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A note on the high abundance of *Syracosphaera marginiporata* HOL in the North Sea in July 1993

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Abstract High numbers of an *Anthosphaera*-like holococcolithophore were found in the northern North Sea in July 1993, close to a large continental shelf bloom of *Emiliania huxleyi*. Recent examination of archive images showed that it can be identified as the holococcolith-bearing stage of *Syracosphaera marginiporata*. The small coccospheres could be counted in samples prepared for immunoassays, because of their positive reaction with antibodies raised against the coccolith polysaccharide of *E. huxleyi* type A cells. Why the holococcoliths showed this unexpected cross-reaction with *E. huxleyi* antibodies cannot be explained. Large numbers of *S. marginiporata* HET have been reported from areas close to or within *E. huxleyi* blooms and the BLOOM 93 samples showed a similar distribution pattern for *S. marginiporata* HOL. It is possible that high abundances of *S. marginiporata* HOL and other small holococcolithophores were previously overlooked and occur far more often than currently documented.

Keywords Calcareous nannoplankton, holococcolithophore, *Syracosphaera marginiporata* HOL, *Emiliania huxleyi*, bloom, immunoassays

1. Introduction

In July 1993, very high abundances (~1.4 × 10^6 cells/litre) of an undescribed holococcolithophore were recorded from the North Sea near Shetland, close to a large continental shelf bloom of *Emiliania huxleyi* (presently known as *Gephyrocapsa huxleyi*; Bendif et al., 2019, 2023). The presence of these small (<5 µm) specimens became apparent during the usual examination of the samples with light microscopy (LM), although they could not be counted due to the small size of the constituent crystals that showed very little birefringence. They could, however, be counted in the samples that were prepared for immunoassays to distinguish between *E. huxleyi* type A and type B cells via fluorescence microscopy. Immunostaining results were completely independent of observations on coccolith morphology, but exactly paralleled them, and both suggested that the taxa were different (van Bleijwijk et al., 1991). In these samples, the small coccospheres were immunostained by their unexpected, positive reaction with the antibodies raised against the coccolith polysaccharide of *E. huxleyi* type A cells (van der Wal et al., 1995).

The coccospheres were additionally studied using scanning electron microscopy (SEM). At that time, the holococcolithophore was identified as a member of the genus *Anthosphaera* Kamptner emend. Kleijne, following Kleijne (1991); however, identification to species level was not possible due to poor quality of the examined images. Later, high resolution images of a similar holococcolithophore from the Balearic Sea were published as *Anthosphaera* sp. type B, including a combination coccosphere with coccoliths of the heterococcolithophore *Syracosphaera marginiporata* Knappertsbusch, 1993 (Cros & Fortuño, 2002, figs. 88B, 112C–D). More recently, a second combination coccosphere was reported from the Adriatic Sea, and the holococcolithophore was officially introduced as *Syracosphaera marginiporata* HOL, the alternate life-cycle phase of *S. marginiporata* HET (Šupraha et al., 2018, figs. 4a–c).

Archive images of better quality than the originally examined prints that were recently made available by Paul van der Wal, showed that the holococcolithophore occurring in such high numbers in the North Sea can be identified as *S. marginiporata* HOL (Cros & Fortuño, 2002; Šupraha et al., 2018; Archontikis et al., 2020).

This note reports on the high abundance of *S. marginiporata* HOL, illustrated with images of the BLOOM 93 specimens. It addresses the unexpected immunolabeling of the holococcolithophore and gives an overview of the presently known distribution of the species. For taxonomic references to the taxa mentioned in this note, see Young et al. (2003), Archontikis et al. (2020), and the Nannotax website (Young et al., 2023). Based on the phylogenomic

