Effects of unbalanced nutrient regime on coccolith morphology and size in *Emiliania huxleyi* (Prymnesiophyceae)

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Single coccoliths of *Emiliania huxleyi* grown in mesocosm enclosures (60°16′N, 05°14′E, May–June 1991) under different N:P regimes were analysed in a scanning electron microscope. The results indicate that only *E. huxleyi* with Type A coccoliths was present in the enclosures. Approximately 80–90% of the total coccolith assemblages had developed normally, whilst the remainder were malformed, incompletely grown or dissolved. Severely under-calcified specimens were rare and dissolution and breakage less than 5%. The coccoliths were of larger size than normal, as has been found previously in fjords of southwestern Norway, supporting the conclusion that a local population of *E. huxleyi* has developed, specific to these waters. Both phosphorus and nitrogen stress caused significant changes in coccolith size and evidence of malformation was clear, particularly in the low-phosphate enclosure. Although the observations presented here concern only Type A coccoliths and it is not known how nutrient stress may affect the coccoliths of the other types of *E. huxleyi*, they do serve to stress the fact that environmental conditions may possibly obscure genetically determined features. Following our observations on coccolith morphology in relation to nutrient status in enclosures, it will be of interest to test whether a similar correlation can be detected in the natural environment.

Key words: coccoliths, *Emiliania*, morphology, size, nutrient regime

Introduction

Coccolithophorid taxonomy is based primarily on the morphology of the coccoliths themselves and, in the majority of species, cells with only a single type of coccolith or a particular combination of different types of coccolith have been reported, other life-cycle stages being unknown. In a few instances, cells with different types of coccolith, and thus originally described as different species, have been shown to be part of a single life-cycle and, therefore, conspecific (e.g. Parke & Adams, 1960; Thomsen *et al.*, 1991), but in general that information is not available.

In the case of *Emiliania huxleyi* (Lohmann) Hay *et* Mohler it is known that the haploid cell is an unmineralized scaly flagellate (Klaveness, 1972; Green *et al.*, 1996) and that the diploid coccolith-bearing cell carries coccoliths of only one form, i.e. placoliths (Jordan *et al.*, 1995). However, it is known that the coccoliths of *E. huxleyi* can exist in one of several distinct morphotypes and these have been referred to as Types A, B, C and *E. huxleyi* var. *corona* (Okada *et* McIntyre) Jordan *et* Young (e.g. van Bleijswijk *et al.*, 1991; Young & Westbroek, 1991). Types A, B and C have also now been recognized as distinct taxonomic varieties (*E. huxleyi* (Lohmann) Hay *et* Mohler var. *huxleyi*, *E. huxleyi* var. *pujosae* (Verbeek) Young *et* Westbroek *ex* Medlin *et* Green, *E. huxleyi* var. *kleijniae* Young *et* Westbroek *ex* Medlin *et* Green respectively; Medlin *et al.*, 1996), but for brevity and convenience we will continue to use the terms 'Type A, B and C' in this report.

Brand (1981, 1982) described a number of physiological variants of *E. huxleyi*, but at that time it was not appreciated that stable morphological types existed and there is no information, therefore, concerning which variety he was working on. Within Type A particularly it has been noted that minor morphological variation can occur, for example as a response to changes in salinity (Paasche *et al.*, 1996), perhaps leading to local ecotypes. There are indications that Type A coccolith morphology can also be altered under nutrient stress (Young & Westbroek, 1991; Young, 1994). However, there are few confirmed examples of this type of variation, possibly in part because of a lack of controlled experiments and partly because assessment of the degree or extent of calcification is subjective (i.e. it is dependent on individual judgement and is not always easy to quantify). Okada & Honjo (1975) and Kleijne (1990), studying the coccolithophorid flora in the Indonesian Seas and surrounding waters, noted several types of malformed coccoliths. They believed that there was a relationship between the deformation and the nutrient balance, while Young & Westbroek (1991) and Young (1994) suggested that the *E. huxleyi* morphotypes

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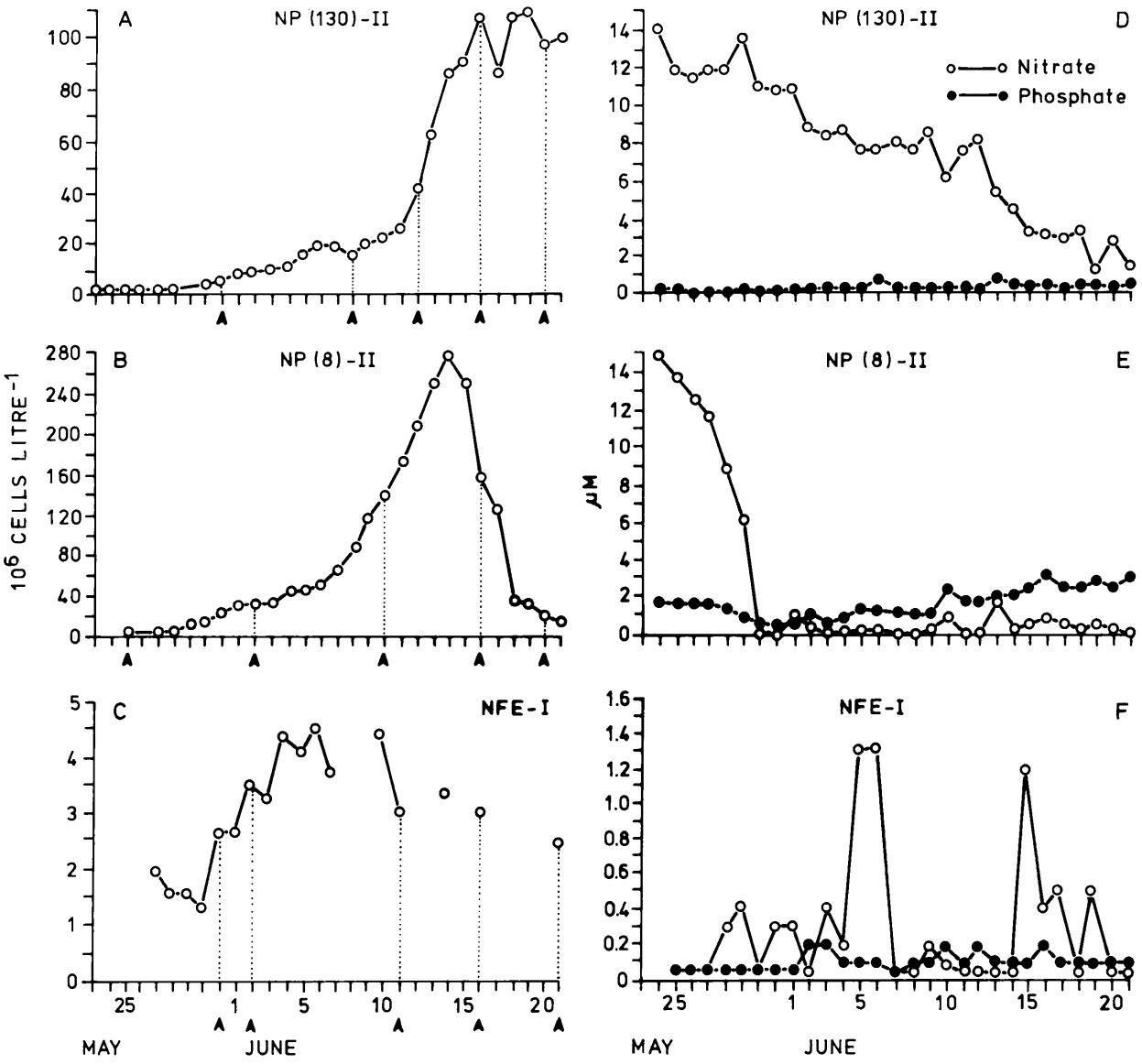

