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Scanning electron microscope analysis of *Emiliania huxleyi* samples revealed the presence of a single morphotype in the Dardanelles Strait, Turkey

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ABSTRACT

The cosmopolitan coccolithophorid, *Emiliania huxleyi* form populations composed of different morphotypes distinguished based on coccolith ultrastructure. The relative abundance of these morphotypes varies along the gradients of several environmental factors, including temperature, pH and nutrients, with significant ecological and biogeochemical outcomes as morhotypes differ in the calcite content, hence in their contributions to the downward carbonate transport. A scanning electron microscope examination of *Emiliania huxleyi* cells and coccoliths was conducted on samples from an *Emiliania huxleyi* dominated coastal phytoplankton community formation captured on the 29th and 31th of May 2019, performing a morphological and morphometric analysis and an assessment of the environmental nutrient characteristics. The main aim of the study was to describe the morphotype from a highly important ecosystem with *E. huxleyi* blooms, the Dardanelles Strait, Turkey and contribute to the present scientific understanding of their ecological preferences. The satellite-derived chlorophyll *a* and particulate inorganic carbon concentrations data were also included to expand the spatio-temporal coverage of the study. The nutrient data suggested nitrogen limitation of the phytoplankton community in general and an additional silicate limitation of the diatoms. The microscopic observations of samples, coccosphere/coccolith counts and the morphologic and morphometric examination of the coccoliths showed the presence of an *E. huxleyi* bloom solely composed of morphotype A. Furthermore, the satellite data showed the coccolithopore bloom started in the interconnected basin of the Black Sea and progressed into the Dardanelles via the Sea of Marmara.

Descriptors: Coccoliths, Electron microscopy, Morphometry, Nutrients.

INTRODUCTION

Coccolithophores are calcifying primary producers within the phylum Haptophyta. The presence of calcium carbonate plates called coccoliths on their cell surfaces bolster their role in the downward transport of inorganic carbon through a ballast effect. They are cosmopolitan (Raven 2012) with higher diversity and abundance in phytoplankton of low latitudes (O'Brien et al., 2016), though also forming extensive blooms at high latitudes (Cerino et al., 2017). They contribute *ca*. 10 % of marine phytoplankton biomass (Tyrell and Young, 2009) and between 5 % and 40 % of marine primary production (Poulton et al., 2007; 2013). Coccolithophores have biogeochemical significance through production and contribution to downward transport of both organic and inorganic carbon, as well as release of CO_2 during calcification (Rost and Riesebell, 2004). Their contribution

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to downward calcite flux varies between 60 – 80 % in different parts of the ocean (Menschel et al., 2016 and *refs. therein*). They are also an important source of the volatile organic sulphur compound dimethyl-sulfoniopropionate, the precursor of dimethly sulphide (DMS), cloud condensation nuclei in the atmosphere (Charlson et al., 1987) that hence contribute to the albedo effect (Holligan et al., 1993; Brown and Yoder, 1994; Tyrell et al., 1999). Among the 200 extant coccolithophore species, only two species, *Emiliania huxleyi (*Lohmann) Hay and Mohler and *Gephyrocapsa oceanica* Kamptner, both in the Noelaerhabdaceae family, form frequent blooms, the former being the most abundant and cosmopolitan (ex. Winter and Siesser, 1994). Despite the wealth of scientific studies on *E. huxleyi*, there is no common agreement on the environmental factors that trigger its blooms (Lessard et al., 2005; Tyrell and Merico, 2004; Tyrell et al., 2008; Menschel et al., 2016; Hopkins et al., 2019). The existing findings suggest presence of a stratified water column, high solar radiation levels, reduced grazing, low NO_3^-:PO_4^{3-} ratios and silicate concentrations (ex.,Tyrell and Merico 2004 and *refs therein*) as well as high carbonate concentrations (Merico et al., 2006) as the possible factors favouring the formation of *E. huxleyi* blooms. The exceptionally good ability of *E. huxleyi* for the uptake of both organic and inorganic forms of nitrogen and phosphorous as well as for iron, distinguishes it from other coccolithophore species (Riegman et al., 2000; Benner and Passow 2010) and can help explain its success as the dominant and most ubiquitous coccolithophore species in the oceans.

