Calcification and transparent exopolymer particles (TEP) production in batch cultures of *Emiliania huxleyi* exposed to different pCO_2

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INTRODUCTION

Coccolithophores, among which *Emiliania huxleyi* (*Ehux*) (Figure 1) is the most abundant and widespread species, are considered to be the most productive calcifying organism on Earth. They play a key role in the marine carbon cycle because of their **calcite production** (in the form of coccoliths) and their subsequent sinking to the ocean floor. Like other phytoplanktonic species, the coccolithophores produce **transparent exopolymer particles (TEP)** (Figure 2) that promote the aggregation of biogenic particles produced in surface oceans, and therefore contribute to the **export of carbon to deep waters.**

Figure 1 : SEM images of *Ehux* for the second experiments at an initial pCO_2 of 630 µatm (Courtesy of N. Van Oostende, UGent).

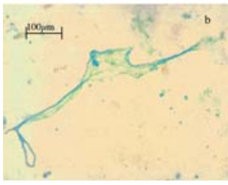

Figure 2 : Microscopic view of a large web-like TEP stained with Alcian Blue [1].

The **rise of atmospheric pCO_2** and the consequent ocean acidification could modify the ecology of coccolithophores, which in turn would have an impact on the production, transformation and fate of carbon in the surface layer of the ocean.

MATERIAL AND METHODS

Three laboratory **batch experiments** (each in duplicate) were conducted with monospecific cultures of *Ehux* exposed to different initial pCO_2, at a temperature of **13°C**. The pCO_2 was not controlled and was let to evolve in these experiments. Cultures were grown in sterilized filtered seawater enriched with nitrate and phosphate (Table 1). Incident irradiance was **150 µmol m^{-2} s^{-1}** and the light/dark cycle was **14h/10h** for the three cultures. Various parameters related to primary production and calcification (**Fluorescence and turbidity, chlorophyll *a* concentration, cell density, nutrient dynamics, total alkalinity, pH, TEP,** particulate inorganic carbon **PIC,** particulate organic carbon **POC**) were followed.

Table 1 : Initial parameters for the 3 batch culture experiments.

Initial parameters	Culture 1	Culture 2	Culture 3
pCO_2 (ppmV)	490	630	930
Initial volume (L)	2	8	8
NO_3 (µM)	f/2 culture medium (Guillard and Ryther, 1962 [2])	32	32
PO_4 (µM)		1	1

REFERENCES

(1) Engel et al., Aquat Microb Ecol, 34: 93-104, 2004; (2) Guillard and Ryther, Can. J. Microbiol., 8: 229-239, 1962; (3) Chisholm and Gattuso, Limnol. Oceanogr., 36 (6) : 1232-1139, 1991; (4) Delille et al., Global Biogeochemical Cycles, 19, GB2023, 2005.

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RESULTS

Phytoplankton growth

The evolution in chlorophyll *a* (chl *a*) concentrations, indicative of ***Ehux* growth**, during the culture experiments is shown in Figure 3. The increase in initial pCO_2 does not lead to a higher biomass. The time lag observed for the onset of the *Ehux* growth may be partially related to the time necessary for the initial pCO_2 to equilibrate with the ambient pCO_2. The cells were not pre-adapted to the initial pCO_2 conditions.

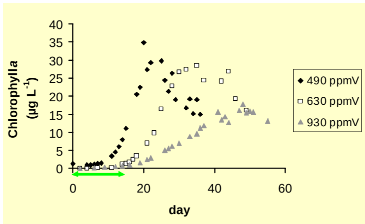

Figure 3 : Evolution of the chl *a* concentrations.

Calcification

The accumulation of calcite was calculated using the alkalinity anomaly technique[3] (Figure 4). The initial pCO_2 does not seem to influence the final concentration of calcite. However, the onset of calcification is delayed in time with increasing initial pCO_2. There is also a **time lag between the onset of organic carbon production and that of the inorganic carbon production** (see the green and red arrows in figures 3 and 4 respectively), which has already been observed in mesocosm studies[4].

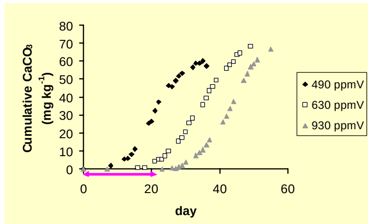

Figure 4 : Evolution of the calcite concentrations.

