures of *E. huxleyi* growing under nutrient-replete conditions, a total of 36 coccoliths were present per cell (attached plus detached). These number rose to 70–120 when either N or P became strongly limiting (Paasche, 1998).

Balch et al. (1993) observed in a laboratory study continually detaching coccoliths during active growth at about the same rate as cells divide. When cell division was inhibited during stationary phase, the coccolith detachment increased 2- to 3-fold until all the cells were naked. Although in addition to increasing coccolith number per cell, also the mean organic C content of the cells increased during nutrient limitation, the molar calcium to C ratio, showed an increase from 1.07 to about 1.38 in a P-limited chemostats study by Paasche (1998). However, this ratio did not change significantly under different growth rates in the N-limited cyclostats of Fritz (1999). Because at least under P-limitation, the POC content per cell also increases, it can be argued that nutrient limitation influences the $CaCO_3$:POC ratio less than the $CaCO_3$:cell ratio (Paasche, 1998).

It should be noted that Berry et al. (2002) and Shiraiwa et al. (2003) observed in their experiments that the increased calcification to photosynthesis ratios in *E. huxleyi* and *G. oceanica* cultures starved by N or P reflected an absolute increase in the rates of calcification, and not simply inhibition of photosynthesis or cell division. It is also noteworthy that under P-limitation *E. huxleyi* cells show a marked increase in cell size (organic C content), which is not observed under N-limitation (Paasche and Brubak, 1994; Paasche, 1998; Riegman et al., 2000; Shiraiwa et al., 2003). Sciandra et al. (2003) observed a decreasing trend in cell volume (probably concomitant with decreasing POC) after N-exhaustion in chemostat cultures. Riegman et al. (2000) reported that the cell volume was about 40% lower under severe N-limitation than under P-limitation and contained 50% less organic and inorganic C.

Also, N or P-limitation may have opposing effects on calcification of individual coccoliths. In the study by Paasche (1998), the mean calcium content of a coccolith decreased from a normal value of 0.60 pg to 0.46–0.49 pg under N-limitation, and increased to 0.67–0.73 pg under P-limitation. The mean length of coccoliths produced in an N-limited chemostat was 5.2% smaller than when grown under a maximum growth rate. These effects were accompanied by corresponding modifications of coccolith morphology. Coccoliths produced under N-limitation appeared to be undercalcified, while those produced under P-limitation tended to be overcalcified (Paasche, 1998). However, Fritz (1999) observed a general increase in coccolith C

content at lower growth rates, while coccolith length and width dimensions remained constant and there were no noticeable differences in the appearance of coccoliths from cultures growing at different rates.

In agreement with laboratory observations, Balch et al. (1991) observed in the Gulf of Maine that the ratios of coccoliths to coccolithophores were highest in the centre, the older parts of the bloom where nutrients were exhausted, and lowest at its periphery where cells were still actively growing. Fernández et al. (1993) suggested that the presence of super-calcifying cells at the later stages of a bloom in the northeast Atlantic may have been the result of ceased cell division rather than enhanced calcification rates. This is in agreement with the findings of Bleijswijk et al. (1994a), Wal et al. (1994), and Delille et al. (2005) in mesocosm bloom experiments, where the *E. huxleyi* population continued calcifying even at the very end of the bloom, where nutrients were exhausted and organic C fixation and division rates decreased markedly. This partly resulted in decreasing cell numbers, but also in an increasing calcite load per cell, the calcite content being inversely correlated with growth rate (Fritz, 1999).

Because coccoliths intensely backscatter light (Voss et al., 1998; Tyrrell et al., 1999; Balch et al., 2001), causing high surface reflectance, blooms of *E. huxleyi* can be observed by remote sensing techniques. Several field observations have been used to verify that many areas of bright waters in satellite images indeed correspond to areas where very high *E. huxleyi* cell numbers are observed (Tyrrell and Merico, 2004, their Table 1). Most of the scatter is probably due to detached coccoliths, increasing in number towards the end of blooms (Holligan et al., 1983; Balch et al., 1991; Wal et al., 1995).