E. huxleyi exist as different morphotypes, distinguished by coccolith morphology, hence in the degree of calcification (Young and Westbroek, 1991, Young et al., 2003). Initially five different *E. huxleyi* morphotypes called as A, B, C, B/C and R were identified by Young et al. (2003) and a further morphotype called 'Type O' was identified by Hagino et al. (2011). The morphotypes can be genotypes or/and ecotypes (Medlin et al., 1996, Iglesias-Rodriquez et al., 2006, Cook et al., 2011, Read et al., 2013). The relative abundance of each type varies along the gradients of environmental factors, most notably temperature, pH, salinity and nutrients which reflected in the global biogeography of different morphotypes (ex., Hagino et al., 2005, Henderiks et al., 2012, Malinverno et al., 2016, Poulton et al., 2011, Díaz-Rosas et al., 2021) with significant biogeochemical consequences (Rigual-Hernandez et al., 2020). Data on the distribution of *E. huxleyi* and identification of morphotype composition of its populations from different marine environments contribute to the scientific understanding of its distributional patterns and ecological niche (Tyrrell et al., 2008**)** also help to predict its contribution to downward carbon transport under variations of relevant environmental factors. However, there is still a scientific need for studies investigating the link between the distribution and abundance of different *E. huxleyi* morphotypes and the environmental factors (ex., temperature, pH, nutrients, salinity) particularly in the coastal ecosystems (Godrijan et al., 2018). Here, the electron microscope images of *E. huxleyi* coccospheres, coccoliths and their morphological and morphometric analysis are presented along with a snapshot of environmental factors during an *E. huxleyi* dominated phytoplankton community formation, captured on the 29th and 31th of May 2019 at a coastal site located along the shoreline of the Dardanelles Strait. The remotely sensed chlorophyll *a* and particulate inorganic carbon data were also included to expand the spatio-temporal coverage of the study. The major goal of the study was to make morphotype characterisation of *E. huxleyi* and contribute to the present scientific understanding of the ecological preferences of *E. huxleyi* in the Dardanelles Strait, a unique waterway in terms of its flow regime, as well being a highly important site of its blooms (ex., Turkoglu, 2008).

METHODS

Study site and sample collection

The interconnected basins of Dardanelles (Canakkale) Strait, the Sea of Marmara (SOM) and the Strait of Istanbul (SoI) form a hydrological continuum, the Turkish Straits System (TSS), enabling exchange of water masses between the Mediterranean Sea (the Aegean Basin) and the Black Sea (ex. Oguz and Sur, 1989). The hydrology of TSS is primarily characterized by

a permanent two-layered counter flow system formed by the flow of brackish Black Sea water over salty Mediterranean water that enters the system at the southern end of the Dardanelles (Figure 1) (Kanarska and Maderich, 2008). The Dardanelles is a 74.1 km long water channel whose width varies between 1.3 km and 7.5 km. The maximum depth of the Dardanelles is 113 m (Gokasan et al., 2008). Previous studies have shown that *E. huxleyi* blooms occur in the Black Sea and the TSS (ex., Cokacar et al., 2001, Aktan et al., 2003, Turkoglu, 2016, Kubryakova et al., 2019). In the Dardanelles, *E. huxleyi* blooms were detected in late spring-early summer (Turkoglu, 2008) and in winter periods (Turkoglu, 2010a), and can attain densities as high as $2.55x10^8$ cells L^{-1} (Turkoglu, 2008). This is above the density of 1.15×10^8 cells L^{-1} observed during an *E. huxleyi* bloom in a Norwegian fjord (Berge, 1962), which had been previously reported as the most intense bloom of this species (Tyrell and Merico, 2004).

The sampling point is located along the southern (Anatolian/Asian) side of the Dardanelles Strait (Figure 1) ~5 m away from the shoreline (40°07'10.71" N - 26°24'34.87" E) and was accessed by walking on a wooden pier on the 29th and 31st of May 2019. Samples were collected in triplicate from the surface with a clean bucket tied