Fig. 1. Changes in cell numbers of *E. huxleyi* (*A–C*) and nutrient concentrations (*D–F*) during May–June 1991 in the NP(130)-II, NP(8)-II and NFE-I enclosures. Arrowheads indicate sampling dates; for exact dates of collection see Table 1. Note the different scales in cell numbers (*A, B* and *C*) and nutrient concentrations (*D/E* and *F*). After Egge (1993). For the initial nutrient status of the NP(130)-II and NP(8)-II enclosures, see text.

described as malformed by Okada & Honjo (1975) and Kleijne (1990), as well as by Verbeek (1989) and Giraudeau *et al.* (1993), were a product of dissolution rather than of primary teratological malformation, and Young (1994) termed them 'collapsed coccoliths'. In samples from the 1992 Norwegian *Emiliania huxleyi* experiment in fjords of southwestern Norway, Young (1994, figs 5, 6) showed that there can be a continuous variation from normal, complete coccoliths to severely affected collapsed specimens. He argued that they are products of dissolution and noted that it can be difficult to distinguish collapsed forms from extremely under-calcified specimens. In the latter case, however, all the basic elements of the coccolith and their internal location should remain intact even if their arrangement may be disturbed. It is our intention here to use the term 'malformed' in the sense of coccoliths that are deformed or misshapen apparently due to primary teratological effects rather than due to the secondary effects of dissolution and collapse.

The aim of the present study was to investigate the effects of unbalanced nutrient conditions on coccoliths of *E. huxleyi* grown in mesocosm enclosures under different N:P regimes. There is no previously published work primarily directed at nutrient effects on *E. huxleyi* coccolith morphology and the 1991 mesocosm experiment provided an excellent opportunity to examine over a period of time some of the intermediate-scale complexities inherent in the natural environment and to relate them to general concepts in coccolithophorid ecology. In a wider context, it is important to know how environmental factors may affect coccolith morphology in general. This information is relevant to work on systematics and identification of living species and in the interpretation of micropalaeontological data.

Materials and methods

Sampling

Coccoliths of *Emiliania huxleyi* were harvested from preserved water samples collected during an experiment conducted from 23 May to 21 June 1991 at the Marine Biological Field Station of the University of Bergen (60°16′N, 05°14′E) using floating enclosures supplied with nitrate and phosphate fertilization in different ratios. Each mesocosm consisted of a floating frame to which was attached a 2 m wide and 4 m deep enclosure with walls of 0·15 mm thick polyethylene with 90% light transmission. The enclosures were mixed continuously using an airlift and the enclosure water was exchanged with surrounding fjord water (1 m depth) at a rate of 10% per day. To compensate for the loss by exchange, one-tenth of the initial nutrient concentrations was added to the enclosures each day. For a detailed description of the construction, mooring and handling of the enclosures, see Egge & Aksnes (1992).

The surface photon irradiance during the experimental period averaged 30·7 mol m^{-2} d^{-1} (Egge, 1993), whilst the water temperature and salinity increased from 8·8 to 11·9 °C and 31·52‰ to 34·08‰, respectively. Relatively small changes in salinity of this kind have been shown to have little effect on coccolith form (Paasche *et al.*, 1996). Pronounced blooms of *E. huxleyi* developed in the fertilized enclosures during the second half of the experiment, but not in the controls.

For the present study the sampling was carried out in two of the fertilized enclosures, NP(130)-II and NP(8)-II, with initial concentrations of 13·9 μM NO_3^{1-} and 0·1 μM PO_4^{3-} and 14·7 μM NO_3^{1-} and 1·6 μM PO_4^{3-} respectively, and in one enclosure with no added nutrients (NFE-I). For ease of cross-referencing we use the same mesocosm reference codes as Egge (1993). Five samples were collected at different time intervals from each enclosure. However, the precise timing of the sampling (Fig. 1, Table 1), although based on cell number and growth phase, was heavily dependent on the requirements of other experiments being carried out simultaneously. More details of cell concentrations and growth in the bags have been summarized by Egge (1993). In summary, she found that in the NP(130)-II enclosure (high N:P ratio) the concentration of *E. huxleyi* measured between $0{\cdot}8 \times 10^6$ and $25{\cdot}5 \times 10^6$ cells l^{-1} until 11 June. Exponential growth then took place for 3–4 days, after which the population appeared to have entered a stationary phase (concentration *c.* 110×10^6 cells l^{-1}; Fig. 1*A*). In the NP(8)-II enclosure (low N:P ratio) the exponential phase appeared to have begun earlier (6 June), so that by 14 June the concentration was *c.* 270×10^6 cells l^{-1}. However, this was followed by a rapid decline so that by 21 June concentration was down to 10×10^6 cells l^{-1} (Fig. 1*B*). In the control enclosure (NFE-I) the *E. huxleyi* population never really bloomed, fluctuating between $1{\cdot}3 \times 10^6$ and $4–5 \times 10^6$ cells l^{-1} (Fig. 1*C*).

