

Elemental composition of coccoliths: Mg/Ca relationships

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SUMMARY: Coccolithophores produce calcium carbonate platelets, the coccoliths, and play a significant role in the C and Ca cycles. Coccoliths are important components of marine biogenic carbonate sediments and their chemical analysis can provide tools for paleoceanographic investigation. In particular, the Mg/Ca ratio of coccoliths has been proposed as a paleotemperature proxy. The present study uses X-ray microanalysis to evaluate the Ca and Mg composition of heterococcoliths and holococcoliths of different coccolithophore species. Our measurements indicate that the Mg values in heterococcoliths do not exceed a low threshold and do not show any consistent relationship with the Ca content, while the Mg content of holococcoliths spans a wider range, can reach much higher values and shows a linear relationship with the Ca content. Several heterococcolithophore species tend to form separate clusters according to their Mg and Ca values. Within each cluster, there were no consistent differences in the Mg/Ca ratios of specimens sampled at different temperatures or seasons, suggesting that using the Mg/Ca ratio as a paleothermometer may be problematic. Our findings could have implications for the interpretation of the fossil record because Mg-rich calcite dissolves more easily.

Keywords: coccolithophores, X-ray microanalysis, calcium, magnesium, holococcoliths, heterococcoliths.

RESUMEN: COMPOSICIÓN ELEMENTAL DE COCOLITOS. RELACIONES Mg/CA. – Los cocolitóforos producen unas plaquitas de carbonato de calcio denominadas cocolitos, y juegan un papel significativo en los ciclos biogeoquímicos del C y el Ca. Los cocolitos constituyen un componente importante del registro sedimentario y su análisis químico puede proporcionar herramientas para la investigación paleoceanográfica. En particular, se ha sugerido que la relación Mg/CA podría ser usada como un indicador de paleotemperatura. En este trabajo presentamos un estudio, realizado mediante la técnica de microanálisis por rayos X, de la composición elemental de holo- y heterococolitos pertenecientes a diferentes especies de cocolitóforos. Se ha observado que el contenido de Mg en los heterococolitos no supera un umbral muy bajo y no presenta relación con el de Ca, mientras que en los holococolitos el contenido de Mg es en general más elevado, abarca un rango más amplio y guarda una relación lineal con el de Ca. Diversas especies de heterococolitóforos tendían a formar grupos separados en función de su contenido en Ca y Mg. Dentro de cada especie, no se detectaban diferencias consistentes en la relación Mg/CA de especímenes de muestras de agua de distintas temperaturas o tomadas en diferentes estaciones del año, lo que indica que el uso de la relación Mg/CA como paleotermómetro puede ser problemático. Nuestras observaciones pueden tener implicaciones para la interpretación del registro sedimentario, ya que la calcita rica en Mg se disuelve más rápidamente.

Palabras clave: cocolitóforos, microanálisis de rayos X, calcio, magnesio, holococolitos, heterococolitos.

INTRODUCTION

Coccolithophores are a phytoplankton group with the ability of secreting an exoskeleton composed of small calcified platelets or coccoliths at least once in their life cycle. There are two main types of coccoliths. Heterococcoliths are formed by crystal units of variable shape and size, and their biomineralization occurs intracellularly inside vesicles of the Golgi apparatus

(Manton and Leedale 1969, Fresnel 1989, Pienaar 1994). Holococcoliths consist of numerous minute crystallites and their calcification appears to occur extra-cellularly (Manton and Leedale 1963, Rowson *et al.* 1986). Both hetero- and holococcolith-bearing phases may be involved in the life histories of coccolithophores (Parke and Adams 1960, Cros *et al.* 2000, Cros and Fortuño 2002, Billard and Inouye 2004). These organisms are major pelagic primary producers

TABLE 1. – Position and some environmental characteristics of the sampling locations.