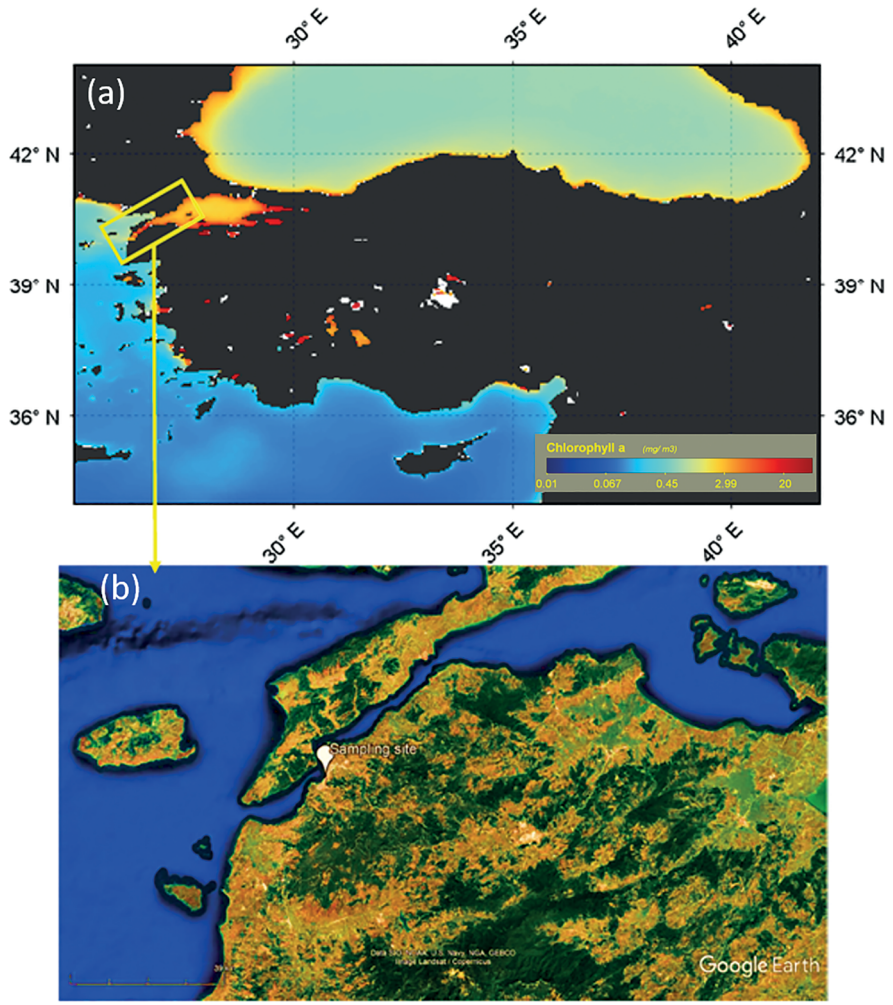

Figure 1. (a) AQUA-MODIS image of study site showing the composite surface chlorophyll *a* concentration for the period between 2017 and 2021 (http://oceancolor.gsfc.nasa.gov) (b) the location of the sampling site.

to a rope and carried back to the laboratory in 5 L clean HDPE containers, placed in black plastic bags, within ~30 minutes of collection.

Physico-chemical and phytoplankton variables

Temperature, salinity and pH of the samples were measured with an alcohol thermometer, a hand-held refractometer (Atago S/ Mill-E, Japan) and a pH meter (Hanna HI 8314, Romania), respectively.

Dissolved nutrients were analyzed using standard colorimetric analysis, with references and details of the methods found in Kocum (2020). 1 L of the samples was filtered through glass fiber filters (Whatman, GF/F, UK, ⌀ = 0.7 µM) in triplicate for bulk pigment analysis. For the measurement of pigments in micro- and nano- phytoplankton size fractions, 1 L of the samples was filtered through 20 µm Nylon filters (Millipore, Ireland), then through 2µm pore-sized nucleopore polycarbonate (PC) filters (Whatman, UK). The material collected on the Nylon and PC filters represents the micro- and nano-plankton size fractions, respectively. All filters were processed following the protocol given in Arar (1997) and concentrations of chlorophyll *a* (chl *a*), chlorophyll *b* (chl *b*) and chlorophyll *c1+c2* (chl *c1+c2*) were calculated using the trichromatic equations of Jeffrey and Humphrey (1975). Absorbance was read on a double-beam UV–VIS spectrophotometer (PG T+80 model, UK) for the nutrient and pigment analysis. For broad taxonomic analysis of samples, whole samples and samples that were filtered through 20 µm (nano- + pico-plankton) and then through 2 µm (pico-plankton) PC filters were examined under an Olympus BX 51 model microscope on the day of sampling. Additionally, on each sampling day fixed volumes of whole and size-fractionated water samples were left to settle directly on to the surfaces glass microscope slides that were horizontally placed next to each other in equal sized plastic containers, as described in Kocum (2020). The slides were then examined under the microscope after 2 hours and then twice a day for another 48 hours.

Scanning electron microscopy

For the scanning electron microscope (SEM) examination of samples; 1L of the sample was filtered through 47 mm, 2 µm PC filter backed with a 12 µm PC filter to achieve an even distribution of cells on the filter surface. The 2 µm PC filter was air-dried and kept in a sealed petri dish in a fridge until analysis. A portion of the filter was cut and mounted on a stub with carbon tape, then sputter coated with Au-Pd. The filters were observed under SEM (JEOL SEM 7100-EDX, Japan) at the Science and Technology Application Center of Canakkale Onsekiz Mart University on 11/07/2019. Morphometric measurements of coccospheres and coccoliths were made on the SEM images using GIMP 2.10.22 image processing software. All measurements on coccoliths were made on flat lying, fully exposed coccoliths seen in distal view. The terms used to describe coccolith morphology were adopted from Young et al., (1997). The measured morphometric characteristics of coccoliths were; distal shield length (DSL), distal shield width (DSW), length (CAL) and the width (CAW) of the central area, number of distal shield elements (NDSE) on the coccoliths and the internal tube width (ITW), measured at both long (ITWLa) and the short axes (ITWSa) of the coccolith, then averaged to give an average internal tube width, ITWa. The morphotype characterization of *E. huxleyi* samples followed methods in Young and Westbroek (1991) and Young et al., (2003). The shapes of the coccoliths were classified with respect to their axial ratio (AR), calculated by dividing the DSL by DSW (Young et al., 1997). A size-independent dimensionless parameter called relative tube width (RTW) was calculated as described by Young et al., (2014) to estimate relative degree of calcification of the observed coccoliths.

The density of *E. huxleyi* cells and detached coccoliths were calculated using SEM images. The formula 'CD = A*N / a*v' of Bollmann et al. (2002) was used in the calculations, where CD= cell/coccolith density (per liter of the sample), A= Effective filtration area, N= total number of cells/ coccoliths counted; a = analyzed area of the filter under the SEM; and v= volume of sample filtered (in liters).

In order to calculate the calcite content of the coccoliths, first the volume of the coccoliths was calculated using the ks model developed by Young and Ziveri (2000), where ks represents a shape specific factor for coccoliths. The suggested ks value of 0.02 for *E. huxleyi* morphotype A was multiplied by the cube of DSL of the coccoliths to obtain coccolith volume. Then, calcite content of each coccolith was calculated using its volume, the density (2.7 pg μm^{-3}), and the molecular weight (100.09 g mol^{-1}) of calcite following Poulton et al. (2011) and D'amario et al. (2018). The total number of attached and detached coccoliths per liter of the sample was multiplied by the average coccolith calcite content to obtain PIC concentration. To estimate the number of attached coccoliths, the number of coccoliths per coccosphere was calculated by counting the coccoliths on the visible side, then doubling (Boeckel and Baumann, 2008). The average coccolith number per coccosphere was multiplied by the number of coccospheres per liter of the sample to obtain attached coccolith density.