TEP production

TEP concentrations increase until the end of the experiment when photosynthesis stops and calcification slows down (Figure 5). TEP production is more intense after nutrient exhaustion (data not shown). **POC and chl *a* concentrations are well correlated during the exponential growth phase** (data not shown). After the nutrient exhaustion and during the decline phase, **POC concentrations** continue to increase; they are **fairly well correlated with TEP** that could contribute significantly to the pool of organic matter. TEP accounted for an increase of POC concentrations of 61% in the second experiment (Figure 6). The **concentrations of TEP** were **also strongly correlated with those of calcite** when the saturation state of calcite is above 1 (Figure 7). The formation of coccoliths requires the intracellular production of acidic polysaccharides, which are TEP precursors. These polysaccharides accompany the exocytosis of calcite plates and contribute to their aggregation in the coccosphere. Thus, calcification may be considered as a source of TEP.

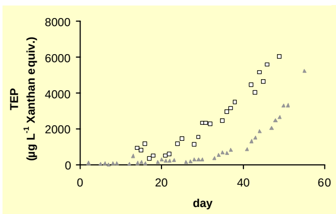

Figure 5 : Evolution of the TEP concentrations. Initial pCO_2 of 630 ppmV (open squares) and 930 ppmV (solid triangles).

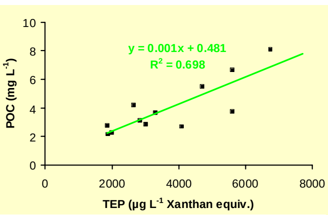

Figure 6 : POC in relation to TEP concentrations after nutrient exhaustion. (Initial pCO_2 of 630 ppmV).

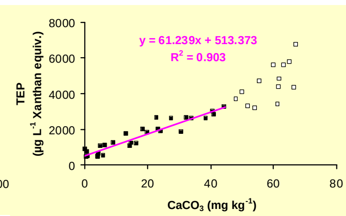

Figure 7 : TEP concentrations in relation to $\Omega_{calcite}$ for the second experiment at an initial pCO_2 of 630 µatm. $\Omega_{calcite}>1$, solid squares and $\Omega_{calcite}<1$, open squares.

Calcification and the saturation state with respect to calcite ($\Omega_{calcite}$)

The rate of calcification has been examined as a function of $\Omega_{calcite}$ (Figure 8). During the course of the experiments, $\Omega_{calcite}$ decreases due to the consumption of CO_3^{2-} ions by calcification. Near the end of the experiment, the net calcification slows down; when $\Omega_{calcite}$ falls below 1, dissolution can take place. For the first and the second experiments, the calcification kicks off when $\Omega_{calcite}$ is around 3 close to initial value, which slows down when it falls below 2. For the third culture, $\Omega_{calcite}$ increases first until 3, due to degassing and primary production, before the onset of calcification. It appears that **3 is a key value below which the calcification is hampered in this species**.

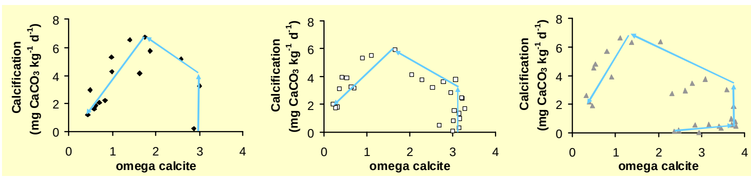

Figure 8 : Calcification in relation to calcite saturation state for each experiment. Initial pCO_2 of 490 ppmV (solid diamonds), 630 ppmV (open squares) and 930 ppmV (solid triangles).

CONCLUSIONS

Ehux growth, calcification and related processes are sensitive to changes in initial pCO_2. Our results show that the development of the *Ehux* cultures is delayed with increasing initial pCO_2. TEP accumulate until the end of the experiment and are enhanced after nutrient exhaustion. TEP contribute significantly to POC concentrations after the exponential growth phase. The very good correlation between TEP and calcite concentrations suggests that the calcification acts as a potential source of TEP in coccolithophore blooms. Finally, an $\Omega_{calcite}$ of 3 seems to be necessary for *Ehux* to calcify.