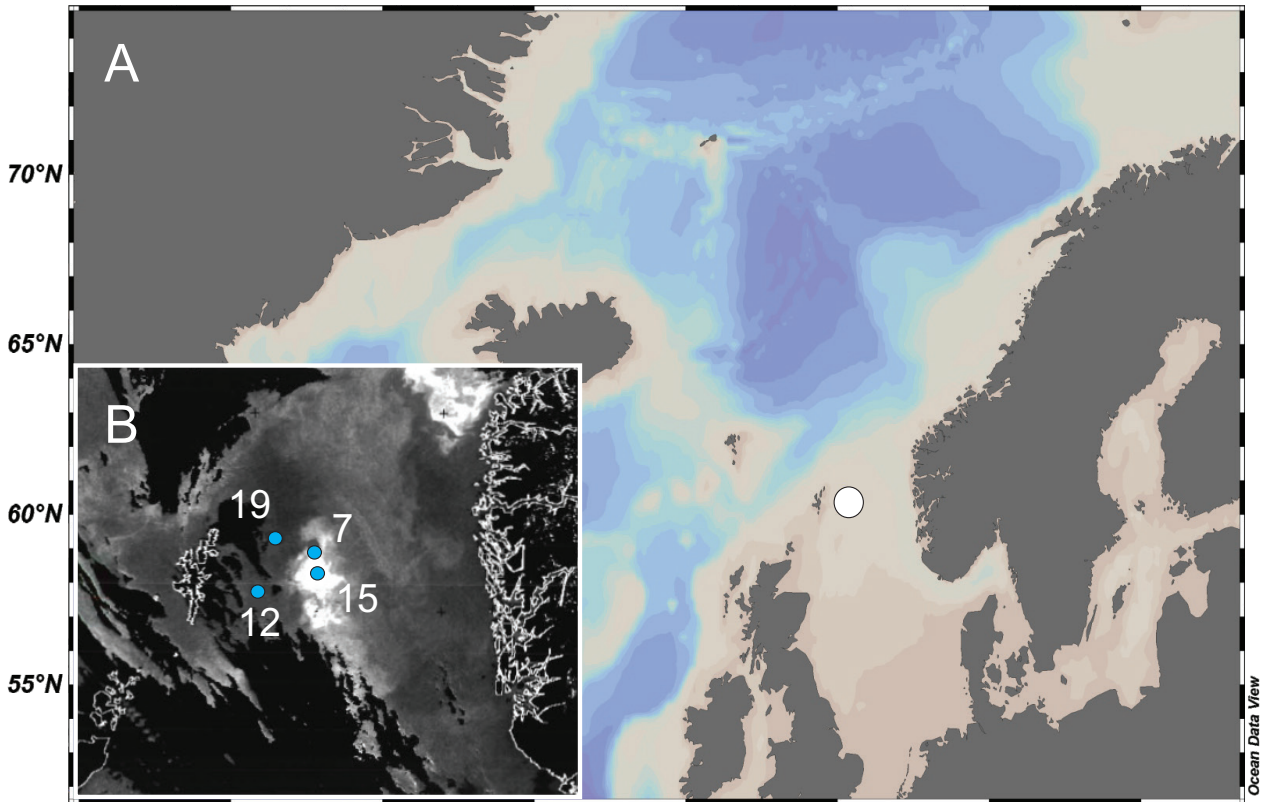

Figure 1: **A.** Map of the sampling location in the North Sea (white circle). The figure was created using the software Ocean Data View v. 5.5 (Schlitzer, 2021). **B.** Map of sampling stations (blue circles) inside (7 and 15) and close to (12 and 19) the *Emiliania huxleyi* bloom. AVHRR visible band image recorded on 27 June 1993 covering an area of the northern North Sea and adjacent Atlantic Ocean. The high reflectance waters (lighter shades) off the east coast of Shetland demarcate the studied bloom. Land and clouds are marked in black. Modified from van der Wal et al. (1995).

study of Bendif et al. (2019), *E. huxleyi* was shown to nest within the *Gephyrocapsa* genus. The *E. huxleyi* morphogroups A and B were separated into three *Gephyrocapsa* species, although for practical reasons these three species can be accommodated into a 'superspecies' concept under the name *G. huxleyi* (Bendif et al., 2023). However, using *G. huxleyi* when referring to the BLOOM 93 samples proved impractical and led to confusing and inconsistent names for *E. huxleyi* types A and B. Therefore, the name *E. huxleyi* has been used here.

