

## Article

# Coccolithophore Distribution in the Western Black Sea in the Summer of 2016

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**Abstract:** Coccolithophores are an important component of phytoplankton abundance and biomass in the brackish environments of the Black Sea. Here, the abundance, composition, and distribution of coccolithophores were investigated in water samples taken from the first 50 m at 18 stations in the western Black Sea during a coccolithophore bloom, in June 2016. The total cell abundances ranged from 2 to  $763 \times 10^4$  coccospheres  $L^{-1}$ ; *Emiliania huxleyi* was the most dominant species, but also *Syracosphaera* spp. (*S. dilatata* and *S. molischii*), *Acanthoica* (*A. acanthifera* and *A. quattrosolina*), and *Algirosphaera robusta* displayed remarkably high concentrations. The formation of the seasonal thermocline significantly affects the vertical distribution of coccolithophores. *Emiliania huxleyi*, *Syracosphaera* spp., and *Acanthoica* spp. were restricted to the upper part of the water column, whereas high abundances of *Algirosphaera robusta* occurred below the thermocline. Overall, our results show significant differences in the vertical (ANOSIM  $R = 0.50$ ,  $p = 0.0001$ ) and spatial (ANOSIM  $R = 0.18$ ,  $p = 0.0006$ ) distribution of coccolithophores. Higher abundances of *E. huxleyi* and *Syracosphaera* spp. were recorded in the northwestern inner shelf region when compared to the open-sea samples. The observed coccolithophore spatial distribution is suggested to be mostly associated with the influx of less saline river water with high nutrient concentrations.

**Keywords:** living coccolithophores; summer blooms; species composition



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## 1. Introduction

Coccolithophores are small, single-celled phytoplankton less than 30  $\mu\text{m}$  in diameter with an external skeleton (coccosphere) formed by multiple calcium carbonate plates called coccoliths. Two structurally different types of coccoliths are produced during the alteration of the haplodiplontic life cycle of coccolithophores: heterococcoliths formed by a radial array of complex crystal units, during the diploid stage (heterococcolithophore phase, HET), and holococcoliths formed of numerous minute euhedral crystallites during the haploid stage (holococcolithophore phase, HOL) [1].

Coccolithophores are widely distributed in the oceanic photic zone from polar to tropical waters. There are approximately 300 extant coccolithophore species [2] and some of these, such as *Emiliania huxleyi*, have the potential to produce large seasonal blooms in both oceanic and marginal environments [3–5]. These blooms cause an increase in complex biogeochemical activity [5,6]. In particular, coccolithophores contribute to the marine biological carbon pump by fixing large amounts of inorganic carbon through photosynthesis and consequently exporting and sequestering particulate organic carbon [7]. Furthermore, they contribute to the carbonate counter pump through the biocalcification of

their coccoliths [8,9]. During this process, seawater alkalinity decreases, CO<sub>2</sub> is released, and inorganic carbon is exported to the seafloor. Coccolithophores also affect the global sulfur cycle; they act as a source of dimethyl sulfide (DMS), a bioactive gas that enhances cloud formation in the atmosphere [10]. Because of their role in global biogeochemical cycles and their sensitivity to forthcoming climate change, coccolithophores have attracted much attention in recent decades [11–13].

