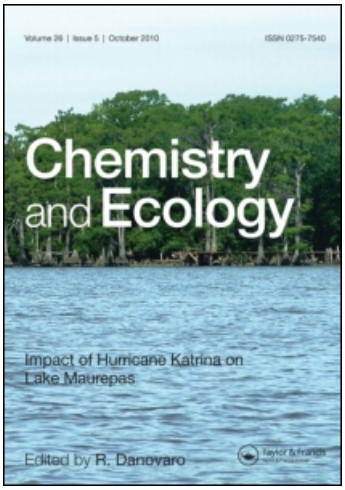

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COCCOLITHOPHORIDS, NUTRIENTS AND THE GREENHOUSE EFFECT

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Despite the fact that coccolithophorids such as *Emiliania huxleyi* are suspected to play an important role in carbon-cycling, there are few data from which to deduce how these organisms may respond to CO_2-induced global warming. The nitrogen and phosphorus nutrient physiology of these organisms, together with its interaction with photosynthesis, needs to be studied especially in connection with the selection for coccolith forming individuals and the quantity and quality of the $CaCO_3$ deposited in the coccoliths. Without such data, it will not be possible to model the contributions that these microalgae may play in arresting the increase in levels of atmospheric CO_2.

KEY WORDS Coccolith, coccolithophorid, *Emiliania huxleyi*, 'greenhouse effect', carbon cycle, nutrient limitation

INTRODUCTION

Although the magnitude and effects of any change in global temperature are still subject to debate, it is now generally accepted that the 'greenhouse effect' is a real phenomenon and that global temperatures are rising or will rise. It may be argued that a situation of high atmospheric CO_2 and associated high temperatures has been encountered before and that various feed-back processes will again occur, returning the planet to a new quasi steady-state. One of these processes is the conversion of atmospheric CO_2 into limestone, mediated by the biota. One organism, the marine alga *Emiliania huxleyi* (formerly called *Coccolithus huxleyi*), has been described as one of the most important lime-secreting organisms on this planet (Westbroek *et al.*, 1984).

Emiliania huxleyi forms frequent extensive blooms in the North Atlantic (Holligan *et al.*, 1983; Holligan and Groom, 1986), an area of particular importance in the global carbon-cycle (Brewer, 1983). These organisms may represent an important sink of atmospheric CO_2 in the ocean, for example at the continental shelf-break (Holligan *et al.*, 1983), and play a crucial role in the carbon-cycle. Furthermore, coccolithophorids such as *E. huxleyi* are suspected to be a source of atmospheric dimethylsulphide (DMS) (Charlson *et al.*, 1987). On oxidation in the atmosphere, DMS contributes to non-sea-salt sulphate, a primary source of cloud condensation nuclei, and hence the biota may have an important effect on cloud formation (Savoie and Prospero, 1985) which in turn affects global warming.

If we are to model the outcome of the greenhouse effect then it is clearly

important that we understand how rapid and effective such biological regulatory systems may be. In order to do that we need to understand the physiology of these organisms, and its relationship to ecological processes. However, although there have been extensive studies of the interaction of carbon metabolism with $CaCO_3$ nucleation and crystallization, little is known about the general physiology of coccolithophorids. The purpose of this paper is to consider what may be pertinent about the physiology of these organisms to a response to global warming, with an aim to high-lighting areas in which, in the view of the author, further research is needed.

COCCOLITHOPHORIDS AND COCCOLITH FORMATION

The coccolithophorids (Prymnesiophyceae) are a group of marine phytoplankton which may produce scales or plates of $CaCO_3$ called coccoliths. The ecologically important species, *Emiliania huxleyi,* has been a subject of research for over 25 years (Paasche, 1964) and numerous strains, uncalcified and calcified, are available in culture. The life cycle of this organism includes a motile uncalcified (S-cell type) and a non-motile calcified form (C-cell type) (Klaveness and Paasche, 1971). However, the degree of calcification in the latter is very variable and, especially apparent in cultured isolates, another form exists, the naked (N-cell) type which, although possessing the basic apparatus for coccolith synthesis, bears no coccoliths. N-cells and C-cells do not appear to be stages in a sexual life cycle (Paasche and Klaveness, 1970) and the abundance of any one form in the population is determined, presumably, by eco-physiological factors (Klaveness and Paasche, 1979). Unfortunately, the distinction between S-cells and N-cells is not often clear in the literature. Much of the work to examine factors promoting calcification in laboratory cultures has probably made use of both N and C-cell types, thus enabling (perhaps unwittingly) a more direct study of the interactions between calcification and other metabolic processes without the complication of cell-cycle changes implicit in comparing C/N-cells with S-cells. However, the underlying questions concerning selection for calcification remain the same.