Satellite data acquisition

A synoptic view of the sea surface chlorophyll-a and PIC concentrations at the study zone was obtained from the Visible and Infrared Imager/ Radiometer Suite (VIIRS), an instrument on the Suomi-National Polar Orbiting Partnership Spacecraft (SNNP). In 8-day intervals, composite observations were obtained corresponding to the time period between 23/04/2019 and 08/06/2019 for the Black Sea and TSS (Available from the Distributed Active Archive Center, DAAC, at the National Aeronautics and Space Administration (NASA) Goddard Space Flight Center (at https://oceancolor.gsfc.nasa.gov/showimages/VIIRS/IMAGES/).

The NASA SeaDAS 8.0 software package (Baith et al., 2001) was used to capture and display the images. The PIC concentrations obtained were used to infer the contribution of coccolithophores to the chl *a* signal of phytoplankton biomass, as this parameter is a reliable indicator of the abundance and distribution of coccolithophores in surface waters (ex., Hopkins et al., 2015; Mikaelyan, 2020).

Data analysis

Pearson bi-variate correlation analysis was used to test the significance of relation among measured morphometric variables after log (x +1) transformation of the data. The coefficient of variations (C.V.) of the morphometric parameters were calculated as a measure of variability and reported as a percentage (Zar, 1984).

RESULTS

Physico-chemical and phytoplankton data

The values of temperature, pH, salinity, concentrations of dissolved inorganic nutrients and pigments measured on two sampling days are displayed in Table 1, using mean ± standard error (s.e.) values for the latter two. NO_3^- formed the > 70 % of DIN pool on both sampling days. The pattern of temporal change in nutrients and pigments was a marked decrease over two days (Table 1). The decreases in NO_3^-, NH_4^+, PO_4^{3-} and $Si(OH)_4$ were equal to 51.26 %, 23.53 %, 14.29 % and 33.33 % of their first sampling day concentrations, respectively. The molar DIN:PO_4^{3-} ratios were below the Redfield N:P ratio of 16:1 on both sampling days. There were also decreases in DIN:PO_4^{3-} and $Si(OH)_4$:PO_4^{3-} ratios (by ~33 % and ~19 %, respectively). $Si(OH)_4$:DIN ratios were well below the 1:1 ratio required by diatoms on both sampling dates, increasing by ~23 % over two days. The low availability of $Si(OH)_4$ were also reflected in the negative Si* values (Table 1), which are the difference between the molar concentrations of $Si(OH)_4$ and NO_3^-, used to infer the nitrate utilization efficiency of diatoms (Ragueneau et al., 2000; Bibby and Moore, 2011). The phytoplankton biomass measured as bulk chl *a* concentration was 6.98 µg chl a L^{-1} on 29/05/2021 and dropped by ~79 % to 1.49 µg chl a L^{-1} on the second sampling date. There were comparable declines in the bulk chl *b* (by ~82 %) and chl c_1+c_2 (by ~70 %) concentrations, as well (Table 1). The decreases in pigments measured in nanoplankton size fraction were much lower (~76 % in chl *a*, ~61 % in chl *b*, ~49 % in chl c_1+c_2) than those that occurred in microplankton (94 % in chl *a*, 86 % in chl *b*, ~89 % in chl c_1+c_2) fractions.

Table 1. Physico-chemical and phytoplankton variables measured in the study site on two sampling dates as mean+s.e. values (n=3) for the nutrient and pigment concentrations (DIN=dissolved inorganic nitrogen, the sum of NH_4^+, NO_3^-, NO_2^- concentrations, s.e.=standard error of the mean).

Variables	1	2
Temperature (oC)	24	21
pH	8.42	8.08
Salinity (ppt)	21	22
NH_4^+ (µM)	0.59 ± 0.24	0.45 ± 0.03
NO_3^- (µM)	3.97 ± 0.64	1.93 ± 0.12
NO_2^- (µM)	0.33 ± 0.12	0.27 ± 0.12
PO_4^{3-} (µM)	0.39 ± 0.05	0.33 ± 0.03
$Si(OH)_4$ (µM)	0.17 ± 0.05	0.11 ± 0.03
DIN: PO_4^{3-}	12.66 ± 0.73	8.07 ± 0.60
$Si(OH)_4$:DIN	0.03 ± 0.01	0.04 ± 0.01
$Si(OH)_4$: PO_4^{3-}	0.42 ± 0.08	0.34 ± 0.08
Bulk chl *a* (µg/L)	6.98 ± 0.03	1.49 ± 0.05
Bulk chl *b* (µg/L)	1.57 ± 0.12	0.28 ± 0.04
Bulk chl c_1+c_2 (µg/L)	1.10 ± 0.07	0.33 ± 0.03
Microplankton chl *a* (µg/L)	5.94 ± 0.01	0.34 ± 0.01
Microplankton chl *b* (µg/L)	0.57 ± 0.05	0.08 ± 0.01
Microplankton chl c_1+c_2 (µg/L)	0.66 ± 0.04	0.07 ± 0.03
Nanoplankton chl *a* (µg/L)	1.95 ± 0.05	0.47 ± 0.02
Nanoplankton chl *b* (µg/L)	0.48 ± 0.06	0.19 ± 0.04
Nanoplankton chl c_1+c_2 (µg/L)	0.49 ± 0.08	0.25 ± 0.023