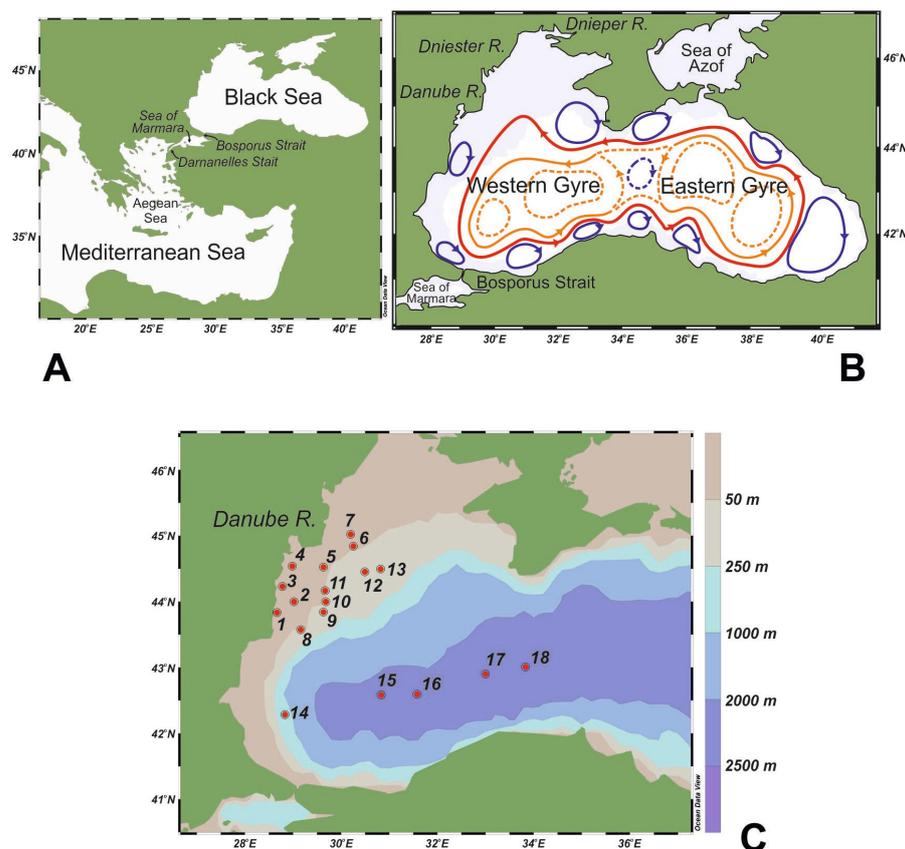
As a marginal ecosystem, the Black Sea is very sensitive to environmental changes, in particular to changes caused by climatic factors and anthropogenic pressures [14,15], which can have significant impacts on the phytoplankton biomass and taxonomic structure [16,17]. Today, coccolithophores, mainly represented by *Emiliana huxleyi*, constitute a significant component of phytoplankton communities in the Black Sea (e.g., [18–20]). Over the past four decades, this species has shown an increasing trend in phytoplankton abundance and biomass [16,17] and is also responsible for intense summer and winter blooms, with cell concentrations of up to  $10 \times 10^6$  cells L<sup>-1</sup> common in coastal and deep waters [16–27]. The most extensive and regular *E. huxleyi* blooms occur from May to July, covering 45–75% of the Black Sea surface [23]. Particularly strong *E. huxleyi* blooms are related to colder winters with deep vertical mixing that increases nutrient availability in the surface layer [16,17,26,28]. In late winter, phytoplankton blooms occur as the seasonal thermocline starts to develop. *Emiliana huxleyi* blooms begin when inorganic nutrients are recycled in waters following diatom blooms and are linked with high summer irradiance conditions, high phosphate availability, and low nitrate-to-phosphate ratios [16,26,29].

Despite the significant role of living coccolithophores in Black Sea phytoplankton communities, detailed investigations of their species composition and cell abundances are limited. Available studies on the water column plankton assemblages (e.g., [22,30]) and the sinking material (e.g., [31,32]) have shown that *E. huxleyi* can be strongly dominant in terms of cell abundance, but some species of *Syracosphaera*, *Acanthoica*, *Calciosolenia*, and holococcolithophores are also present in the communities. Further studies on the distribution of these taxa will contribute to a better understanding of the ecology and diversity of living coccolithophores in the Black Sea. This study focused on the coccolithophore assemblages from the western Black Sea during the *E. huxleyi* bloom in June 2016. The main objectives are (1) to investigate the vertical ecology and distribution of coccolithophore communities and (2) to document differences in the coccolithophore communities between the continental shelf and open-sea environments.

## 2. Study Area

The Black Sea is a semi-enclosed basin with an area of  $4.2 \times 10^5$  km<sup>2</sup> and a volume of  $5.3 \times 10^5$  km<sup>3</sup> [33], connected to the eastern Mediterranean Sea through the narrow (0.76–3.60 km) and shallow (<93 m) Bosphorus Strait, the Sea of Marmara, and the Dardanelles Strait (Figure 1A). According to the bathymetrical and morphological features, the central deep basin occupies more than 60% of the total area, with a maximum depth of around 2250 m and an average depth of 1240 m. At the periphery of the basin, the continental slope is steep, and the continental shelf is broad, up to 200 km wide in the northwest, narrow, and rarely more than 20 km wide along the rest of the basin. Numerous rivers flow into the Black Sea, particularly into the northwestern part of the basin, including some of the longest and largest European rivers (Danube, Dnieper, and Dniester).

Wind stress and thermohaline forcing drive the general cyclonic circulation of the Black Sea [34–36]. The surface circulation primarily consists of the quasi-permanent cyclonic jet “Rim current” surrounding the basin along the continental slope, multi-cell cyclonic circulation gyres in the interior of the eastern and western sub-basins, and several anticyclonic mesoscale eddies located between the Rim Current and the coastline (Figure 1B) [33,35,36].



**Figure 1.** (A) Location map of the study area; (B) main pattern of the upper layer circulation (based on data from Oguz et al. [35] and Korotaev et al. [36]); (C) locations of the sampled stations.