Coccoliths originate intracellularly but are transported to the outside of the cell (Klaveness and Paasche, 1979; Westbroek *et al.,* 1984). Nucleation and crystal growth occurs on an organic matrix within an endoplasmic-reticular membrane system—the coccolith vesicle. One component of this matrix is a water soluble polysaccharide whilst another is a water-insoluble complex of a positively charged protein and carbohydrate (DeJong *et al.,* 1976, 1979; van der Wal *et al.,* 1983; Westbroek *et al.,* 1984). Dorigan and Wilbur (1973) showed that calcification was inhibited by cycloheximide, an inhibitor of protein synthesis, but the mechanism of this inhibition is not clear. The polysaccharide binds Ca^{2+} and inhibits crystal growth, unless protein is present and then $CaCO_3$ precipitation is stimulated. The polysaccharide also promotes crystallization of $CaCO_3$ as calcite, even in the presence of Mg^{2+} which normally induces aragonite formation (Okazaki and Furuya, 1985). Through the requirement for both carbohydrate and protein in the coccolith matrix, there is scope for interaction between carbon and/or nitrogen metabolism and coccolith synthesis.

In addition, through ionic effects, cell metabolism may have other influences on

coccolith formation. Borowitzka (1982) suggests that algae can initiate and control calcification by altering and modulating the ionic composition of subcellular compartments. For example, various molecules including amino acids affect the crystallization of different morphs of $CaCO_3$, possibly by complexing with interfering ions such as inorganic phosphate which inhibits growth of $CaCO_3$ crystals. Also, intracellular pH is likely to be a factor, first by affecting the equilibrium of dissolved inorganic carbon in the cytoplasm, and secondly (perhaps more important) by altering the proton electrochemical gradient across the coccolith vesicle. The nutrient status of the cells, and use of different sources of nitrogen, are likely to affect intracellular pH and the ability of cells to regulate intracellular pH.

The rate of coccolith production is variable. Overproduction of coccoliths in natural populations can be very common, resulting in the accumulation of coccoliths at the cell surface and shedding of coccoliths (Westbroek *et al.*, 1983). As a consequence, a significant proportion (37–64%) of coccoliths in natural waters may be cell-free (Holligan *et al.*, 1983). However, cultured isolates tend to produce fewer coccoliths; often only a proportion of the cell may be covered. With time the ability to produce coccoliths is often lost (the culture becomes dominated by N-cells), the implication being that the condition(s) required for the stimulation or selection of coccolith formation is/are not met in culture. A satisfactory method for the long term maintenance of calcified forms, or for the conversion of a naked to a calcified culture, does not exist, although some attempts have been made (Wilbur and Watabe, 1963; Sikes and Wilbur, 1982).

ENVIRONMENTAL VARIABLES AND THE SELECTION FOR CALCIFICATION

The role that coccoliths play in the physiological ecology of the algae is not clear, although various proposals have been put forward (Sikes and Wilbur, 1992). A suggestion that the presence of coccoliths gives some degree of protection against predation, has not been substantiated (Sikes and Wilbur, 1982). Other suggestions centre on possible physiological advantages for coccolith synthesis. Among factors suggested to affect selection or differentiation between uncalcified and calcified forms are temperature, nutrient supply and the prevailing rates of photosynthesis and protein synthesis. Some of these factors may also affect the form of $CaCO_3$ in the coccoliths.

In the natural environment, an algal population encounters complex variations of temperature, photosynthetically active radiation (PAR—a composite of diurnal, depth and turbidity interactions), and concentrations of nutrients. With changes in climate associated with global warming, different conditions may develop. As the partial pressure of CO_2 (pCO_2) increases in the atmosphere it will also increase in the sea, but because of the Revelle factor (buffer factor of sea water) this increase will be relatively small (say 10% of the atmospheric rise—Brewer, 1983). Locally in the water column, pCO_2 may be lowered by photosynthesis (Codispoti *et al.*, 1982; Takahashi *et al.*, 1985), perhaps even to levels which may limit the rate of C-fixation. Furthermore, as temperatures rise, stratification may become stronger so that internal wave breaks, which may

introduce nutrients from below the euphotic zone, may become less frequent and nutrient limitation more likely. Rapidly changing weather patterns also affect match and mismatch relationships between predators and prey such that mismatch, and hence nutrient limitation, may become more frequent (see Flynn, 1989). It is not clear what will happen to levels of PAR but it may be lowered as a result of increased cloud cover.