Microscopic and morphometric examination of the samples

The light microscope and SEM analysis of samples showed that coccospheres and detached coccoliths of *E. huxleyi* dominated the samples on both sampling dates. The diameter of the coccospheres varied between 5 µm to 6.22 µm with a mean value of 5.62 ± 0.16 µm (Figure 2). All the observed coccolith specimens confirmed the morphological features of the *E. huxleyi* morphotype A, with slits between the distal shield elements, rod-like, curved central area elements and larger distal shields than proximal shields (Figure 3). The DSL and DSW varied between 2.18 – 3.38 µm (mean ± s.e. = 2.92 ± 0.08, n=23) and 1.94 and 2.76 µm (mean ± s.e. = 2.40 ± 0.05, n=23), respectively. The mean length (CAL) and width (CAW) of the central area were 1.44 ± 0.04 µm (0.95 µm – 1.69 µm, n=23) and 0.92 ± 0.03 µm (0.70 µm – 1.33 µm, n=23), respectively. The mean AR of the observed coccoliths was 1.21 ± 0.01 (range: 1.07 – 1.39 n=23) and the majority of the observed coccoliths (18 out of 23) confirmed a "broadly-elliptical" shape. The RTW and ITWa:DL values varied between 0.11 and 0.24 (mean ± s.e. = 0.17 ± 0.008, n=23) and between 0.047 and 0.098 (mean ± s.e. = 0.069 ± 0.003, n=23), respectively. The NDSE on observed coccoliths were between 25 and 40 (mean ± s.e. = 32.15 ± 0.74, n=21). The variation in the measured/calculated coccolith morphometric parameters were smallest in the AR (C.V.= 6.47 %) and highest in INTWs (C.V.= 29 %). The DSL values correlated to DSW, CAL, CAW, AR, ITWa and NDSE while CAL also correlated to CAW, AR and NDSE. There was also a good agreement between AR and the CAL:CAW ratio (Table2).

The coccosphere and detached coccolith concentrations were 1.24 x 10^6 L^{-1} and 2.75 x 10^7 L^{-1}, respectively. The average calcite mass of a single coccolith mass was 1.41 ± 0.10 pg (n=23) while

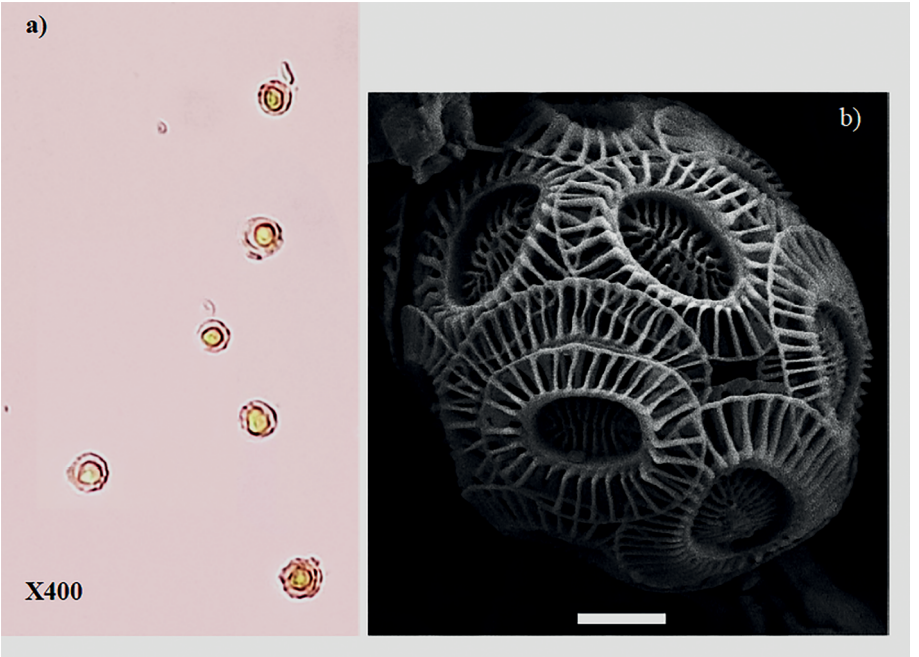

Figure 2. (a) light and (b) scanning electron microscope images of samples (scale bar = 1 µm).

the PIC (calcite) concentration was calculated as 0.79 µmol L^{-1}.