4. Trace metals

4.1. Field situation

Several studies have demonstrated that *E. huxleyi* requires metals for its metabolism, such as iron (Fe) (Brand et al., 1983; Brand, 1991; Sunda and Huntsman, 1995a), zinc (Zn) and cobalt (Co) (Brand et al., 1983; Sunda and Huntsman, 1992, 1995b), selenium (Se) (Danbara and Shiraiwa, 1999), cadmium (Cd) (Sunda and Huntsman, 2000) and manganese (Mn) (Brand et al., 1983; Muggli and Harrison, 1996). Sunda and Huntsman (1995b) showed that the primary requirement for Co in *E. huxleyi* is partially (~70%) replaceable by Zn. The ability to replace Co by Zn or vice versa is shared by some other eukaryotic algae (Sunda and Huntsman, 1995b) but not by all (Timmermans et al., 2001).

Unlike that for macronutrients, the stoichiometry of trace metals in phytoplankton does not resemble the composition of seawater. This is most obvious for Fe, which is the most abundant trace metal in phytoplankton but the least abundant in seawater. Therefore, atmospheric deposition plays an important role in fulfilling the trace metal requirements for phytoplankton growth. The rates for both atmospheric Fe and Zn deposition are probably up to 10 times lower in today's ocean compared to the glacial ocean (Petit et al., 1999; Schulz et al., 2004, and references therein). At present, Fe concentrations limit phytoplankton productivity in large parts of the world's ocean that are known as high nitrate-low chlorophyll (HNLC) areas (Martin and Fitzwater, 1988; Baar et al., 2005). Although the significance of open-ocean Zn-limitation awaits further assessment, prevailing Zn concentrations have in a few instances been found to limit phytoplankton growth (Coale, 1991), particularly coccolithophores (Crawford et al., 2003). The focus will be here on the role of Zn and Fe for *E. huxleyi* growth.

The cellular requirement of *E. huxleyi* for Fe is lower than for typical coastal diatoms, and at a similarly low level as for other oceanic coccolithophores, especially *G. oceanica* (Brand, 1991; Sunda and Huntsman, 1995a; Muggli and Harrison, 1996, 1997). It has been suggested that its low Fe requirement permits *E. huxleyi* to grow in parts of the world ocean where diatoms are limited by Fe (Brand, 1991; Townsend et al., 1994; Muggli and Harrison, 1997) or by Zn (Sunda and Huntsman, 1992). Townsend et al. (1994), following Mitchell-Innes and Winter (1987), suggested that the depletion of macronutrients and trace metals might facilitate the occurrence of episodic *E. huxleyi* blooms in the Gulf of Maine. Sunda and Huntsman (2000) calculated that *E. huxleyi* would be able to grow at Fe-limited rates of $\sim 0.5\,d^{-1}$ in the North Pacific, calculated from Fe:P ratios below the nutricline and assuming no aeolian Fe input. According to their calculations, in the North Atlantic *E. huxleyi* growth also would be Fe-limited if growth depended only on Fe input from below the nutricline. However, as the North Atlantic

receives much more dust input, growth will not be Fe-limited in these areas.

Datasets that confirm or disprove Fe-limitation of *E. huxleyi* in the field are still rare. In an Fe-enrichment experiment in the HNLC region of the Subarctic Pacific, Lam et al. (2001) found that organic C production under Fe enrichment in short-term incubation experiments were significantly different from the control experiments, probably due to a preferential stimulation of non-calcifying phytoplankton. In contrast, inorganic C production was not significantly affected by the Fe treatment in any of the incubations (their Table 1), supporting the hypothesis that coccolithophores respond little to Fe additions. A similar result has been found from taxon-specific pigment analyses of Fe-addition experiments in a coastal upwelling regime (Hutchins et al., 2002). In the Fe fertilization experiment EisenEx in the Southern Ocean, the initially small cell numbers of *E. huxleyi* decreased after Fe fertilization and did not differ significantly from cell numbers outside the fertilized patch (Assmy et al., 2006). In spite of the ability of *E. huxleyi* to grow at very low Fe concentrations, the distribution of *E. huxleyi* blooms is not at all correlated with regions of Fe deficiency in the ocean (Tyrrell and Merico, 2004), and it is therefore questionable whether this ability has any ecological significance.