2. Material and methods

Samples were collected from a large coccolithophore bloom in the North Sea, halfway between the Shetland Islands and Norway, during Cruise BLOOM 93 of the Royal Netherlands Institute for Sea Research (NIOZ), from 28 June until 13 July 1993 (Figure 1). Prior to and during the cruise, the location and development of the bloom were recorded via satellite imaging by the Advanced Very-High-Resolution Radiometer (AVHRR) aboard the National Oceanic and Atmospheric Administration (NOAA) satellite (Figure 1B; Groom & Holligan, 1987). Van der Wal et al. (1995) reported on the hydrography, production, and sedimentation of particulate organic carbon (POC) and calcite carbon at four stations: Stations 7 (60.489°N, 1.203°E, 1 July 1993) and 15 (60.459°N, 1.326°E, 7 July 1993) were located within the *E. huxleyi* bloom; and Stations 12 (60.193°N, 0.049°E, 4 July 1993) and 19 (60.568°N, 0.397°E, 10 July 1993) were located west of the bloom (Figure 1B). At these stations, water samples were collected from various depths at 04:00 h (UTC + 2) using a rosette sampler with 10.5 L NOEX bottles.

To measure concentrations of coccospheres and loose coccoliths by LM, 0.1 to 1.0 L of the sea water samples were fixed in buffered formalin and filtered through cellulose acetate filters with a pore diameter of 0.45 µm (HAWP Millipore filters). For immunoassays to enable identification of *E. huxleyi* types A and B, water samples of 0.25 to 1.0 L were fixed on buffered formalin and filtered through Sudan Black stained, 47 mm polycarbonate filters with 0.8 µm pore size. All filters were stored at –50°C. For sampling procedures, see van der Wal et al. (1995).

The samples from Stations 12 and 19 yielded large numbers of unknown coccospheres that could not be

counted with LM due to their small size and very poor birefringence. However, cell counts could be performed in the samples that were prepared for immunoassays because of their positive reaction with antibodies raised against the coccolith polysaccharide of *E. huxleyi* type A cells. Samples taken from Station 19 at a depth of 5 m were additionally examined by SEM to identify this highly abundant holococcolithophore. At the Vrije Universiteit Amsterdam, pieces of filter were mounted on aluminium stubs, coated with a thin layer of gold, and examined in a JEOL 6400 SEM at 25 kV.

Images of the BLOOM 93 coccospheres are presented here, together with an image of *Syracosphaera marginiporata* HOL from the North Atlantic Ocean, as well as images of the heterococcolith-bearing phase of the species from the northeastern Indian Ocean (identified as *Syracosphaera* sp. type H; Kleijne, 1993, p. 259, pl. 5, fig. 6) and the North Atlantic Ocean. For detailed information, see Kleijne (1991).

3. Morphology

The coccoliths shown in Plate 1 are described following the terminology introduced in Kleijne (1991), Knappertsbusch (1993), Young et al. (1997), Archontikis et al. (2020), and Young et al. (2023). *Syracosphaera marginiporata* is one of the ~42 extant *Syracosphaera* species that can be divided into four species groups (SY) and subdivided into a total of sixteen types by their heterococcolith morphology (Young et al., 2023), based on the work of Cros (2000), Young et al. (2003), and Kleijne & Cros (2009). Several *Syracosphaera* species are known to have a holococcolith-bearing stage in their life cycle (e.g. Archontikis et al., 2020 and references therein; Godrijan et al., 2023; Young et al., 2023). *Syracosphaera marginiporata* is accommodated in the SY *molischii* group, because its body coccoliths (BC) are muroliths with proximal and distal flanges, without a spine in the central area. Within this group the species is placed in the *molischii* type, because its exothecal coccoliths (XC) are undulating planoliths, confined to an imbricate circlet around the flagellar pole.