Cruise	Date	Position	Station	Depth (m)	Temperature °C	Salinity	No. HET	No. HOL
FAMOSO-1	17/03/2009	41°16.37'N, 3°49.11'E	19	5	13.42	38.28	10	--
FAMOSO-1	17/03/2009	41°16.37'N, 3°49.11'E	19	35	13.12	38.28	10	2
FAMOSO-3	18/09/2009	41°55.76'N, 4°2.67'E	12	5	23.94	38.18	2	7
FAMOSO-3	18/09/2009	41°55.76'N, 4°2.67'E	15	5	22.72	38.23	20	15
FAMOSO-3	18/09/2009	41°55.76'N, 4°2.67'E	15	60	13.50	38.33	27	1

and calcifiers and play a fundamental role in global biogeochemical cycles (Westbroek *et al.* 1993). Coccoliths are an important component of many marine sediments and their elemental composition could have important potential uses as a tool for paleoecological investigation. However, the small size of coccoliths has made studying them difficult. Mann and Sparks (1988) used energy-dispersive X-ray analysis coupled with transmission electron microscopy to show that *Emiliana huxleyi* coccoliths gave peaks of Ca, C, O and trace amounts of S. Some years later, Fagerbakke *et al.* (1994) showed that growth conditions can explain large differences in the molar proportions of Ca/C in *Emiliana huxleyi* coccoliths, and Stoll *et al.* (2007) concluded that there was a positive relationship between the Sr/Ca ratio of some coccolithophore species and their productivity, thus providing a potential tool for evaluating paleoproductivity. Using the Mg/Ca ratio as a paleotemperature proxy, suggested by Stoll *et al.* (2001), has proved more difficult. In general, heterococcoliths have been found to consist mainly of low-Mg (<4% Mg) calcite (Siesser 1977, Siesser and Winter 1994, Stoll *et al.* 2007), although Stanley *et al.* (2005) presented data indicating that the *Pleurochrysis carterae* and *Ochrosphaera neapolitana* coccolithophores secreted high-Mg calcite when cultured in modern seawater, which has a higher Mg/Ca content than late Cretaceous and early Cenozoic seawater. Stoll *et al.* (2007) observed a significant correlation between Mg/Ca and sea-surface temperature for *Helicosphaera carteri* coccoliths in the Arabian Sea, but not for *Calcidiscus leptoporus* coccoliths in either the Arabian Sea or the Sargasso Sea. Ra *et al.* (2010) found that Mg/Ca values of the exponential and stationary phases of cultured *Emiliana huxleyi* and *Gephyrocapsa oceanica* were positively related to temperature, without statistically significant differences in slope, but they concluded that the difference in absolute Mg/Ca values of the two species complicated the use of the Mg/Ca ratio as a paleothermometer and stated that further research into the differences between species was needed before the Ca/Mg could be used as a paleotemperature tool for paleoclimatic reconstructions. To the best of our knowledge, the first measurement of the elemental composition of holococcoliths was carried out by Cros (2001), who examined three species of both holococcolithophores and heterococcolithophores and noticed that the Mg content was higher in the holococcolithophores. These findings highlight that further investigation should be addressed not only to different species, but also to both holo- and heterococcolith crystallization types.

Within this framework, the objective of the present work was to study the elemental composition of heterococcoliths and holococcoliths from a varied group of species sampled in three coccolithophore-rich stations of the northwestern Mediterranean in order to ascertain whether these two coccolith types differ in their relative concentrations of Ca and Mg and to consider the potential use of the Ca/Mg ratio as a paleoceanographic proxy for temperature.

MATERIALS AND METHODS

The studied specimens were sampled (Table 1) from station 19 (5 and 35 m depth) of the FAMOSO-1 cruise (March 2009), and from stations 12 (5 m depth) and 15 (5 and 60 m depth) of the FAMOSO-3 cruise (September 2009). Both cruises were carried out in the Catalano-Balearic Sea (NW Mediterranean) within the framework of projects of the Institut de Ciències del Mar (CSIC, Barcelona). The number of specimens analysed from each sample is indicated in Table 1. In order to avoid confusion, the term “HOL” will be added to the species name when referring to the holococcolithophore phase.