Table 1. Summary of information on samples studied from the 1991 mesocosm experiment

Enclosure	Initial conc. added NO_3^{1-} (μM)	Initial conc. added PO_4^{3-} (μM)	Sampling date (1991)	Cell conc. (10^6 cells l^{-1})
NP(130)-II (high N:P ratio)	13·9	0·1	31 May	5·6
			8 June	15·0
			12 June	40·9
			16 June	106·5
			20 June	97·3
NP(8)-II (low N:P ratio)	14·7	1·6	25 May	5·0
			2 June	33·4
			10 June	136·1
			16 June	155·8
			20 June	15·2
NFE-I (control)	No added nutrients		31 May	2·7
			2 June	3·6
			11 June	3·1
			16 June	3·0
			21 June	2·5

Note that the five samples from each enclosure were collected at different times. For further explanation, see text.

Scanning electron microscopy

Water samples used for scanning electron microscopy (SEM) analyses were fixed with 20% formaldehyde freshly neutralized with hexamethylene tetramine (pH 8·00) and the bottles left unopened until the SEM preparation started. Coccoliths were collected on 0·4 μm Nuclepore polycarbonate filters, gold/palladium coated and examined using a JEOL JSM 35 scanning electron microscope to document variation in coccolith morphology and to estimate the number of normal and abnormal forms. Observations were also made on whole coccospheres. Incompletely grown coccoliths or coccoliths deviating from normal form in size and calcification were not regarded as malformed, nor were specimens which had been altered during secondary processes such as dissolution, overgrowth and breakage after having been extruded outside the cell.

Specimens were examined using zero tilt of the specimen stage and electron micrographs were taken of coccoliths that were lying flat on the supporting filter, at a magnification of × 8600 to × 20000. The magnification was calibrated using polystyrene latex spheres (Agar Scientific, mean diameter $1{\cdot}090 \pm 0{\cdot}0331$ μm) as a standard. It was found that the absolute magnifications deviated from the nominal magnifications given by the manufacturer, necessitating correction of the observed coccolith dimensions. This emphasizes the importance of calibrating the microscope when undertaking any biometric analysis.

According to van Bleijswijk *et al.* (1991) *Emiliania huxleyi* Types A and B can be determined by coccolith morphology and immunofluorescence, but at the time we collected our samples in May–June 1991 the immuno-

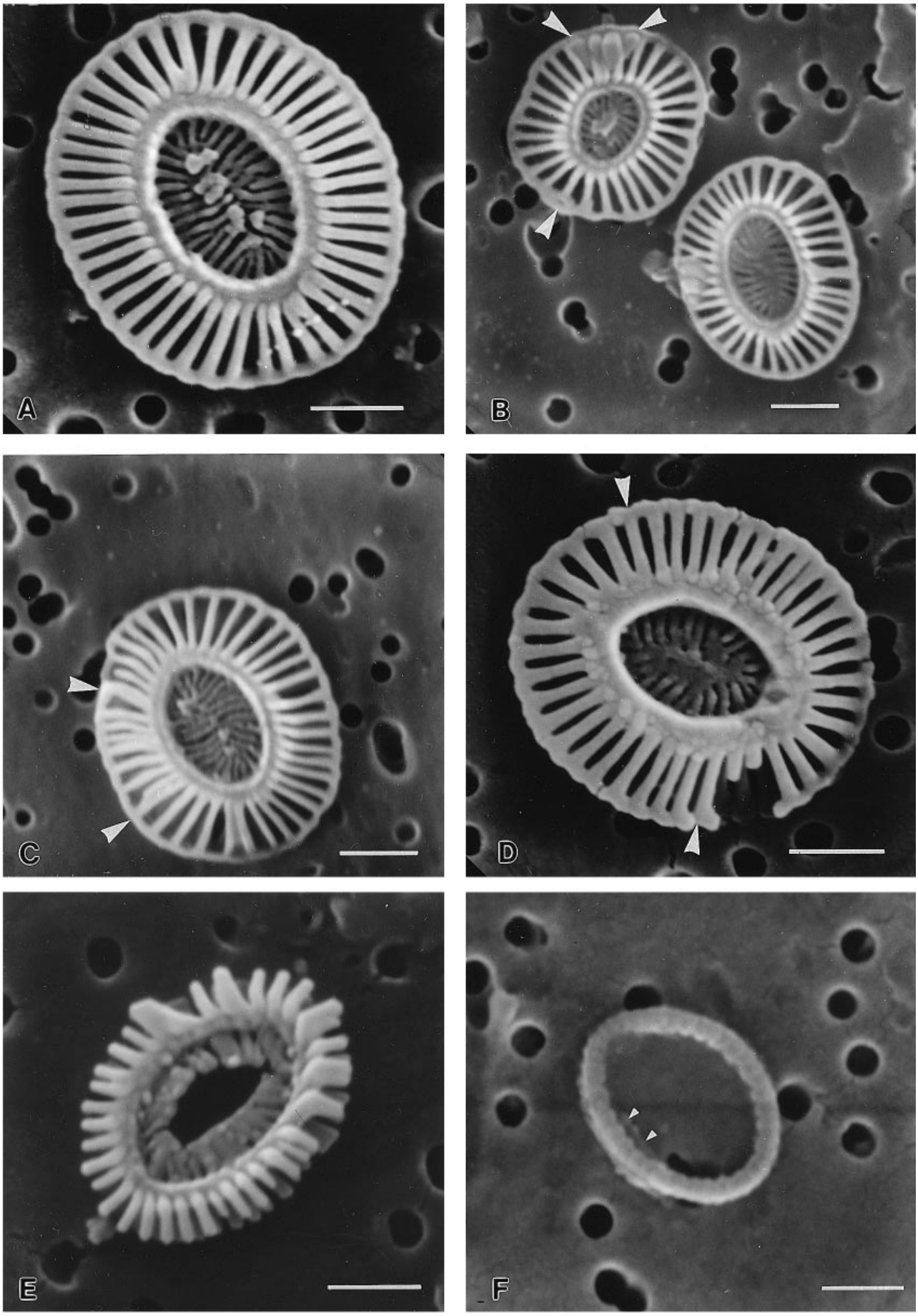

Fig. 2. *E. huxleyi* Type A coccoliths, distal views. (*A*) Normally developed coccolith. (*B*, left), (*C*) Malformed coccoliths, characterized by a reduced symmetry and changed shape of some of the elements (arrowheads). (*D*) Coccolith with two reduced distal shield elements, showing signs of malformation (arrowheads). (*E*), (*F*) Incompletely formed coccoliths, the specimen in (*F*) consisting only of the protococcolith ring with localized minimal development of the central area elements (small arrowheads). Scale bars represent 1·0 μm.

fluorescence technique had not been published and therefore there is no information in this paper about the immunological properties of the coccoliths with which we worked.