Phytoplankton may therefore have to endure conditions of different temperature, illumination, pCO_2 and nutrient supply compared to those at present. Certain of these changes may promote calcification, with possible release of coccoliths, whilst others may inhibit or select against it. However, in order to predict what may happen, it is important to understand the mechanisms underlying the interaction between these environmental parameters and cell physiology (specifically interactions with coccolith formation).

High temperature appears to favour the growth of calcified forms of coccolithophorids in nature, with uncalcified forms predominating in Arctic waters (Klaveness and Paasche, 1979). Generally, nutrient concentrations (from upwelling of cold bottom waters) and pCO_2 may be expected to be greater in colder waters. Carbonic anhydrase appears to play a minor role in these algae (Sikes and Wheeler, 1982) although photosynthesis in coccolithophorids requires CO_2, rather than HCO_3^- or CO_3^{2-}, as the substrate for C-fixation. Photosynthesis in coccolithophorids may therefore be rate limited by the supply of CO_2(aq) (Sikes and Wheeler, 1982), a condition more likely in warmer waters where the solubility of CO_2 is lessened. Coccolith formation has been suggested to be a mechanism for concentrating intracellular CO_2(aq). One model predicts that in calcified forms, for every 2 molecules of HCO_3^- taken up, one is converted to CO_2 in the cytoplasm and the other is deprotonated to form CO_3^{2-}, eventually precipitated as $CaCO_3$ (Sikes *et al.*, 1980; Borowitzka, 1982). By this scheme, deprotonation and precipitation of $CaCO_3$ effectively aids photosynthesis (Westbroek *et al.*, 1983) by drawing the reaction

$$HCO_3^- \rightarrow CO_2 + OH^-$$

to the right. De Jong *et al.* (1979) say that high rates of photosynthesis are required for calcification; there appears to be a circular argument here as calcification should, by this model, be a prerequisite for high rates of CO_2-fixation. The effectiveness of this 'CO_2-concentrating mechanism' has been questioned by Klaveness and Paasche (1979) and it is also possible that some other factor is responsible for the domination of calcified forms in warmer waters.

Nitrogen-deprivation induces calcification in some naked strains (Wilbur and Watabe, 1963; Baumann *et al.*, 1978) and phosphorus-deprivation has a similar effect (Klaveness and Paasche, 1979). It has been suggested that when cells are starved of nutrients, the stimulation of calcification, resulting in increased density of individual cells, leads to sinking of the organisms into nutrient-rich waters (Baumann *et al.*, 1978). Evidence linking nitrogen-status and the regulation of calcification also comes from the work of Brown and Romanovicz (1976), who found that calcification in the coccolithophorid *Pleurochrysis* was stimulated by growth with serine (which is not a very good source of nitrogen) as a sole nitrogen-source.

From such observations, one may conclude that the loss of the ability for calcification in laboratory cultures (typically growing in the presence of nutrients

at two orders of magnitude more concentrated than in nature and under unnatural illumination, temperature and pCO_2) is either due to an inhibition of calcification or more probably (in view of the time scale involved) a loss of any physiological advantage in calcification, a process which may clearly exert a considerable drain on metabolic demands. Unfortunately, very little is known about nitrogen metabolism in these organisms, and in particular how nitrogen-deprivation affects the enzymes of nitrogen and carbon metabolism. Results obtained from studies with other algae (Hipkin and Syrett, 1977; Hipkin *et al.*, 1983; Everest *et al.*, 1986) show that nitrogen-deprivation promotes considerable changes in photosynthetic capacity and in the activities of certain enzymes of carbon and nitrogen metabolism. From the work of Eppley *et al.* (1971), there is undoubtably a diurnal interaction with carbon-nitrogen metabolism as well.

The suggestions for the selection of coccolith forming individuals, or the triggering of a gene switch for coccolith formation, as a response to carbon-stress or nitrogen-stress, conflict. Carbon-stress (caused by rate limitation of photosynthesis) and nitrogen-stress may be considered mutually exclusive (Flynn *et al.*, 1989; Flynn 1990a). An alternative may be that calcification arises in response to general metabolic stress; carbon, nitrogen and phosphorus-stress each result in a reduction in the rate of amino acid synthesis, for example, and in growth rate. However, as synthesis of coccoliths is itself a metabolically demanding process, there is perhaps a gene switch for calcification which once triggered stays active through many generations through positive feed-back. It is also probable that both carbon-stress and nitrogen/phosphorus-stress will be encountered in nature.