The satellite data

The satellite derived surface chl *a* concentrations were 2- 3 µg chl *a* L^{-1} over the first two 8-day intervals (Figure 4 a, b). The PIC signals were < 0.3 µmol L^{-1} over the same time intervals in TSS with a patchy spatial distribution (Figure 4 g, h). The decrease in chl *a* signals occurred in the TSS between 09-16/05/2019, followed by a period of increase (to 2-3 µg chl *a* L^{-1} range in the Dardanelles) over the next 8-day interval (Figure 4 c, d). This change was accompanied by a > 10-fold increase in PIC concentrations from < 0.1 µmol L^{-1} to > 1 µmol L^{-1} (Figure 4 i, j). The satellite derived PIC signals further increased to > 2 µmol L^{-1} and a slight decline occurred in chl *a* (to 1.5- 2 µg chl *a* L^{-1} range) between 25th of May and 1st of June (Figure 4 e, k). Both chl *a* and PIC declined slightly over the following 8-day interval in the TSS (Figure 4 f, l).

DISCUSSION

The high density of *E. huxleyi* cells and detached coccolith density (> 1 x 10^6 cells L^{-1}) and the temporal pattern of change in satellite- derived PIC concentrations clearly showed the sampling coincided with the late phase of an *E. huxleyi* bloom. Although the measured PIC concentration was much lower than the satellite derived 8-day composite concentration that includes the sampling dates it was still indicative of a coccolithophore bloom (Terrats et al., 2020).

The size of the observed coccospheres were typical for *E. huxleyi* diploid, coccolith bearing C-cells of morphotype A (Paasche, 2002 and refs. therein), similar to ones observed in the Black Sea during an *E. huxleyi* bloom that occurred in May 2013 (Stelmakh and Gorbunova, 2018), in the Aegean Sea (Triantaphyllou et al., 2010), in the Benguela coastal upwelling system (Henderiks et al., 2012). The size was also comparable to the most common coccosphere size observed in the northwestern Mediterranean (Cros and Fortuno, 2002), but smaller than the ones observed in the Black Sea during June- July 2004 (Mikaelyan et al., 2005). However, much greater coccospheres sizes were observed on two occasions in the Dardanelles during a summer (9.05 ± 1.05 µm) and a winter (11.20 ± 1.38 µm) *E. huxleyi* bloom (Turkoglu, 2010a). These differences could be due to the variations in the growth phase (Young and Westbroek, 1991, Gibbs et al., 2013) or in the

Figure 3. Detached coccoliths of *Emiliania huxleyi* type A morphotype seen in both the distal and proximal view; showing the T-shaped distal shield elements with slits between them and the central area formed by the curved rods (scale bar = 1 µm).

Table 2. Pearson correlation coefficients among environmental and phytoplankton variables at two sampling sites. (Only significant correlations were displayed, (*p<0.05, **p<0.01, the abbreviations are as explained in the text).

	DSL	DSW	CAL	CAW	ITWavr	NDSE	AR	CAL/CAV	RTW
DSL	1								
DSW	0.88**	1							
CAL	0.91**	0.81**	1						
CAW	0.62**	0.67**	0.77**	1					
ITWavr	0.42*				1				
NDSE	0.85**	0.85**	0.83**	0.68**		1			
AR	0.61**		0.53*				1		
CAL:CAW				-0.48*			0.46*	1	
RTW					0.92**				1