Biometric work and data analyses

To identify and enumerate the various components of the coccolith community, coccoliths were grouped into the following categories: normal, incomplete, dissolved, and malformed, the latter characterized by a reduced symmetry or a change in the shape of the individual elements (Young & Westbroek, 1991; see also Introduction above).

About 30 normal coccoliths ($n = 26–34$) from each sample were measured from electron micrographs and digitized on a 2400 Digitable instrument. This gave a statistically usable data set, whilst at the same time keeping the work within manageable proportions. The data were processed by computer and stored in a Procomm computer database. Analyses of variance (ANOVA) were performed with the Analysis statistical package (1991).

Results

Morphotype

All the normally formed specimens showed the basic morphology of *Emiliania huxleyi* with Type A coccoliths as defined by Young & Westbroek (1991): a low profile, curved and rod-like central area elements and wide distal shield elements (Fig. 2*A*). Because the coccoliths had a tendency to lie on the proximal face, it was difficult to distinguish the size and the structure of the proximal shields. However, a limited number of pictures of coccoliths in proximal view indicated that the distal shield was somewhat larger than the proximal shield, a characteristic feature also mentioned by Young & Westbroek (1991). Secondary projections linking adjacent central elements to form a grill (Young & Westbroek, 1991) were observed occasionally, even if the coccoliths did not show any particular evidence of being heavily over-calcified. In some of our specimens the central elements tended to fuse to form a low ridge along the long axis, but this was never as distinct as documented by Young (1994, fig. 4*C*) in his samples from the 1992 Norwegian mesocosm experiment from the same fjord area.

Malformation, dissolution and mechanical breakage

Most coccoliths observed varied from normally calcified to slightly over-calcified. Severely under-calcified specimens were rare. The over-calcified specimens differed from the normal ones in terms of an increasing thickening of the distal shield elements and an extended collar around the central area outwards towards the margin of the coccolith. In most of the over-calcified specimens the central area structure remained unaltered with no extra calcification.

Approximately 80–90% of the coccoliths harvested from the enclosures had developed normally (Fig. 3), the remainder being malformed, incompletely grown or dissolved (Fig. 2*B–F*). Dissolution affected less than 7% of the coccoliths sampled and most were undamaged. Protococcolith rings (Fig. 2*F*) were present during the whole experimental period, but were never abundant.

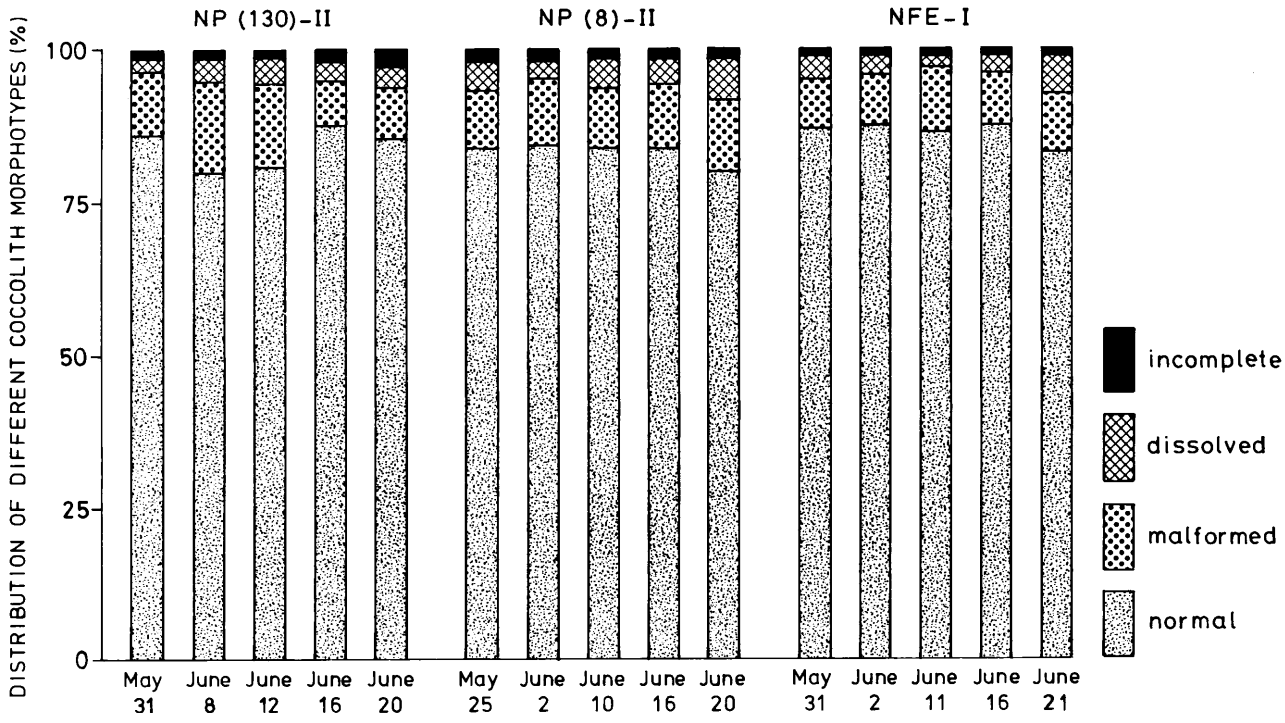

Fig. 3. Distribution of different morphotypes in the NP(130)-II, NP(8)-II and NFE-I enclosures, May–June 1991.