Sea water samples were collected with Niskin bottles attached to a Rosette device. Samples were filtered through polycarbonate filters of 0.8 µm pore size to retain the coccolithophores. The filters were subsequently rinsed with about 1 ml of bottled water. The specimens were analyzed with a Hitachi S-3500N SEM, fitted with a EDS Si(Li) detector (Bruker AXS). A small part of the filters was attached on aluminium stubs and coated with Au-Pd; both Ca and Mg signals, are clearly separated of the Au and Pd peaks in the spectrum. The detector processes X-rays from Z=5 including light elements, with a resolution ≤129 eV. Spectra were acquired at an accelerating voltage of 15 kV. The results for Mg and Ca were expressed as a number of counts per channel. The software employed was QUANTAX Esprit 1.8 (Bruker AXS).

RESULTS

The Ca and Mg counts obtained in the X-ray microanalysis of the FAMOSO-1 and FAMOSO-3 specimens are shown in Figure 1. The Ca counts ranged from slightly more than 4000 to about 58000 in heterococcolithophores and from 7700 to about 42000 in holococcolithophores. The Mg counts did not exceed 800 in most heterococcolithophores; the exception was three specimens from *Algirosphaera robusta* (marked with A in Fig. 1) that had between 1500 and 2100 counts.

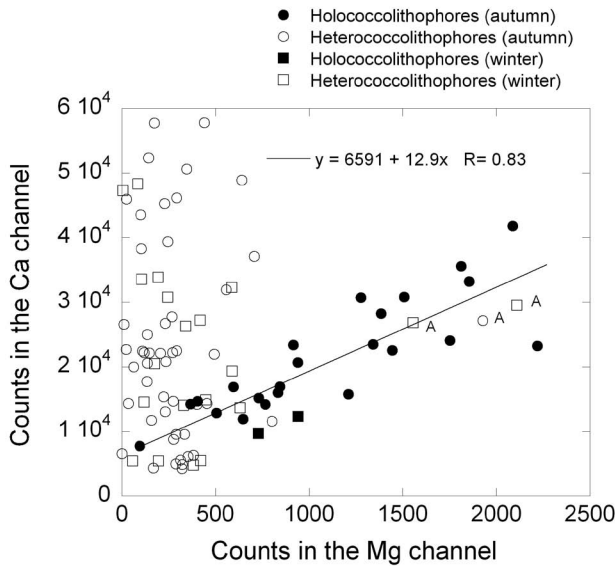


FIG. 1. – Mg and Ca X-ray microanalysis counts for holococcolithophores (black symbols) and heterococcolithophores (open symbols) from cruises FAMOSO-1 (winter) and FAMOSO-3 (autumn). The line and the equation correspond to the linear regression of Ca on Mg counts for holococcolithophores. The points marked with “A” correspond to *Algirosphaera robusta*.

In holococcolithophores, the Mg range went from 100 to about 2200. There was a significant linear relationship between the Ca and Mg counts of the holococcolithophores ($\text{Ca} = 12.9\text{Mg} + 6591$, $R^2 = 0.69$), while there was no relationship between the Ca and Mg counts of heterococcolithophores. Only a few species were present in both seasons (*Emiliana huxleyi*, *Algirosphaera robusta*, *Helicosphaera carteri* and *Syracosphaera molischii*), or in surface and deep samples of the same season (*Emiliana huxleyi*, *Helicosphaera carteri*). Except for *Emiliana huxleyi* (Fig. 2), the points corresponding to different heterococcolithophore species tended to group together, as can be seen in Figure 3, which includes species with four or more specimens