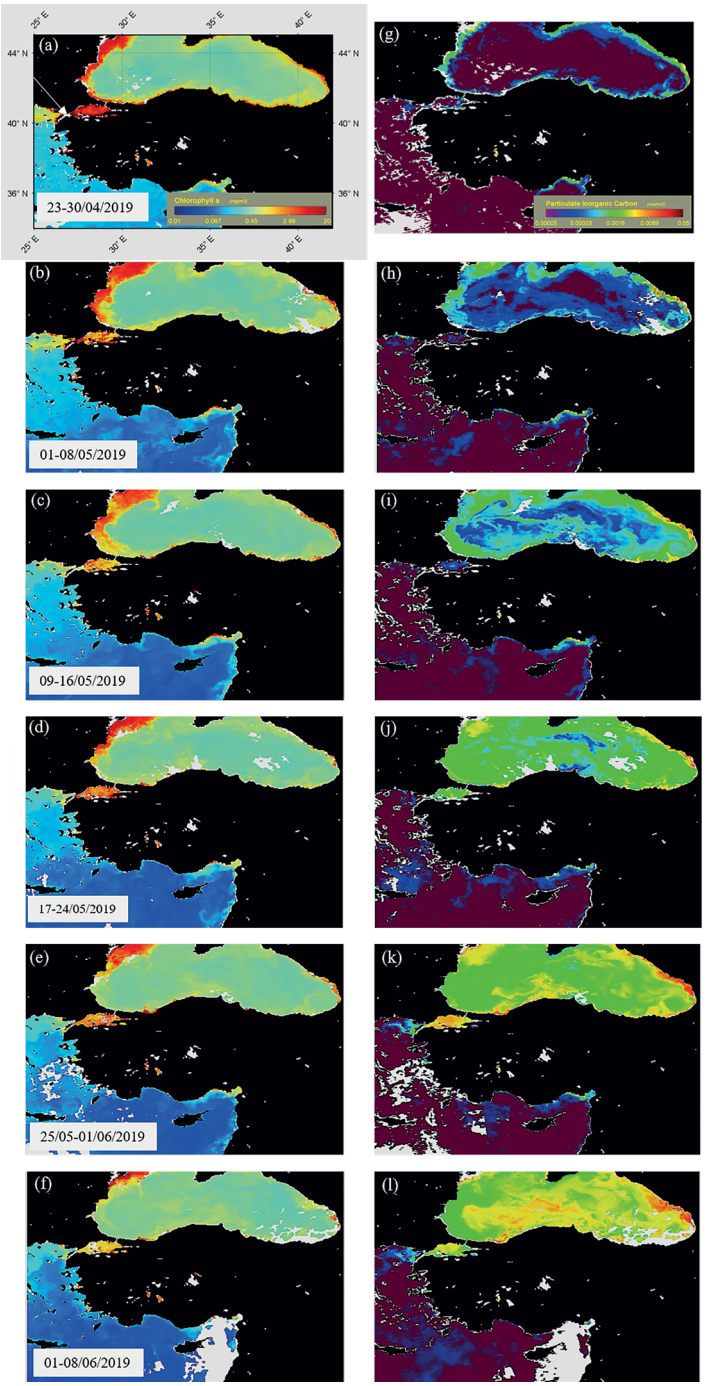

Figure 4. The spatio-temporal distributions of satellite derived chl *a* and particulate inorganic carbon concentrations in the Black Sea and the TSS. (The arrow indicates the Dardanelles).

dominating morphotype of the sampled populations (Poulton et al., 2011). The environmental factors also contribute to the size variation of the *E. huxleyi* cells and the coccoliths. For example, an inverse relation between the seawater temperature and the coccosphere size was observed in the Aegean Sea (Triantaphyllou et al., 2010). In the same study the DSL, DSW, RTW, INTW values

measured on *E. huxleyi* morphotype A coccoliths, collected in August were comparable to those of the present study suggesting a similarity in the degree of calcification.

The variation in the morphometric features of coccoliths is a good indicator of that in their calcite content. Among the different coccolith morphometric variables, the RTW provides a more direct and comparable assessment of the degree of calcification in *E. huxleyi* coccoliths (Young et al., 2014) which is significant on the magnitude of downward flux of calcite in any given marine locality (Poulton et al., 2007). Nonetheless a recent study showed that the variations in RTW (called 'CT:L ratio') was a poor indicator of *E. huxleyi* Type A calcite content (Linge and Bollmann, 2020).

The RTW values observed in this study were similar to those measured in lightly calcified morphotypes collected at two different sites (39° 15.00' N - 25° 26.76' E and 39° 06.48' N - 25° 26.10' E) in the Aegean Sea (Karatsolis et al., 2017). However the RTW values were greater than those measured (mean ± s.e. = 0.07 ± 0.01, n=30) on type A coccoliths collected in samples at a coastal site near Canary Islands (Linge and Bollmann, 2020). However, both ITWLa and ITWSa values were smaller than those observed in the Aegean Sea samples collected during the cold season but similar to those observed in samples collected in the warm season at the same location (Triantaphyllou et al., 2010). The coccolith morphology and morphometric characteristics of *E. huxleyi* populations result from the dominating morphotype and mainly reflect the prevailing temperature, salinity, nutrient and carbonate chemistry of the seawater (Poulton, 2011 and refs. therein, Von Dasow et al., 2018). Besides, within the same morphotype, the degree of calcification may also vary along the gradients of several environmental factors (D'Amario et al., 2018). Therefore the differences between the measured calcification parameters, and those in other studies, could be due to the variations in several environmental factors, such as temperature (Sorrosa et al., 2005, Poulton et al., 2011), salinity (Bollmann and Herrle 2007), nutrients (ex., Paasche et al., 1994, Batvik et al., 1997, Muller et al., 2012, 2015), or carbonate chemistry (ex., Bach et al., 2015, Rigual-Hernandez et al., 2020).

The nutrient concentrations and ratios measured in the present study were indicative of a nitrogen limitation of the phytoplankton community in general and an additional limitation by silicate for diatoms which were further supported by negative Si* values, pointing to the inability of efficient utilization of nitrate by diatoms (Raguneau 2000; Brzezinski et al., 2003). Negative Si* values have been observed during coccolithophore blooms in other parts of the world (Smith et al., 2017) and were suggested as giving coccolithophores a competitive edge over large-celled diatoms (Balch, 2014). Lower NO_3^- and PO_4^{3-}, higher $Si(OH)_4$ concentrations and $Si(OH)_4$:DIN, $Si(OH)_4$: PO_4^{3-} ratios, and similar N:P ratios were measured (at surface layer) at a site located in the Dardanelles (40°09' N - 26°24' E) during an early summer (07/06/2007-11/07/2007) mixed bloom of *E. huxleyi* with 3 dinoflagellate species (Turkoglu, 2008). This bloom was reported to be preceded by a diatom bloom in Turkoglu (2008) and by a *Noctiluca scintillans* bloom in Turkoglu (2013). In the Black Sea, N:P ratios were identified as the cause behind the switch between a diatom- or *E. huxleyi*-dominated phytoplankton community, low (<16:1) ratios being associated with the dominance by the latter (Silkin et al., 2014; Oguz and Merico, 2006). Hence N- and Si-limited conditions are commonly observed nutrient characteristics observed during *E. huxleyi* blooms both in the TSS and the Black Sea. However the same species is also able to gain dominance under high nitrate-low phosphate concentrations (Tyrrell and Taylor, 1996) or under N or P/deficiency (Lessard et al., 20005).