Rate limitation of photosynthesis (by the disassociation of CO_3^{2-} and HCO_3^- in sea water replacing CO_2(aq) used by the algae) is a stress which still allows the growth of an organism. In contrast, nutrient limitation in an algal bloom tends to be absolute rather than rate limiting. Cadée (1985) reports the formation of macroaggregates of *Emiliania huxleyi* at the end of a bloom, saying that only through the formation of such aggregates could a rapid sedimentation of carbon occur. Individual cells or coccoliths sink very slowly, raising a question over the usefulness of coccoliths as an aid to sinking into nutrient-rich waters (Baumann *et al.*, 1978). Such aggregates are often indicative of nutrient-limited algae which continue to photosynthesize, releasing dissolved organic carbon into the water, and are often associated with bacterial activity (Newell *et al.*, 1981). Thus coccolithophorids may bloom and then senesce under a combination of first carbon and then nutrient (nitrogen or phosphorus) stress both of which may be associated with coccolith formation.

THE FORM OF $CaCO_3$ IN COCCOLITHS

Significantly, nitrogen-deprivation may result in the deposition of coccolith $CaCO_3$ in crystalline forms other than calcite, such as aragonite and vaterite (Wilbur and Watabe, 1963). Calcite may comprise only 10–30% of coccolith $CaCO_3$ in these cultures. This phenomenon has been suggested to result from an inadequate supply of intracellular amino acids for protein synthesis which are required for matrix formation (Borowitzka, 1982). The protein associated with coccolith formation contains a high percentage of basic amino acids (Westbroek *et al.*, 1984) which would normally be in short supply in nitrogen-deprived cells

(Flynn, 1990b). Furthermore, the proportion of aragonite may increase in nitrogen-stressed cultures at elevated temperatures (Wilbur and Watabe, 1963). The nitrogen-stressed cultures of Wilbur and Watabe (1963) grew in media containing approximately 100 times more NO_3^- than natural waters. Only in cultures with a vast excess of nitrogen did calcite form the only component of coccoliths, yet it is widely claimed that in nature coccoliths are of calcite (usually quoting Watabe, 1967).

Aragonite, unlike calcite, dissolves at low pressure in shallow waters, leading to an increase in concentration of CO_3^{2-} (and so in alkalinity). This in turn can have significant consequences for the carbon-cycle, since increased alkalinity enhances the absorption of atmospheric CO_2 into surface waters (Whitfield, 1984). The data of Holligan *et al.* (1983) show that *Emiliania huxleyi* blooms in surface waters containing $<2.3\ \mu M\ NO_3^-$, with a suggestion that coccolith release, and hence presumably production, may be correlated with low NO_3^-. In the absence of good data for the kinetics of nutrient transport, and the efficiency of nutrient assimilation (NO_3^- reduction could be rate limiting for example) it is not possible to say when nutrient stress may become significant. However, as temperatures rise, stratification of the water column may become stronger and nutrient limitation more likely. The possible consequences of such events on the formation of aragonite or calcite coccoliths need to be examined. Clearly if aragonite is a major component of coccolith $CaCO_2$ then atmospheric CO_2 may be removed into the oceans more rapidly than considered at present. It should, however, be said that this line of questioning stems from work done over 25 years ago. It is important that it be repeated to verify the results of Wilbur and Watabe (1963).

DISCUSSION AND CONCLUSIONS

Clearly, there are grounds to suspect an important interaction between carbon, nitrogen and phosphorus metabolism in the regulation of coccolith production, yet such interactions have been little studied. We must first understand how coccolithophorids react to controlled conditions before analysing data for natural populations of unknown history growing in a complex, ever-changing, environment. Some questions which need to be answered are:

(1) Under which conditions does calcification confer a competitive advantage, and what conditions influence transitions between uncalcified and calcified forms?

(2) What factors influence the crystalline form and quantity of $CaCO_3$ deposited?

and

(3) What factors affect the release of coccoliths?

Once we understand more about the underlying physiology of these organisms we can then ask:

(4) How do changes in the environment, such as those that may be induced by the 'greenhouse effect', interact with the physiology of the organisms? The environmental factors which need to be considered are: availability of carbon

(pCO_2 and illumination), nitrogen and phosphorus, pH, alkalinity (pCO_2, pH and total alkalinity are interdependent) and temperature.

We also need to know if there are synergistic interactions between any of these factors, and how any of these factors also affect dimethysulphide production.

Only through a better understanding of the physiology of ecologically important groups of organisms, such as coccolithophorids and other important bloom forming Prymnesiophyceae such as *Phaeocystis* and *Chrysochromulina*, can we hope to model the biotic response to changes in the environment caused by CO_2-induced global warming. The fact that we do not understand how the process of coccolith synthesis is regulated does not augur well for predicting how, and how rapidly, coccolithophorids may respond to the effects of global warming. All we can say at present is that the type of changes to be expected as a consequence of the greenhouse effect also appear to be those which stimulate or select for coccolith formation.

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