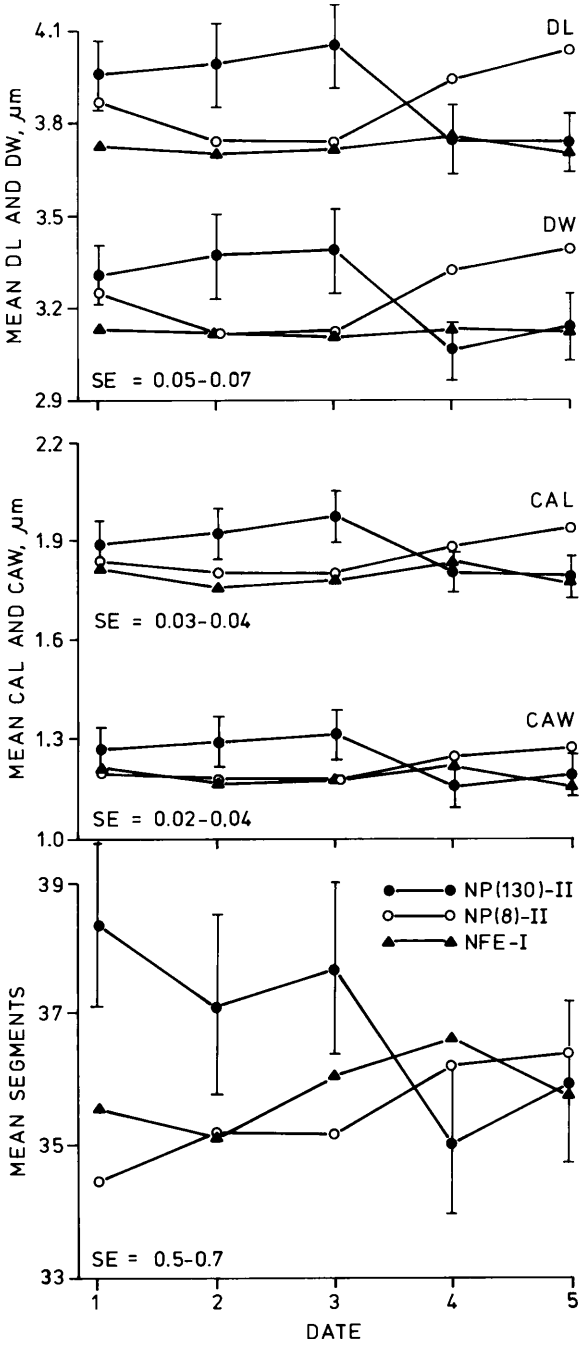

Fig. 4. Variation in the mean length and width of the distal shield (DL, DW) and the central area (CAL, CAW), and the number of segments from the NP(130)-II, NP(8)-II and NFE-I enclosures, May–June 1991. The mean values are based on 26–34 measurements; standard error bars are shown only on the uppermost curves to avoid confusion. For sampling dates and full statistical details summarizing the variation in coccolith size and number of segments, see Tables 1 and 2.

Malformed coccoliths (Fig. 2*B–D*) were most abundant at the onset of the bloom (31 May–12 June) in the NP(130)-II enclosure (Fig. 3), where they made up 7–15% of the assemblages. In the NP(8)-II enclosure and in the control there was only moderate variation in the occurrence of such coccoliths. Relative to coccolith size the size of the central area appeared to be much more variable in the malformed specimens than in normal coccoliths. The central area pattern was similar to normal, but often with some evidence of dissolution.

Coccolith size

Data on size variation in coccoliths harvested from all the enclosures are presented in Fig. 4 (for further details of the statistics summarizing the variation in coccolith size and number of segments, see Table 2). Changes in the distal shield length (DL) were reflected in the distal shield width (DW), central area length (CAL), central area width (CAW) and number of segments. Moreover, the ratios between the length and the width of the distal shields, and the length and width of the central areas showed the same trend (see Fig. 4), so that the overall coccolith profile did not change even though absolute sizes could vary.

In the NP(130)-II enclosure (low in phosphate) the coccoliths ranged from 2·97 μm to 4·64 μm long (Fig. 5) and from 2·42 μm to 4·05 μm wide. The results of ANOVA analyses showed that distal shield length decreased significantly ($p = 0{\cdot}001$) between date 3 and date 4 (Fig. 4; 12–16 June). This correlated well with the decrease in distal shield width and was also reflected in the central area measurements (Fig. 6) and in the number of segments. Also, the coccoliths were less uniformly sized in the NP(130)-II enclosure in late May and early June than towards the end of the experiment. During the peak of the bloom (16–20 June) about 80% were between 3·0 μm and 4·0 μm long (Fig. 7) and more than 60% were between 3·5 and 4·0 μm (see also Fig. 5).

The highest cell concentration of *E. huxleyi* was found on 14 June in the NP(8)-II enclosure (Egge, 1993; our Figs 1, 7). In this enclosure distal shield length ranged from 3·02 μm to 4·85 μm (Fig. 5) and distal shield width from 2·50 μm to 4·24 μm. Mean distal shield length increased significantly (ANOVA, $p = 0{\cdot}011$) from 10 to 16 June (date 3 to date 4, Fig. 4). In the early phases of the bloom 64–77% of the coccoliths were between 3·0 μm and 4·0 μm long (Fig. 7), compared with 53–46% after the peak of the bloom (16 and 20 June). The 3·5–4·0 μm fraction constituted about one-half of the total assemblage at the onset of the bloom and less than 40% towards the end (see also Fig. 5).

In the control enclosure (NFE-I) coccoliths varied moderately in size during the experiment and the size of the central area and the number of segments correlated well with overall coccolith size (Fig. 4). The distal shield lengths ranged from 2·94 μm to 4·68 μm (Fig. 5) and widths from 2·48 μm to 3·99 μm; 82–87% of the measured coccoliths were between 3·0 μm and 4·0 μm long (Fig. 7) and, of these, the majority was even more uniform in size with a distal length between 3·5 μm and 4·0 μm. It is noteworthy, however, that this size fraction constituted about 60–65% of the total assemblage in late May and early June and only about one-half towards the end of the experiment.

Table 2. Variation in coccolith size (μm) and number of segments in the NP(130)-II, NP(8)-II and NFE-I enclosures in May–June 1991

	Date 1		Date 2		Date 3		Date 4		Date 5	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
NP(130)-II										
DL	3·96	0·06	3·99	0·07	4·06	0·07	3·75	0·06	3·74	0·05
DW	3·31	0·05	3·37	0·07	3·39	0·07	3·07	0·05	3·14	0·06
CAL	1·89	0·04	1·92	0·04	1·98	0·04	1·81	0·03	1·80	0·03
CAW	1·27	0·03	1·29	0·04	1·32	0·04	1·16	0·03	1·20	0·03
Seg.	38·31	0·60	37·07	0·68	37·65	0·63	35·03	0·56	35·94	0·60
n	32		27		26		34		34	
					NP(8)-II					
DL	3·87	0·06	3·74	0·06	3·74	0·06	3·94	0·05	4·04	0·06
DW	3·25	0·06	3·12	0·06	3·12	0·06	3·32	0·05	3·39	0·07
CAL	1·84	0·04	1·80	0·04	1·80	0·04	1·88	0·04	1·94	0·03
CAW	1·20	0·03	1·18	0·03	1·18	0·03	1·25	0·03	1·28	0·03
Seg.	34·45	0·59	35·18	0·56	35·18	0·56	36·21	0·62	36·41	0·53
n	33		34		34		34		33	
					NFE-I					
DL	3·72	0·05	3·70	0·06	3·72	0·06	3·76	0·06	3·71	0·07
DW	3·13	0·05	3·12	0·05	3·11	0·05	3·13	0·06	3·12	0·07
CAL	1·81	0·04	1·76	0·04	1·78	0·04	1·84	0·04	1·78	0·03
CAW	1·21	0·02	1·17	0·03	1·18	0·03	1·22	0·03	1·16	0·03
Seg.	35·55	0·55	35·12	0·54	36·06	0·61	36·62	0·57	35·77	0·55
n	28		30		28		30		29	