The distribution of total dissolved Zn [Zn] and of free Zn [Zn^{2+}] in the ocean is presently even less constrained than that of free (i.e. not organically complexed) Fe concentration ([Fe′]) (e.g. Bruland, 1989). Although Zn has been shown to limit coccolithophore growth in the subpolar North Pacific (Crawford et al., 2003) the general question of Zn-limitation of *E. huxleyi* at present cannot be judged.

4.2. Trace metal quotas, growth rate, $CaCO_3$:particulate organic nitrogen (PON) ratio

The most extensive dataset on the effects of Fe concentrations on cellular Fe quotas and growth rate of *E. huxleyi* has been compiled by Sunda and Huntsman (1995a) in a series of laboratory experiments, with [Fe′] controlled by complexation with EDTA. They show that *E. huxleyi* can down-regulate its Fe:C and Fe:cell composition under Fe-limitation. The intracellular Fe:C quota vary from about 3–4 μmol:mol at a [Fe′] of circa 5 pmol l^{-1} (when the cells were still able to grow at about 70% of their maximum growth rate) to a plateau of about 25–30 μmol:mol at [Fe′] > 80 pmol l^{-1}. The cells reached half their maximum growth rate at [Fe′] values below 3 pmol l^{-1}, at a cellular Fe:C ratio below 3 μmol:mol.

Sunda and Huntsman (1995a) also showed that the surface-normalized Fe uptake rate at the lowest [Fe′] values were a factor of 2–3 times higher in *E. huxleyi* than in all other species investigated (that included several open-ocean diatoms), possibly indicating a high-affinity Fe-uptake mechanism. Mean cell size decreased with decreasing [Fe′], while the C:volume ratio increased by up to 30% (Sunda and Huntsman, 2000). Sunda and Huntsman (2000) also found increased Fe-limitation of growth rate at reduced (although non-limiting) light irradiance, but that effect was fully explainable by the reduced [Fe′] in the medium due to less photoreduction of organically complexed Fe.

Similar experiments also were carried out by Muggli and Harrison (1996) in EDTA-buffered artificial seawater at three different Fe levels and with either nitrate or ammonium as a N source. Unfortunately, their publication only gives the total concentration of Fe added and not free inorganic Fe [Fe′], so that a comparison with Sunda and Huntsman (1995a) is difficult. However, the Fe:C ratios found in Muggli and Harrison (1996) are in a similar range, although somewhat higher, than those found by Sunda and Huntsman (1995a), ranging from circa 6–14 μmol:mol at the Fe-stressed (total [Fe] $\ll$ 0.8 nM) level to 40–80 μmol:mol at low Fe (total [Fe] = 0.8 nmol l^{-1}) and to 80–160 μmol:mol at high Fe concentrations.

The Fe:C cell quotas found recently by Ho et al. (2003) in *E. huxleyi* (46 μmol:mol, calculated from Fe:P and C:P ratios) and *G. oceanica* (62.5 μmol:mol) under [Fe'] = 0.2 nmol l^{-1} agree well with the findings by Sunda and Huntsman (1995a) and Muggli and Harrison (1996) under non-limiting Fe conditions. Schulz et al. (2004) cultivated *E. huxleyi* over a range of [Fe′] of 0.06–360 pmol kg^{-1} at two different CO_2 concentrations, 20 and 7 μmol kg^{-1}. The instantaneous growth rate decreased with decreasing [Fe′] and was half-saturated at about 0.7 pmol kg^{-1}.