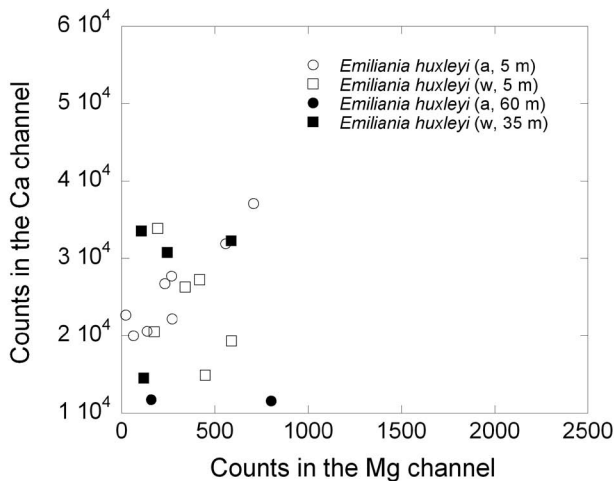


FIG. 2. – Mg and Ca counts for specimens of *Emiliana huxleyi* taken from different depths of the cruises FAMOSO-1 (winter, “w”) and FAMOSO-3 (autumn, “a”).

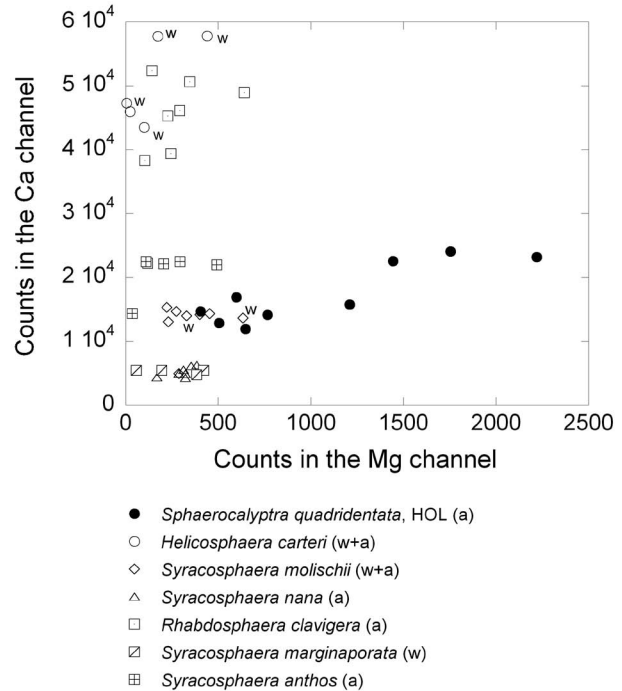


FIG. 3. – Mg and Ca counts of species for which more than four specimens from the cruises FAMOSO-1 (winter, “w”) and/or FAMOSO-3 (autumn, “a”) were analysed. The letter “w” next to a point indicates that it belongs to a winter sample.

in the analyses. However, these species did not show any consistent differences among specimens sampled in different seasons or depths (Figs. 2 and 3).

DISCUSSION

The X-ray microanalysis showed a striking difference between the Mg/Ca relationships of hetero- and holococcoliths. In heterococcoliths, with the exception of *Algirosphaera robusta*, which will be discussed later, Mg counts did not exceed a relatively low threshold and were uncorrelated with the Ca counts. In holococcoliths, however, the Mg counts showed a wider range, could reach substantially higher values than in heterococcoliths and were significantly correlated with Ca.

The lack of heterococcoliths with high Mg counts is consistent with previous data (Siesser 1977, Cros 2001, Stoll *et al.* 2001, Stoll *et al.* 2007, Ra *et al.* 2010). It must be noted, however, that according to our measurements, the actual Mg/Ca ratio in heterococcoliths can be highly variable and, in heterococcoliths with low Ca counts (like those at the bottom left of Figure 1), can even exceed the Mg/Ca count ratio of holococcoliths, which can be adjusted to a line of slope = 12.9. It would be interesting to consider in which part of our Figure 1 would fit the heterococcolith Mg/Ca data of Stanley *et al.* (2005). However, the lack of absolute quantification of the Ca and Mg coccolith content in the two studies precludes this comparison.