DL, distal shield length; DW, distal shield width; CAL, central area length; CAW, central area width; Seg., number of segments; *n*, number of coccoliths measured.
The data are presented as mean and standard error of the mean (SE) of the results. For exact sampling dates, see Table 1.

Discussion

E. huxleyi *cell type and size*

All the coccoliths harvested from the enclosures showed the basic morphology of *Emiliania huxleyi* Type A (see the paragraph on morphotype in Results; van Bleijswijk *et al.*, 1991; Young & Westbroek, 1991), which is widespread in the Atlantic region (van Bleijswijk *et al.*, 1991) and, in contrast to Types B and C and *E. huxleyi* var. *corona*, appears to have a worldwide distribution (van Bleijswijk *et al.*, 1994*a*). Our identification agrees well with Young (1994), who has studied numerous samples from mesocosm experiments in the same fjord area from three successive years (1992–4) and recorded Type A coccoliths only, and are also in accord with the immunological and genetic data from 1992 (Barker *et al.*, 1994; van Bleijswijk *et al.*, 1991).

Coccolith size can be influenced by environmental conditions (Young & Westbroek, 1991). Distal shields of Type A coccoliths are normally 2–4 μm long (Young & Westbroek, 1991, p. 21), exceptionally up to 5 μm (J. R. Young, personal communication). Within the mesocosm samples from 1992–4 from the same fjord area the length of the coccoliths was about 15% larger than normal (J. R. Young, personal communication). In addition some of his specimens had a low ridge along the long axis of the central area. After having studied numerous mesocosm and natural samples from three successive years from this area covering a wide range of ambient conditions, nutrient regimes and population densities, Young (personal communication) found only this morphotype, although with variation in the degree of calcification and malformation, suggesting, therefore, that it does represent a local subtype. Also our Type A coccoliths were consistently larger (2·94–4·85 μm) than originally described (Young & Westbroek, 1991), and there was little contrast in the primary morphology of the coccoliths between Young's samples and our material, which supports the suggestion of Young (1994) that populations on this part of the Norwegian coast represent a local stock of *E. huxleyi*.

Variation in coccolith morphology

Morphological changes of coccoliths can be the result of either a primary process such as malformation as described above or of secondary processes such as overgrowth and dissolution.

Most coccoliths we observed varied from normally calcified to slightly over-calcified. Severely under-calcified specimens were rare and there was little dissolution and breakage. However, various mesocosm experiments have also been conducted that yielded clear evidence of dissolution of *E. huxleyi* coccoliths, particularly in collapsed specimens (Purdie & Finch, 1994; van Bleijswijk *et al.*, 1994*b*; Young, 1994). This apparent discrepancy may represent different development stages of the blooms studied. Van der Wal *et al.* (1995) demonstrated that

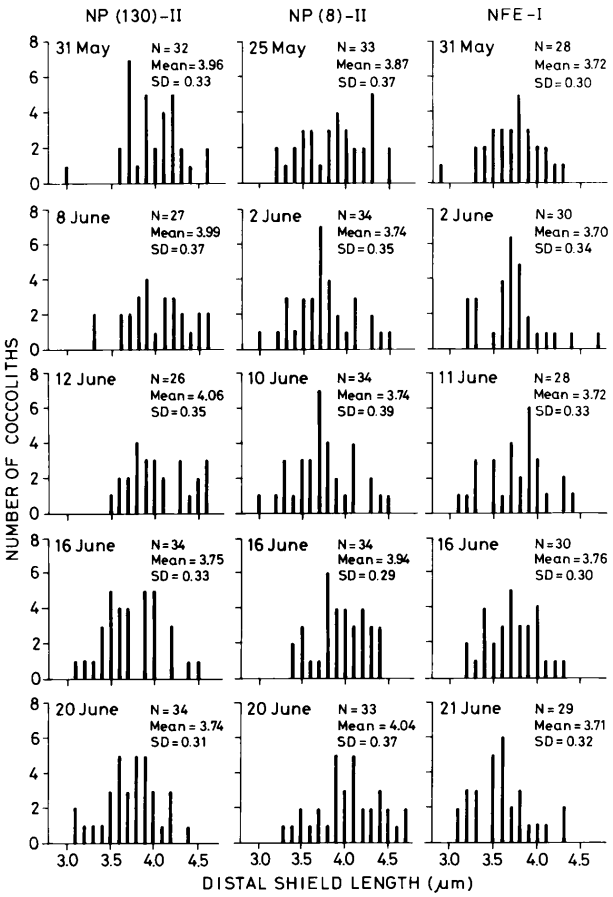

Fig. 5. Distribution of coccoliths of different shield length in the NP(130)-II, NP(8)-II and NFE-I enclosures sampled on five different dates (N = number of coccoliths measured). For more details see Materials and Methods, Tables 1 and 2, and Fig. 4.