The Zn requirement of *E. huxleyi* is also low compared to other phytoplankton species (Sunda and Huntsman, 1992, 1995b). According to Morel et al. (1994), this may be due to the fact that carbonic anhydrase, a Zn metalloenzyme, has no essential function in inorganic C acquisition in coccolithophores, which is confirmed in a study by

Rost et al. (2003). Sunda and Huntsman (1995b) show variations in the Zn:C ratio for *E. huxleyi* from below 1 μmol:mol at $[Zn^{2+}]$ of circa 1 pmol l^{-1} to more than 100 μmol:mol at $[Zn^{2+}] > 1$ nmol l^{-1}. In the presence of circa 10 pmol l^{-1} $[Co^{2+}]$, the cells were able to grow at their maximum rate over the whole $[Zn^{2+}]$ range, indicating that the Zn requirement can be fully substituted with Co in *E. huxleyi*. At values of $[Co^{2+}]$ similar to those observed in the open ocean (about 0.2 pmol l^{-1}, Ellwood and Berg, 2001), growth rates decreased with decreasing Zn:C ratios, reaching about half of the maximum growth rate below Zn:C < 10 μmol:mol. The Zn:C ratios found by Muggli and Harrison (1996) (around 30 μmol:mol, not significantly affected by Fe-limitation or N source) and by Ho et al. (2003) (5 μmol:mol for *E. huxleyi* and 6.4 μmol:mol for *G. oceanica*, both calculated from Zn:P and C:P ratios) fall into the range of values found by Sunda and Huntsman (1995b).

Schulz et al. (2004) also studied the effect of Zn on growth. The free Zn concentrations ranged from 0.3 to 6 pmol kg^{-1} at CO_2 concentrations varying between 30 and 7 μmol kg^{-1}. The growth rate decreased with decreasing $[Zn^{2+}]$ concentrations and was half-saturated at about 0.5 pmol kg^{-1}, in close agreement with the findings of Sunda and Huntsman (1995b), 0.6 pmol l^{-1}.

Over a range of [Fe′] species of 0.06–360 pmol kg^{-1} and at both 20 and 7 μmol kg^{-1} CO_2, neither the $CaCO_3$ content per cell nor the cellular $CaCO_3$ to PON ratio changed significantly (Schulz et al., 2004). However, the $CaCO_3$ production rate per cell decreased about 6-fold with decreasing Fe concentration, proportionally to the growth rate. Thus, Fe depletion reduced growth and the production of cellular PON and $CaCO_3$, causing a constant cellular $CaCO_3$:PON ratio under different Fe concentrations.

Contrary to the findings under Fe-limitation, the amount of cellular $CaCO_3$ content increased from 10 to almost 60 pg with decreasing $[Zn^{2+}]$ from 6 to 0.3 pmol kg^{-1}. This corroborated with scanning electron micrograph images, where cells were shown to be covered with multiple layers of coccoliths under Zn-limitation and a more than 2-fold increase in the $CaCO_3$:PON ratio from 4 to 9. Obviously a de-coupling of cell growth and calcification, where $CaCO_3$ production rate stayed constant over the Zn range tested, caused accumulation of $CaCO_3$ in slow-growing cells of *E. huxleyi*.

Other trace metal quotas for *E. huxleyi* are presented by Muggli and Harrison (1996) (Mn, Cu) and Ho et al. (2003) (Sr, Cu, Co, Cd, Mo). The Mn:C ratio found by Muggli and Harrison (1996) is lower than in other previously reported species (all diatoms). This is in agreement with the finding of Brand et al. (1983), where oceanic coccolithophores have a low requirement of Mn for growth relative to other phytoplankton.

5. CO_2

5.1. Laboratory experiments

Over the last few years, CO_2-related effects on the production of particulate inorganic C and POC in the two related coccolithophore species *E. huxleyi* and *G. oceanica*, as well as on phytoplankton assemblages were tested. The results come from laboratory, mesocosm and field experiments. Riebesell et al. (2000) and Zondervan et al. (2001, 2002) first reported reduced calcite production at elevated CO_2 concentrations in monospecific dilute batch cultures of *E. huxleyi* and *G. oceanica*. Over a CO_2 range of circa 5–33 μmol l^{-1} (corresponding to circa 280–750 ppmV CO_2), under high-light (150 μmol photons m^{-2} s^{-1}) and nutrient-replete conditions, calcification diminished by 10–19% in *E. huxleyi* and by 66% in *G. oceanica*. This was accompanied by a strong increase in cellular POC production of 10–45% in *E. huxleyi* and of 39% in *G. oceanica*, leading to a decrease in the ratio of $CaCO_3$:POC of 27–39% and 76%, respectively. No difference in growth rate was observed with increasing CO_2 concentrations (Zondervan et al., 2002).