It can be hypothesized that the different mineralization behaviour of hetero- and holococcoliths is related to the structural and physiological differences between these two crystallization types. It appears that the process of heterococcolith formation, inside an internal Golgi body vesicle, would allow a stronger biological control of mineralization, resulting in calcite in which the presence of Mg cations does not exceed a certain threshold, in spite of the higher content of Mg relative to Ca in modern seawater (Mg/Ca molar ratio = 5.2). On the other hand, the extracellular calcification process in holococcolithophores, which involves less regulation of crystal growth than that occurring in heterococcolithophores (Young *et al.* 1999), would increase the Mg content in parallel with that of Ca. This process could maintain a relatively constant relationship between the Mg/Ca ratio of the holococcoliths, at least within the calcification range found in this work. In this context, the exceptional behaviour of *Algirosphaera robusta* is very interesting. The heterococcolithophore *Algirosphaera* (Rhabdosphaeaceae) is closely related to *Syracosphaera* (Probert *et al.* 2007), but its coccoliths show a peculiar structure. The formation of the coccolith rim follows the typical heterococcolith biomineralization mode, but the central hood has a unique ultrastructure and its formation appears to follow a different mineralization pattern (Probert *et al.* 2007). These authors went on to suggest that *A. robusta* could be a promising target for comparative biomineralization studies. Our measurements, indicating the high Mg content and Mg/Ca ratio of the coccoliths of *A. robusta*, their outlying position compared to those of the other heterococcolithophores, and their Mg/Ca situation within the holococcolithophore (Fig. 1), support the arguments of Probert *et al.* (2007).

As can be seen in Figures 1 and 3, crystallization mode and taxonomic affinity appear to be in general much more important than temperature in determining the Mg/Ca ratio of the coccoliths. Points of the heterococcolithophore species tend to group together, including those coming from different samples and temperatures. The wide dispersion of Mg and Ca values of *Emiliania huxleyi* could be related to the high genetic heterogeneity of this organism (Iglesias-Rodríguez *et al.* 2006), but again in this case there were no consistent groupings related to season or temperature. Most of our holococcolithophore specimens were found in autumn surface samples with relatively high temperature, but the two winter specimens (Fig. 1), which belonged to unidentified species of *Sphaerocalyptra*, did not appear to depart from the constant Mg/Ca ratio defined by the regression line shown in Figure 1. These observations suggest that using the Mg/Ca ratio as a paleothermometer, even within the same coccolithophore species, could pose many difficulties, as cautioned by previous authors (Ra *et al.* 2010).

The different Mg and Ca biomineralization patterns of hetero- and holococcoliths has paleoceanographical implications. Higher Mg content in calcite increases its

dissolution rate (Chave *et al.* 1962, Rushdi *et al.* 1998). The presence of relatively high Mg concentrations may explain, in addition to their microcrystalline structure, the low preservation potential of holococcoliths, which have very sporadic fossil records (Bown *et al.* 2004). Differences in the Mg and Ca content of holo- and heterococcolithophores could also have consequences in relation to the increasing acidification of the world oceans (Orr *et al.* 2005) because high-Mg calcite dissolves faster than low-Mg calcite after a drop in pH (Tynan and Opdyke 2011).

Major changes in the morphological strategy and ecology of coccolithophores along the geological record have been linked to shifts in the Mg and Ca composition of seawater. Stanley and Hardie (1998) suggested that the rise in the Mg/Ca ratio of seawater and decline in the Ca concentration during the Cenozoic favoured the progressive reduction of coccolith size. Aubry (2007) agreed with this view but concluded that it was also possible that the shift to smaller cells could have been induced by biological factors. In his culture experiments with artificial seawater, Stanley *et al.* (2005), found that heterococcolithophores of three extant species multiplied much faster when the composition of the seawater medium was shifted toward the high Ca concentrations and low Mg/Ca ratios estimated for Cretaceous seas. In this context, it would be interesting to consider how the different biomineralization behaviour of holo- and heterococcolithophores could be integrated into the interpretation of coccolithophore evolution.