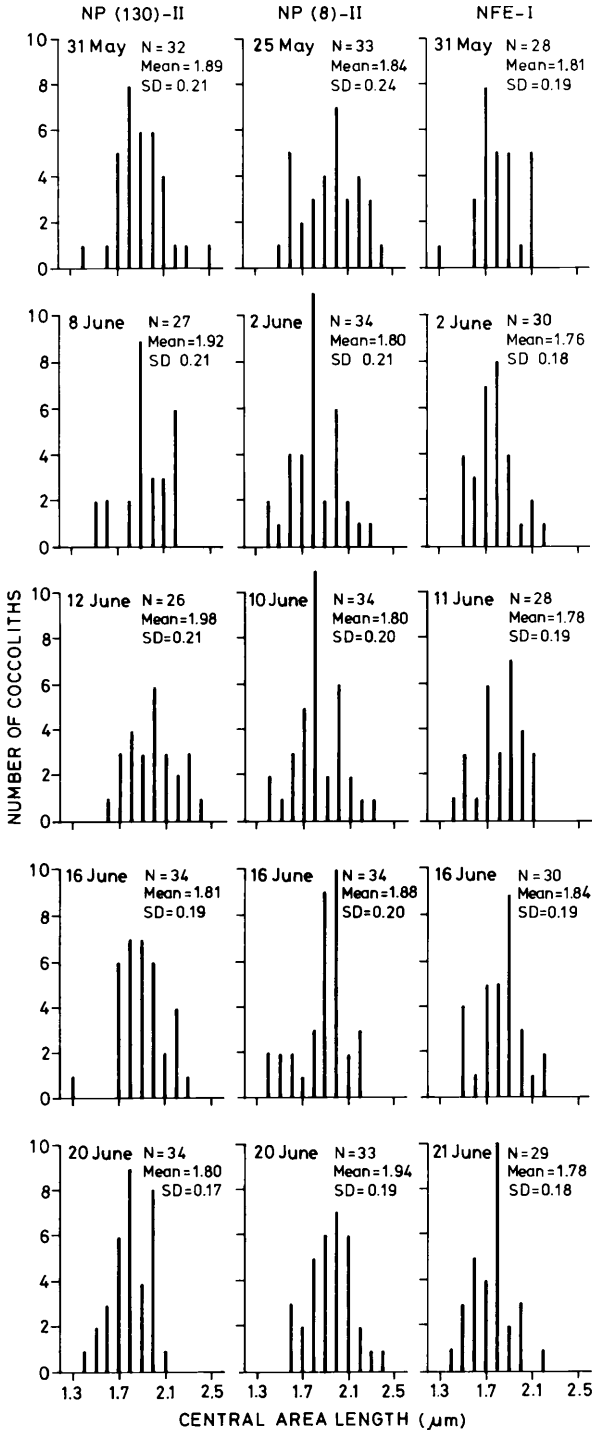

Fig. 6. Distribution of coccoliths of different central area length in the NP(130)-II, NP(8)-II and NFE-I enclosures sampled on five different dates (N = number of coccoliths measured). For more details see Materials and Methods, Tables 1 and 2, and Fig. 4.

dissolution only occurs in old coccoliths and that freshly formed specimens are not affected. This view is consistent with the finding of Young (1994) that the bulk of the dissolution occurred after the bloom had culminated during the 1992 mesocosm experiment. We had few data from the corresponding period in 1991.

Nutrient effects on coccolith morphology and size

Both the *E. huxleyi* cell numbers and the size and morphology of the coccoliths seemed to be influenced by the N:P ratio in the fertilized enclosures. In the low-phosphate enclosure (NP(130)-II) the maximum cell number was only 110×10^6 cells l^{-1}, and the bloom did not terminate during the experimental period. In the NP(8)-II enclosure (high in phosphate) maximum numbers of *E. huxleyi* cells were almost 3 times as high, and the bloom declined under low nitrate concentrations 1 week before the experiment terminated. The decay of this bloom was associated with an increase in the abundance of large virus-like particles (Bratbak *et al.*, 1993).

In the NP(130)-II enclosure, phosphate (measured in fresh samples; Egge, 1993, p. 76) was depleted to $< 0{\cdot}05$ μM on 26 May. The nutrient samples analysed from June were preserved with chloroform and they gave higher values ($0{\cdot}10$–$0{\cdot}80$ μM), probably due to leakage from the cells. Egge (1993) suggested that the NP(130)-II enclosure was phosphate-limited during the whole experimental period as the nitrate concentration never dropped below 1 μM. During the early phase of the bloom (late May to 11 June) when cell numbers increased only from $0{\cdot}8 \times 10^6$ to $25{\cdot}5 \times 10^6$ cells l^{-1}, the phosphorus stress led to the production of an increased number of malformed and less uniformly sized coccoliths in this enclosure (NP(130)-II), in