The BCs of *S. marginiporata* HET are ~1.5 µm in length. Their central area consists of a large, flat central structure surrounded by a narrow lath cycle with characteristic round pores (Plate 1, figures 1–3). The narrow-elliptical circumflagellar coccoliths (CFC) have a delicate distal flange and a tall and narrow central spine (Plate 1, figures 2–3). The XCs are undulating planoliths with a wing-like extension (Plate 1, figure 3).

The holococcolith-bearing phase shows a small, spherical dimorphic coccosphere with a diameter of ~4–4.5 µm (Cros & Fortuño, 2002; Archontikis et al., 2020). Their minuscule (<1 µm) BCs and remarkably larger CFCs (previously termed 'fragarioliths' by Kleijne [1991]) have an elliptical basal rim of one angular crystallite wide (Plate 1, figures 4, 6–7). The central area of the BCs is a low dome-shaped structure of angular crystals with openings in between that appears separated from the rim (Plate 1, figures 4–7). In the BLOOM 93 specimens, the central structure is highly variable in morphology, and ranges from some loosely scattered crystallites (Plate 1, figure 7) to an almost closed structure (Plate 1, figure 6). The CFCs bear a large central arch that extends in the distal direction as a single-layered leaf with rounded to straight sides (Plate 1, figures 4–5). The crystallites of this process are arranged in a characteristically ordered pattern with minute pores. The columns of the arch are connected to the narrow rim. In the coccosphere from the North Atlantic Ocean, the columns appear to consist of a bundle of several irregularly arranged rows of crystallites. The columns become wider and more flattened in the distal direction, where the rows of crystals continue in the upper part of the arch and form the leaf (Plate 1, figure 4; see also Archontikis et al., 2020, pl. 2).

4. Distribution

Syracosphaera marginiporata HOL is known from the North Atlantic Ocean (Plate 1, figure 4; van der Wal et al., 1995; Archontikis et al., 2020) and from the Mediterranean Sea, including the two previously reported combination coccospheres (Cros & Fortuño, 2002; Šupraha et al., 2018; Archontikis et al., 2020; Godrijan et al., 2023). *Syracosphaera marginiporata* HET is mainly known from the North Atlantic Ocean and the Mediterranean Sea (Plate 1, figure 3; Heimdal & Gaarder, 1981; Kleijne, 1993; Knappertsbusch, 1993; Samtleben & Schröder, 1990; Cros & Fortuño, 2002; Guerreiro et al., 2013; Šupraha et al., 2018; Godrijan et al., 2018, 2023; Young et al., 2023). In addition, it was also found in the northeastern Indian Ocean (Plate 1, figures 1–2; Kleijne 1993).

High abundances of the species in its heterococcolith-bearing phase have been reported from the central Portugal margin, outside a bloom that was dominated by

Emiliania huxleyi and *Gephyrocapsa oceanica* (Guerreiro et al., 2013), and from the Mediterranean Sea in a bloom together with *E. huxleyi* (Cros et al., 2017). The BLOOM 93 samples show that *S. marginiporata* may also occur in very high numbers and close to an *E. huxleyi* bloom in its holococcolith-bearing phase.

5. Discussion and Conclusion

The samples from the North Sea bloom were dominated by *Emiliania huxleyi* type A, with cell numbers (living cells and empty coccospheres without detectable traces of chlorophyll) up to 1.2×10^6 cells/litre. *Emiliania huxleyi* type B was not found at Station 19 and occurred at the other three stations in relatively low numbers of loose coccoliths and only a few coccospheres. At the stations outside of the bloom, the *E. huxleyi* cell numbers diminished to 0.2×10^6 cells/litre and the dominant coccolithophore was *Syracosphaera marginiporata* HOL, with counts of up to 1.4×10^6 cells/litre at a water depth of 5 m (van der Wal et al., 1995). Bloom Stations 7 and 15 were confined to North Sea waters, with a low salinity surface layer resulting from river water input into the North Sea basin. At the time of sampling, a stable shallow mixed layer had formed and bloom development had entered the decaying phase as indicated by the relatively low living cell numbers, the high number of loose coccoliths, and the sedimentation of calcite carbon exceeding the production (van der Wal et al., 1995).