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REFERENCES

- Aubry M.-P. 2007. A major Pliocene coccolithophore turnover: Change in morphological strategy in the photic zone. In: Monnechi S., Coccioni R., Rampino M.R. (eds.), *Large Ecosystem Perturbations: Causes and Consequences*. *Geol. Soc. Am., Special Paper* 424: 25-51.
- Billard C., Inoué I. 2004. What is new in coccolithophore biology? In: Thierstein H.R., Young J.R. (eds.), *Coccolithophores: From Molecular Processes to Global Impact*. Springer, pp. 1-30.
- Bown P.R., Lees J.A., Young J.R. 2004. Calcareous nannoplankton evolution and diversity through time. In: Thierstein H.R., Young J.R. (eds.), *Coccolithophores: From Molecular Processes to Global Impact*. Springer, pp. 481-508.
- Chave K.E., Deffeyes K.S., Weyl P.K., Garrels R.M., Thomson M.E. 1962. Observations on the solubility of skeletal carbonate in

- aqueous solutions. *Science* 137: 33-34.
- Cros Miguel M.L. 2001. *Planktonic coccolithophores of the NW Mediterranean*. Ph.D. thesis, Univ. Barcelona, 365 pp. (Publicacions Universitat de Barcelona, 2002).
- Cros L., Fortuño J.-M. 2002. Atlas of Northwestern Mediterranean coccolithophores. *Sci. Mar.* 66(Suppl. 1): 1-182.
- Cros L., Kleijne A., Zeltner A., Billard C., Young J.R. 2000. New examples of holococcolith-heterococcolith combination coccospheres and their implications for coccolithophorid biology. *Mar. Micropaleontol.* 39: 1-34.
- Fagerbakke K.M., Heldal M., Norland S., Heimdal B.R., Batvik H. 1994. *Emiliana huxleyi*. Chemical composition and size of coccoliths from enclosure experiments and a Norwegian fjord. *Sarsia* 79: 349-355.
- Fresnel J. 1989. *Les Coccolithophorides (Prymnesiophyceae) du littoral: Genres: Cricosphaera, Pleurochrysis, Crucioplacolithus, Hymenomonas et Ochrosphaera. Ultrastructure, cycle biologique, systématique*. Ph.D. thesis, Univ. Caen, 281 pp.
- Iglesias-Rodríguez M.D., Schofield O.M., Badley J., Medlin L.K., Hayes P.K. 2006. Intraspecific genetic diversity in the marine coccolithophore *Emiliana huxleyi* (Prymnesiophyceae): the use of microsatellite analysis in marine phytoplankton population studies. *J. Phycol.* 42: 526-536.
- Mann S., Sparks N.H.C. 1988. Single Crystalline Nature of Coccolith Elements of the Marine Alga *Emiliana huxleyi* as Determined by Electron Diffraction and High-resolution Transmission. *Proc. R. Soc. Lond. B* 234: 441-453.
- Manton I., Leedale G.F. 1963. Observations on the microanatomy of *Crystallolithus hyalinus* Gaarder and Markali. *Arch. Mikrobiol.* 47: 115-136.
- Manton I., Leedale G.F. 1969. Observations on the microanatomy of *Coccolithus pelagicus* and *Cricosphaera carterae*, with special reference to the origin and nature of coccoliths and scales. *J. Mar. Biol. Ass. U.K.* 49: 1-16.
- Orr J.C., Fabry V.J., Aumont O., Bopp L., Doney S.C., Feely R.A., Gnanadesikan A., Gruber N., Ishida A., Joos F., Key R.M., Lindsay K., Maier-Reimer E., Matar R., Monfray P., Mouchet A., Najjar R.G., Plattner G.-K., Rodgers K.B., Sabine C.L., Sarmiento J.L., Schlitzer R., Slater R.D., Totterdell I.J., Weirig M.-F., Yamanaka Y., Yool A. 2005. Anthropogenic ocean acidification over the twenty-first century and its impact on calcifying organisms. *Nature* 437: 681-686.