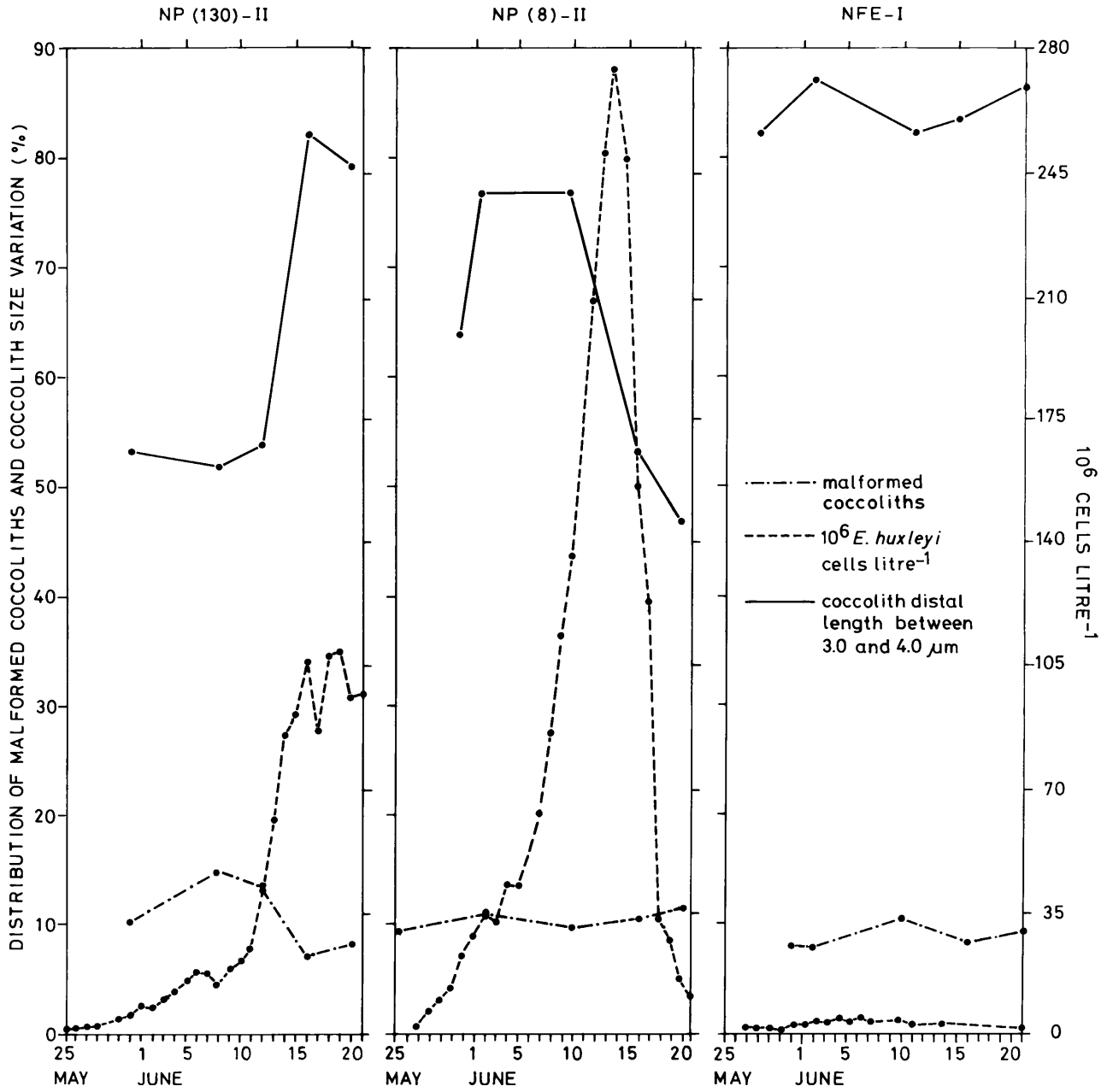

Fig. 7. Coccolith size variation and frequency of malformed coccoliths related to cell growth in the NP(130)-II, NP(8)-II and NFE-I enclosures, May–June 1991.

contrast to the situation later in June when most of the coccoliths were smaller, normal in development and more uniform in size. This shift in dominance might be due to cell physiological processes that are not understood at present, but which should be further investigated owing to their apparently important role in the synthesis of coccoliths. However, it may be noted that the most malformed coccoliths were formed in the samples from 8 June and 12 June when the population was entering logarithmic growth. By 16 June, when the proportion of malformed coccoliths had decreased again, the population had entered the stationary phase when some recycling of nitrogen and phosphorus would be expected.

Andersen (1981) demonstrated that coccolith formation could be induced by phosphorus starvation in a normally non-calcifying *E. huxleyi* strain (the non-motile naked N-cell type; Klaveness & Paasche, 1971), but that these coccoliths were malformed, possibly as a result of insufficient control of the calcifying apparatus. Van Bleijswijk *et al.* (1994*b*), Paasche & Brubak (1994) and Skattebøl (1995) have recently documented that there is a link between phosphorus limitation and calcification in mesocosm and laboratory culture experiments of calcifying *E. huxleyi* cells. The precise effect of phosphate limitation upon calcification and coccolith morphology has yet to be determined.

Although morphogenetic analyses were not included in our study, it seems likely that nitrogen limitation in the NP(8)-II enclosure caused a different development of the coccoliths compared with the phosphorus stress in the NP(130)-II enclosure. Nitrate was depleted to $< 0{\cdot}05$ μM on 30 May in the NP(8)-II enclosure (Egge, 1993, p. 66),

but reached detectable levels (0·1–1·7 μM) in June. Phosphate was never measured at levels lower than 0·2 μM in this enclosure and the coccoliths changed little in size from late May to 10 June. Unfortunately, we had no samples from the peak of the bloom (maximum *c.* 270×10^6 cells l^{-1}, 14 June), but samples from 16 and 20 June showed that the coccoliths increased significantly but were less homogeneous in size towards the end of the experiment than in the low-phosphate (NP(130)-II) enclosure. Malformed coccoliths were seen during the whole experimental period in the NP(8)-II enclosure, but were never abundant.

Electron microscopy of cultures has shown that nutrient conditions can influence the crystal growth and the process of calcification in *E. huxleyi* coccoliths (Wilbur & Watabe, 1963; Young & Westbroek, 1991; Young, 1994). Wilbur & Watabe (1963) found that in full-strength medium the coccoliths consisted entirely of calcium carbonate in the crystalline form of calcite, but that they contained a high proportion of aragonite and vaterite when the cells were grown in a nitrogen-deficient medium. Young *et al.* (1991) attempted to repeat this result, but only found calcite and suggested that the earlier results were spurious. However, Young *et al.* were not using the same strain and their study was undertaken at different nutrient concentrations. There is a possibility that the organic matrix itself which is regulating crystal nucleation (for a detailed description of coccolith biosynthesis see, e.g., Westbroek *et al.*, 1993) may be directly modified under the influence of external variables such as nitrogen, or that one or more systems associated with it (e.g. arrays of organized enzymes) may be modified. Thus, the ability of the organic matrix itself to determine the pattern of calcification of the coccolith may not be as direct or specific as indicated by Young *et al.* (1991), but this is something that remains to be demonstrated.

Young (1994) showed that *E. huxleyi* produced slightly smaller coccoliths during the logarithmic growth phase in a Redfield ratio nutrient-enriched enclosure. We found that coccoliths decreased in size during the logarithmic growth phase, starting about 12 June, in the phosphorus-depleted enclosure (NP(130)-III where the bloom did not terminate during the experimental period. This is in contrast to the situation in the enclosure with the lowest N:P ratio (NP(8)-II), where the phosphate was in excess when the bloom decayed, and the cells produced larger coccoliths towards the end of the experiment. Growth of the population in enclosure NP(8)-II was apparently terminated by a viral infection (Bratbak *et al.*, 1993), but as the nutrients were non-limiting, this population probably had the potential for continued development. This suggests that the populations of *E. huxleyi* from the two fertilized enclosures may well have been in different physiological states at the end of the experiment. According to Egge (1993), the *E. huxleyi* cell numbers in the unfertilized NFE-I enclosure were generally less than $4–5 \times 10^6$ cells l^{-1} during the whole experimental period. Our results suggest that the generally well-formed *E. huxleyi* coccoliths were remarkably homogeneous in size in this enclosure, particularly at the onset of the experiment, indicating a slow-growing population with similar physiological properties.

In summary, our results indicate that phosphorus and nitrogen stress may indeed cause changes in coccolith size. There was also clear evidence of malformation, particularly in the low-phosphate enclosure. It should be remembered that our observations concern only Type A coccoliths (Young & Westbroek, 1991) and it is not known how such conditions may affect the other coccolith types. They do serve, however, to stress the fact that environmental conditions may possibly obscure genetically determined features. Following our observations on coccolith morphology in relation to nutrient status in enclosures, it will be of interest to test whether a similar correlation can be detected in the natural environment.

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