Syracosphaera marginiporata HOL co-occurred with a small, unidentified, pennate diatom species at Stations 12 and 19, and was not found at the bloom stations. Despite the high numbers of coccospheres, the amount of calcite produced per cell was insignificant. Nutrients were present in non-limiting concentrations and production of POC was twice as high as at the bloom stations. This high nutrient availability would have resulted from the high recycling rate of the low weight, loosely packed faecal material in the surface layers (van der Wal et al., 1995). Results from the BLOOM 93 samples show that the holococcolithophore thrived at non-limiting nutrient conditions. However, a possible relation between its high abundance and the proximity of the *E. huxleyi* bloom cannot be concluded from the material at hand.

In the BLOOM 93 samples the immunostaining results were used to distinguish *E. huxleyi* types A and B. The holococcoliths' remarkable cross-reaction with the *E. huxleyi* antibodies cannot be clarified because it is likely that all coccolithophores have coccolith-associated polysaccharides, and it is not known how much these biomolecules vary and how closely variations may track taxonomy. It could be possible that similar changes occur at random in non-related taxa, or there may even be a functional explanation (Jeremy R. Young, pers. comm., 2023). Therefore, a phylogenetic explanation cannot be considered for these taxa that, moreover, belong to different families, and with the holococcolithophore being the alternate life cycle stage of *S. marginiporata* HET. However, the presence of the holococcolithophore was demonstrated perfectly by the applied method. It is very possible that the small coccospheres with their minute holococcoliths have been previously overlooked because of their size, and that high abundances of *S. marginiporata* HOL as well as other small holococcolithophores occur far more often than currently documented.

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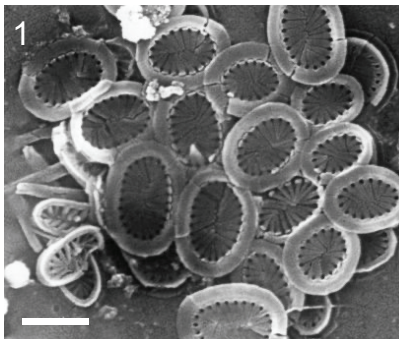
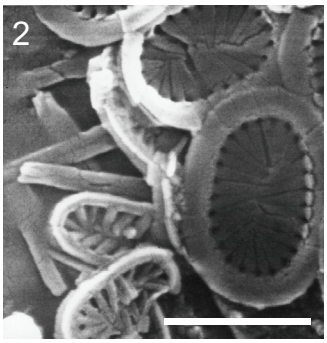
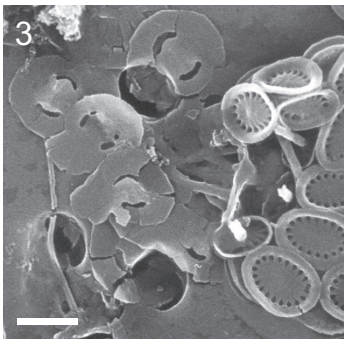
Plate 1



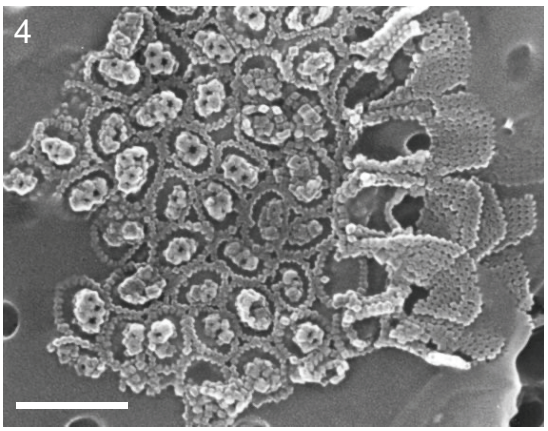
Collapsed coccosphere. Image A42-6. Snellius-II sample Gx-32, 0–5 m, northeastern Indian Ocean (3.38°N, 88.04°E; 21 June 1985).