- Parke M., Adams I. 1960. The motile (*Crystallolithus hyalinus* Gaarder & Markali) and non-motile phases in the life history of *Coccolithus pelagicus* (Wallich) Schiller. *J. Mar. Biol. Ass. U.K.* 39: 263-274.
- Pienaar R.N. 1994. Ultrastructure and calcification of coccolithophores. In: Winter A., Siesser W.G. (eds.), *Coccolithophores*. Cambridge Univ. Press, pp. 13-37.
- Probert I., Fresnel J., Billard C., Geisen, M., Young J.R. 2007. Light Electron Microscope observations of *Algirosphaera robusta* (Prymnesiophyceae). *J. Phycol.* 43: 319-332.
- Rowson J.D., Leadbeater B.S.C., Green, J.C. 1986. Calcium carbonate deposition in the motile (*Crystallolithus*) phase of *Coccolithus pelagicus* (Prymnesiophyceae). *Br. Phycol. J.* 21: 359-370.
- Rushdi A., Chen C.-T., Suess E. 1998. The solubility of calcite in seawater solution of different magnesium concentrations at 25°C and 1 atm total pressure: a laboratory re-examination. *La mer* 36: 9-22.
- Ra K., Kitagawa H., Shiraiwa Y. 2010. Mg isotopes and Mg/Ca values of coccoliths from cultured specimens of the species *Emiliana huxleyi* and *Gephyrocapsa oceanica*. *Mar. Micropaleontol.* 77: 119-124.
- Siesser W.G. 1977. Chemical Composition of Calcareous Nannofossils. *S. Afr. J. Sci.* 73: 283-285.
- Siesser W.G., Winter A. 1994. Composition and morphology of coccolithophore skeletons. In: Winter A., Siesser W.G. (eds.), *Coccolithophores*. Cambridge Univ. Press, pp. 51-62.
- Stanley S.M., Hardie L.A. 1998. Secular oscillations in the carbonate mineralogy of reef-building and sediment-producing organisms driven by tectonically forced shifts in seawater chemistry. *Palaeogeogr. Palaeoclimatol. Palaeoecol.* 144: 3-19.
- Stanley S.M., Ries J.B., Hardie L.A. 2005. Seawater chemistry, coccolithophore population growth and the origin of Cretaceous chalk. *Geology* 33: 593-596.
- Stoll H.M., Ruiz Encinar J., Garcia Alonso I., Rosenthal Y., Probert I., Klaas C. 2001. A first look at paleotemperature prospects from Mg in coccolith carbonate: cleaning techniques and culture measurements. *Geochem. Geophys. Geosyst.* 2, 1047, doi: 10.1029/2000GC000144.
- Stoll H.M., Shimizu N., Ziveri P., Archer D. 2007. Coccolithophore productivity response to greenhouse event of the Paleocene-Eocene thermal maximum. *Earth Planet. Sci. Lett.* 258: 192-206.
- Tynan S., Opdyke B. 2011. Effects of lower surface ocean pH upon the stability of shallow water carbonate sediments. *Sci. Total Environ.* 409: 1082-1086.
- Westbroek P., Brown C.W., van Bleijswijk J., Brownlee C., Brummer G.J., Conte M., Egge J., Fernandez E., Jordan R.W., Knap-pertsbusch M., Stefels J., Veldhuis M., van der Wal P., Young, J.R. 1993. A model system approach to biological climate forcing. The example of *Emiliana huxleyi*. *Global Planet. Change* 8: 27-46.
- Young J.R., Davis S.A., Bown P.R., Mann S. 1999. Coccolith ultrastructure and biomineralisation. *J. Struct. Biol.* 126: 195-215.

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