In nitrate-depleted, high irradiance (circa 575 μmol photons m^{-2} s^{-1}) chemostat cultures of *E. huxleyi*, Sciandra et al. (2003) also observed a significant decrease in $CaCO_3$ production with increasing CO_2 levels. One to two generation times after increasing the pCO_2 levels (from 400 to 700 ppmV), calcification decreased by 11–25% (depending on how calcification was measured). However, in contrast to the findings of Riebesell et al. (2000) and Zondervan et al. (2001, 2002), in this study a decrease in calcification was not accompanied by a significant reduction in the ratio of $CaCO_3$:POC, since POC production decreased roughly in the same proportion to $CaCO_3$ production with increasing CO_2 levels (see their study and references therein for discussion on the physiological mechanisms). This is in contrast to the chemostat observations of Leonardos and Geider (2005), who found enhanced CO_2 fixation

into organic matter also under nitrate limited, high light conditions in a naked strain of *E. huxleyi*. Note that they did not observe an increase in POC production with elevated CO_2 concentrations under P-limitation.

5.2. Field experiments

In a mesocosm bloom experiment dominated by *E. huxleyi*, the response of primary production and calcification to different partial pressures of CO_2 was investigated (Delille et al., 2005) under intermediate nutrient depletion and high-light conditions (mean photon flux densities of 650 μmol photons $m^{-2} s^{-1}$). The partial pressures of CO_2 tested were circa 180, 370 and 700 ppmV. Assessing the day-to-day dynamics of the net community production and calcification (calculated from changes in alkalinity, DIC, as well as oxygen production and ^{14}C incorporation), they observed that the onset of net community calcification occurred 24–48 h later in the mesocosms with 700 ppmV CO_2 than in the mesocosm with 180 and 370 ppmV CO_2. This reduced the duration of calcification of the bloom in the former. Also, at 180 ppmV CO_2, calcification increased steadily from the very beginning of the bloom in parallel to the exponential rise in net community production, while at 700 ppmV CO_2 calcification occurred suddenly when net community production was at its maximum. The rate of calcification was 40% lower at 700 ppmV CO_2 compared to 180 ppmV CO_2, whereas total organic C production rates were similar under the three pCO_2 conditions. This led to a decrease in the mean ratio of calcification to total organic C production, with values of 0.45 at 700 ppmV CO_2 and 0.73–0.78 at 180 and 370 ppmV CO_2. This was confirmed by the data of Engel et al. (2005), where normalized production rates indicated that the rate of calcification per cell and the day-to-day ratio of $CaCO_3$:POC where lowest in the high-pCO_2 treatment and the size of coccospheres and coccoliths, as well as the weight of individual coccoliths were smallest at 700 ppmV CO_2. Finally, Delille et al. (2005) observed enhanced loss of organic C from the surface of the mesocosms under elevated pCO_2 levels, doubling from 180 to 700 ppmV. This is likely due to enhanced particle settling due to relatively higher production of transparent exopolymer particles (TEP) under 700 ppmV CO_2 (Engel et al., 2005). These results suggest a shift in the ratio of calcium carbonate to organic C production and vertical flux with rising atmospheric pCO_2.

Similar results as in the mesocosm experiment by Delille et al. (2005) and Engel et al. (2005) were obtained in incubations of natural plankton assemblages from the North Pacific Ocean when exposed to experimentally elevated CO_2 levels, at 10–30% of surface irradiance (Riebesell et al., 2000). Compared to the low-CO_2 treatments (circa 250 ppmV) the rate of calcification decreased by 36–83% when incubated for 1.5–9 days at high pCO_2 levels (circa 800 ppmV). The production of POC did not differ significantly between treatments. The authors argue that since no significant difference in calcification was observed between shorter- and longer-term incubations, the observed response most probably reflects a reduction in carbonate precipitation of the calcifying organisms in the plankton assemblage, rather than a CO_2-related shift in taxonomic composition, as observed by Tortell et al. (2002).