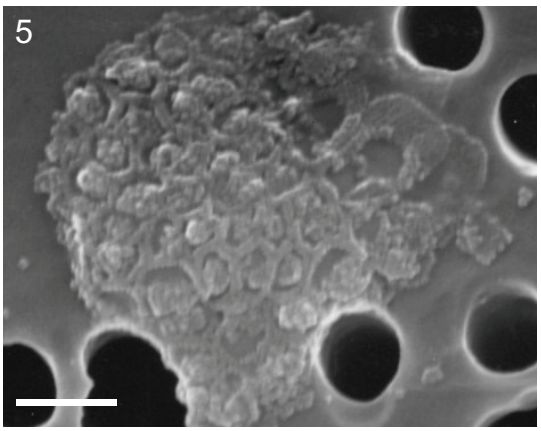
Detailed view of CFCs of Fig. 1. Image A42-5.



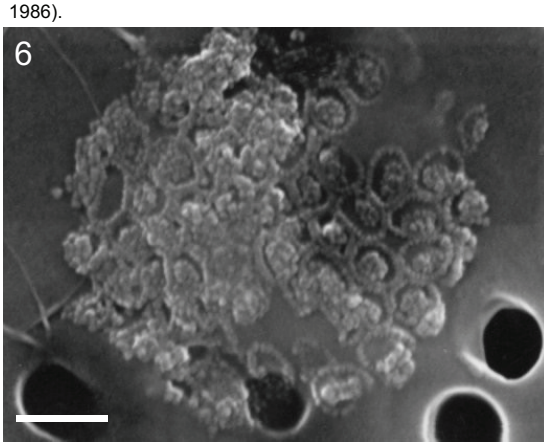
Collapsed coccosphere with XCs. Image A86-1. Cruise APNAP-I sample 17R-45-C-A, 45 m, North Atlantic (28.55°N, 38.74°W; 5 Sep. 1986).



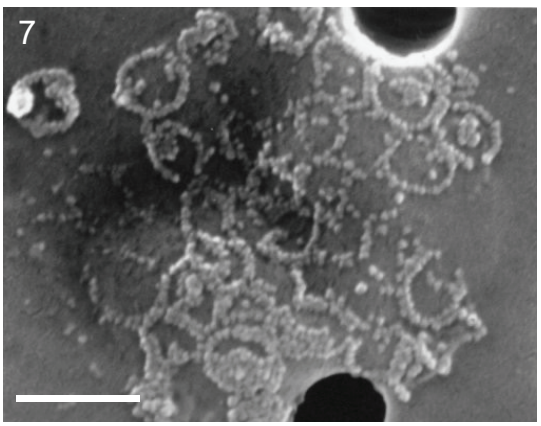
Collapsed coccosphere; the CFCs have an elliptical basal rim and a large process. Image A74-15. APNAP-I sample T86-8R-0-C, 0–1 m, North Atlantic Ocean (42.25°N, 25.67°W; 26 Aug. 1986).



Collapsed coccosphere; the CFCs have a large leaf-like process. Image 487W-01. Sample BLOOM 93-19, 5 m, North Sea.



Variation in BC morphology. Image 487W-02. Sample BLOOM 93-19, 5 m, North Sea.



BCs with a basal rim one crystallite wide and a central area structure of variable morphology. Image 486W-07. Sample BLOOM 93-19, 5m, North Sea.

Plate 1: 1–3: *Syracosphaera marginiporata* HET from the northeastern Indian Ocean and the North Atlantic Ocean. 4–7: *Syracosphaera marginiporata* HOL from the North Atlantic Ocean and the North Sea. Scale bars = 1 μm.