The chl *a* concentrations measured on the first sampling day were higher than those reported by previous research in the Dardanelles during the late spring-early summer period, under non-bloom conditions, which were mostly below 3 µg chl *a* L^{-1} (ex., Turkoglu et al., 2004; Turkoglu 2010b; Buyukates and Inanmaz, 2009; Buyukates et al., 2017; Kocum and Sutcu, 2014; Kocum, 2020). However, the chl *a* concentrations of the second sampling day were more similar to those reported in the same studies and to the values measured at a nearby site (40° 8'31.00"N - 26°23'54.39"E) on the 17th (2.18 ± 0.06 µg chl a L-1) and 24th (2.00 ± 0.03 µg chl *a* L^{-1}) of April, 2019 (data unpublished),

implying that the sampling coincided with the late phase of a phytoplankton bloom. The sharp decreases observed in pigment concentrations coincided with the decreases in the nutrients, which were not equal in magnitude. These implied that the losses might be due to differential utilization of nutrients by the phytoplankton. The increase in Si*, a sign of preferential loss of NO_3^- over $Si(OH)_4$, also supported this possibility and together with the results of microscopic analysis emphasized the role of coccolithophores in the observed declines in nutrients, rather than that of diatoms. The decreases in pigments measured in the microplankton fraction was greater than those in the nanoplankton, raising its relative abundance in the phytoplankton (Table 1). Previous research has shown the significance of autotrophic nanoplankton and their overall impact on the phytoplankton biomass size structure in the Dardanelles, where they tend to dominate phytoplankton in the late spring-summer period (Kocum, 2020). This coincides with the timing of frequently occurring *E. huxleyi* blooms in the Black Sea (ex., Cokacar et al., 2001; Eker-Develi et al., 2003), and in the Dardanelles (Turkoglu, 2008). Therefore, the role of an *E. huxleyi* dominated nanoplankton size fraction on the observed phytoplankton dynamics can be significant in this study. The observed concentrations of *E. huxleyi* cells and coccoliths further supports this possibility.

The temporal changes in the satellite derived sea surface chl *a* and PIC signals were not synchronized. The strong chl *a* signals detected between 23th and 30th of April were not due to coccolithophore development, whereas the increase in chl *a* signals that occurred during 17-24/05/2019 was accompanied by a conspicuous increase in the satellite derived PIC signals. A further rise in PIC signals to peak levels detected during 25th of May and 1st of June corresponded to a slight decrease in chl *a*. Considering the small size and low chl *a* content of *E. huxleyi* cells (ex., Hopkins et al., 2015), chl *a* signals are less reliable compared to PIC signals in tracing its spatio-temporal dynamics. Besides, detached coccoliths also contribute to the PIC signals and remain high even after a coccolithophore bloom (Lehahn et al., 2014). The density of detached coccoliths in comparison to that of coccosphere cells and the temporal change in the measured pigment and nutrient concentrations suggest the samples examined in this study came from the late phase of an *E. huxleyi* bloom. Furthermore, the overall spatio-temporal distribution of satellite-derived surface chl *a* and PIC signals indicated the development of a coccolithopore dominated phytoplankton community in the TSS by the middle of May that persisted into the beginning of June, 2019. As specified by the temporal change in the spatial distribution of PIC signals, the bloom started to form in the Black Sea and progressed into the SOM (via Strait of Istanbul) then into the Dardanelles. The bloom formation of *Emiliania huxleyi* during May-July period is a common phenomenon in the Black Sea (Cokacar et al., 2001; 2004; Mikaelyan et al., 2011) and reported to be carried into the North Eastern Aegean Sea via Dardanelles (Karatsolis et al., 2017).

CONCLUSIONS

The dependence of the distribution and abundance of *E. huxleyi* morphotypes and their degree of calcification on the environmental factors enables prediction of change in the contribution of different morphotypes to the *E. huxleyi* populations. This, in turn, enables prediction hence calcite production by them under ongoing and projected changes in the seawater temperature, pH and nutrient content caused by anthropogenic climate change (von Dassow et al., 2018). Research on the identification and distribution of different *E. huxleyi* morphotypes and how these relate to environmental characteristics in various marine ecosystems is necessary in making such predictions. Although highly limited in its temporal and spatial coverage, this study demonstrated the morphotype composition and morphometric analysis of the *E. huxleyi* samples along with the nutrient characteristics during an *E. huxleyi* bloom observed in a coastal station of the Dardanelles Strait, for the first time. This study identified a bloom composed solely of morphotype A, supporting the previous studies that show morphotype A as the most abundant type in the Mediterranean (D'amario et al., 2018) as well as in various other oceanic regions (Poulton et al., 2011). This prevalence is probably owing to its being the more generalist *E.*

huxleyi morphotype, with a larger niche breadth (Diaz-Rosas et al., 2021). Overall, the study contributes to the understanding of the ecological preferences of *E. huxleyi* in a highly important ecosystem for these blooms. This study also provided an account of the formation and progression of the *E. huxleyi* bloom in the interconnected basins of the Black Sea and the TSS over a time interval of 7 weeks through an analysis of the spatio-temporal dynamics of satellite derived chl *a* and PIC concentrations.

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