The data obtained from experiments testing CO_2-related effects on *E. huxleyi* and *G. oceanica* as well as phytoplankton assemblages are consistent in the sense that under high light conditions, calcification decreases with increasing CO_2 concentration by 10–83% over the CO_2 range of 180–800 ppmV. This is irrespective of whether the DIC system was manipulated by the addition of acid and base (Riebesell et al., 2000; Zondervan et al., 2001), or by adding CO_2 gas mixtures (Riebesell et al., 2000; Sciandra et al., 2003; Delille et al., 2005; Engel et al., 2005). Also, the nitrate concentration did not affect the response of calcification to CO_2-related changes in the growth medium. Calcification decreases due to less production per cell, or due to a delay in the onset of calcification. There is no indication that less coccoliths per cell were produced under elevated CO_2 levels. More likely, coccospheres and coccoliths are smaller and thinner (Engel et al., 2005) or show malformations (Riebesell et al., 2000) at elevated CO_2 concentrations. $CaCO_3$ content and production rates per cell under Fe-limited growth were also higher under low CO_2 concentrations, although not that pronounced, and not detectable under Zn-limitation (Schulz et al., 2004).

Despite the high dissolved inorganic C concentration in seawater, photosynthetic C fixation in *E. huxleyi* and *G. oceanica* seems to be remarkably sensitive and well below saturation at these levels (Paasche, 1964; Nielsen, 1995; Berry et al., 2002; Zondervan et al., 2002; Rost et al., 2003). However, the dependency of POC production to

CO_2 concentration is not straightforward. Whether organic C production in *E. huxleyi* and *G. oceanica* is sensitive to CO_2 concentrations appears to depend on the nutrient status of the cells. It seems that under nitrate replete, high-light conditions, POC production increases strongly with increasing pCO_2 (Riebesell et al., 2000; Zondervan et al., 2001). Under nitrate deplete conditions, organic C production decreases with increasing CO_2 (Sciandra et al., 2003), whereas under intermediate nitrate depletion organic C production remains constant with increasing CO_2 concentrations (Delille et al., 2005; Engel et al., 2005).

When discussing the changes in the ratio of $CaCO_3$:POC production with increasing CO_2 concentrations, it is therefore of importance to distinguish between three scenarios: with increasing CO_2 the $CaCO_3$:POC ratio (1) decreases because $CaCO_3$ production decreases and/or POC production increases; (2) decreases because POC production increases; or (3) stays approximately constant because both $CaCO_3$ and POC production decrease. Which of these scenarios occurs in nature will depend on growth conditions, especially on irradiance and nitrate concentration.

It should be noted that two of the most productive coccolithophore species in terms of $CaCO_3$ export to the sediments, *Coccolithus pelagicus* and *Calcidiscus leptoporus*, do not follow the CO_2-related response in calcification found for *E. huxleyi* and *G. oceanica* (Langer et al., 2006). In *C. leptoporus* an optimum curve with maximum calcification rates at modern surface ocean pCO_2 levels was obtained. This result, together with observations in the sedimentary record of the pre-industrial time and the last glacial maximum, leads to the suggestion that *C. leptoporus* may adapt to changing CO_2 levels. In *C. pelagicus*, calcification rates did not vary significantly at all over the pCO_2 range tested. In both species POC production rates remained constant with varying CO_2.

5.3. CO_2-effects under low light

The results discussed above are all obtained from experiments executed under high-light growth conditions. Riebesell et al. (2000) reported that a similar CO_2-dependant response in a natural plankton assemblage was obtained under reduced light irradiance (10% surface irradiance). However, Zondervan et al. (2002) showed in laboratory experiments that the effect of CO_2 on the production of $CaCO_3$ and POC in *E. huxleyi* strongly depends on the irradiance. In contrast to the findings under high-light (150 μmol photons $m^{-2} s^{-1}$) conditions, no sensitivity to CO_2 in calcification was observed in *E. huxleyi* under light conditions ranging from 15 to 80 μmol photons $m^{-2} s^{-1}$. However, except for the lowest irradiances tested, POC production increased strongly by 53–57% and 43% under 80 and 30 μmol photons $m^{-2} s^{-1}$, respectively, over a CO_2 range of circa 5–31 μmol l^{-1}, where photosynthetic C fixation appears to be co-limited by light and CO_2 at a photon flux density of 30 μmol $m^{-2} s^{-1}$ (Zondervan et al., 2002). Note that in a recent study with a naked *E. huxleyi* strain, no sensitivity of POC fixation to CO_2 was observed under a photon flux density of 80 μmol $m^{-2} s^{-1}$ (Leonardos and Geider, 2005). At a photon flux density of 15 μmol $m^{-2} s^{-1}$, the increase in POC production was only 8% over this CO_2 range, and not very significant (Zondervan et al., 2002). No sensitivity of calcification and a strong increase in POC production with increasing CO_2 concentrations led to a decrease in the $CaCO_3$:POC ratio of 34–41% and 36% under 80 and 30 μmol photons $m^{-2} s^{-1}$, respectively, which was very similar to the decrease in the $CaCO_3$:POC ratio under light replete conditions (Riebesell et al., 2000; Zondervan et al., 2001, 2002). Only under a photon flux density of 15 μmol $m^{-2} s^{-1}$, the change in the $CaCO_3$:POC ratio with CO_2 was not significant (Zondervan et al., 2002).

6. Discussion

The anomalous rapid increase in atmospheric CO_2 concentrations observed since the onset of the industrial revolution, and predicted for the future due to lasting anthropogenic activity (Keeling and Whorf, 2005), is expected to change the environmental growth conditions for marine phytoplankton in a variety of ways. Elevated atmospheric partial CO_2 pressure causes an increase in surface ocean CO_2 concentration, accompanied by oceanic acidification and changes in the $CaCO_3$ saturation state (Brewer, 1978; Caldeira and Wickett, 2003; Feely et al., 2004; Orr et al., 2005). Greenhouse warming may cause a warming of the ocean (Barnett et al., 2005) and a shallowing of the mixed layer (Levitus et al., 2000), changing nutrient concentrations and light conditions (Bopp et al., 2001; Sarmiento et al., 2004). Finally, oceanic trace metal supply by aeolian dust input may be affected

by human land use changes (Jickells et al., 2005). These changes are likely to affect marine phytoplankton physiology and ecology, as well as the pelagic ecosystem as a whole. Conversely, changes in the pelagic ecosystem create the possibility of feedbacks on the climate system itself. One important variable in that respect is the amount of pelagic calcification and the ratio between calcification and organic C production. It is therefore crucial to know the climate change potential of variations in the ratio of marine calcite to organic C production and flux to the sea floor.

Several studies have shown that, depending on season and location, coccolithophores contribute significantly to the pelagic calcite flux (e.g. Steinmetz, 1994; Broerse et al., 2000; Tanaka and Kawahata, 2001; Sprengel et al., 2002). Thus, a change in calcite production by coccolithophores, due to a change in either coccolithophore abundance or in cellular calcite production, may significantly affect the oceanic C cycle, and therefore the partitioning of CO_2 between the surface ocean and the atmosphere. Little is known about the environmental factors important for coccolithophore bloom dynamics, leave alone the effect of changes in causative factors of bloom formation expected for the near future.

This review is a compilation of studies in the laboratory, in mesocosms and in the field on environmental factors important for growth, photosynthethic C fixation and calcification by *E. huxleyi* (and also by *G. oceanica*, where data were available) and is intended of use for modelling studies. The focus is on data relevant to actual oceanographic conditions and growth factors that are most likely to change in the foreseeable future under a changing climate.

It should be noted that the outcome of this exercise is not one general formula (1) (i.e. applicable under all growth conditions) for the dependency of growth, photosynthetic C fixation and calcification on the environmental factors considered. Such a uniform dependency does not exist in nature because of the physiological plasticity of phytoplankton and the existence of a number of various strains (Paasche, 2002). Nevertheless, some general trends have been identified that should be considered when attempting to simulate *E. huxleyi* growth, and modellers will have to decide how to best implement them in their models.

Irradiance seems to be the central factor determining the occurrence of *E. huxleyi* blooms. *E. huxleyi* doubling rates saturate at higher light levels than those of diatoms (with some temperature dependency). POC production as well as calcification are very sensitive to light, but the latter appears to saturate at lower light intensities. This leads to an increase of the $CaCO_3$:POC ratio with decreasing irradiance. At light intensities below 30 μmol photons m^{-2} s^{-1} this trend is reversed.

Phosphate and nitrate limitation lead to an increase in the ratio of calcite to POC. This is mainly because coccolith formation is less dependant on P and N than is cell division, but may also be due to stimulation of cellular calcification rates under nutrient stress. Under nitrate-starvation coccoliths appear to be undercalcified, which may be reflected in the total inorganic C content, whereas under P-starvation coccoliths tend to be overcalcified.

Coccolithophores seem to respond little to Fe addition in the field, but laboratory experiments have shown physiological effects of Fe and Zn-limitation on *E. huxleyi*. This species can down-regulate its Fe:C and Fe:cell composition under Fe-limitation and Fe depletion reduces cell growth, cell volume, and the production of cellular PON and $CaCO_3$. Contrary to the response to Fe-limitation, where the cellular $CaCO_3$:PON ratio remains constant, under Zn-limitation a de-coupling of cell growth and calcification was observed, causing an accumulation of cellular $CaCO_3$.

With increasing CO_2 the $CaCO_3$:POC ratio may decrease because $CaCO_3$ production decreases and/or POC production increases, or this ratio may stay constant because both $CaCO_3$ and POC production decrease under elevated CO_2 levels, depending on irradiance level and nitrate concentration.

In this overview on climate-related effects on coccolithophore growth and calcification a few aspects have not been considered. First, not every growth factor has been discussed. The most important omissions are certainly the effects of temperature and salinity. *E. huxleyi* occurs in waters varying from 2 to 28 °C (McIntyre and Be, 1967) and is known to be one of the most eurythermal and euryhaline phytoplankton species (Winter et al., 1994). Hence, neither temperature nor salinity are likely to be a significant primary factor for bloom formation of this species. However, they appear to have an effect on the regulation of cellular calcification and organic C production. For more information on effects of these factors on *E. huxleyi* growth see Brand (1984), Paasche (1996, 2002), and

Sorrosa et al. (2005). Second, joint effects caused by the simultaneous variation of several environmental factors are only touched in this compilation. Examples discussed are the temperature dependency of the light saturation for *E. huxleyi* growth, or the light and nutrient dependency of the CO_2 effects on $CaCO_3$ production and photosynthetic C fixation. There are a large number of possible further combinations of environmental factors that might be relevant, but for which simply not enough data exist. Third, although *E. huxleyi* forms large blooms over wide areas in the ocean (Iglesias-Rodríguez et al., 2002), because of its small coccolith and cell volume this species is probably not dominant in terms of production and vertical flux of calcite to the deep sea. In contrast, *C. leptoporus* and *C. pelagicus* are two of the heaviest calcifying living coccolithophores and probably the most important species in terms of calcite export to the sediment (Broerse et al., 2000; Ziveri and Thunell, 2000). Hence, it is of major importance to also study effects of environmental growth factors on these species, since at present it is not clear whether the reaction of other coccolithophore species is similar to that of *E. huxleyi*, i.e. whether *E. huxleyi* is a good model organism for coccolithophore calcite production and photosynthetic C fixation. Indeed there are indications that this may not always be the case. Finally, only bottom-up controls are discussed here. However, also top-down control, for example by microzooplankton grazing, mortality or viral lysis, appears to be an important aspect in coccolithophore bloom dynamics (Bratbak et al., 1993, 1996; Nejstgaard et al., 1997; Olson and Strom, 2002). More attention should be devoted to this topic, both in models and in experimental studies.

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