The Black Sea is one of the largest meromictic basins in the world. The large freshwater inputs from the rivers, the high rate of precipitation, and the limited water exchange with the Mediterranean Sea create a low salinity surface layer with values around 18 [37,38]. Therefore, the water column of the Black Sea exhibits strong density stratification, with fresher and lower-density surface waters overlying saltier and higher-density waters of Mediterranean origin [33,39]. The formation of a steep halocline/pycnocline separates the surface from deep waters between 50 and 100 m [38,40]. The permanent stratification prevents vertical mixing, causing continuing anoxia and high hydrogen sulfide concentrations below 100 m depth. In general, the upper well-oxygenated layer ( $O_2 = 8.89 \pm 1.19$  mg/L) is followed by a suboxic zone ( $O_2 < 2$  mg/L) of about 50 m thick [41]. Below this suboxic zone, a sulfide layer extends to the seafloor. The sea surface temperature shows typical seasonal variation, ranging from 8 °C to 30 °C, while the deep-sea temperature is about 8.5 °C [37]. One of the most distinctive features of the thermohaline structure of the Black Sea is the cold intermediate layer (CIL). The water in this layer is characterized by temperatures below 8 °C and is formed by winter convection processes on the northwest shelf and in the center of the western gyre [33,35,42]. The CIL is usually found at depths between 30 and 100 m [43].

### 3. Material and Methods

#### 3.1. Sample Collection and Analysis

Forty-eight samples were collected from eighteen stations, seven on the inner (<50 m depth), six on the outer (50–200 m depth) northwestern continental shelf, and five in the open sea (>200 m depth) of the western Black Sea (between 42°17' to 45°02' latitude N and 28°40' to 33°50' longitude E) by the R/V Akademik during the BIO-OPT-2016 EUROFLEETS expedition on 2–24 June 2016 (Figure 1C, Table S1). Seawater samples were

taken at different depths (1 to 50 m) in the well-oxygenated upper water column using 5 L Niskin bottles attached to a rosette with a CTD (conductivity–temperature–depth) system. Water column temperature, salinity, density, and chlorophyll (chl-*a*) fluorescence were measured using the SBE 9/11plus CTD (Sea-Bird Electronics Inc., Bellevue, WA, USA).

For coccolithophore analysis, seawater subsamples (0.2–2 L in volume; Table S1) were filtered under low pressure (<200 mm Hg) onboard onto cellulose nitrate membrane filters (47 mm diameter, 0.45 µm pore size), dried, and stored in plastic Petri dishes. In the laboratory, a quarter of each filter was cut and mounted between the microscope slide and cover slip and made optically translucent with immersion oil. The filter was examined under a Leica DMLSP polarized light microscope (PLM) at 1250× magnification. Coccolithophore concentrations were estimated by counting a field area of 4–7 mm<sup>2</sup> along the radial transects. The coccosphere abundance was calculated using the following equation of Bollmann et al. [44]:

$$CD = N \times S / (A \times V), \quad (1)$$

where CD = coccosphere abundance (number of coccospheres L<sup>-1</sup>); N = total number of coccospheres counted; S = filtered area (mm<sup>2</sup>); A = analyzed area (mm<sup>2</sup>); and V = filtered seawater volume (l).

Further species identification and documentation of small-size species in the coccolithophore assemblages was performed on selected samples via scanning electron microscopy (SEM). For the analysis, a small portion of the dried filter, approximately 8 × 8 mm<sup>2</sup>, was cut and attached to a copper electron microscope stub with double-sided adhesive tape, and then coated with Au. The samples were examined with a Jeol JSM 6360 SEM (National and Kapodistrian University of Athens, Department of Geology and Geoenvironment) at a magnification of 2000× or 5000× as needed. All the individual coccospheres in the studied filter area were identified at the species level according to the taxonomic references of Young et al. [1], Cros and Fortuño [45], and the electronic guide to the biodiversity and taxonomy of coccolithophores Nannotax 3 [46].

The small and rare holococcolithophores were difficult to identify and enumerate using light microscopy. Their presence was documented via SEM, but their concentrations were not counted. Counting from SEM allows for a more accurate analysis of the total coccolithophore biodiversity and cell counts [44], but this technique could not be useful for studying the high abundances of the Black Sea, where the high concentrations of shed coccoliths and biogenic particles make individual coccospheres difficult to distinguish.

CTD measurements and coccolithophore abundances were plotted with Ocean Data View (ODV 5.6.7, <https://odv.awi.de>, accessed on 15 November 2023) software [47].

### 3.2. Statistical Analysis

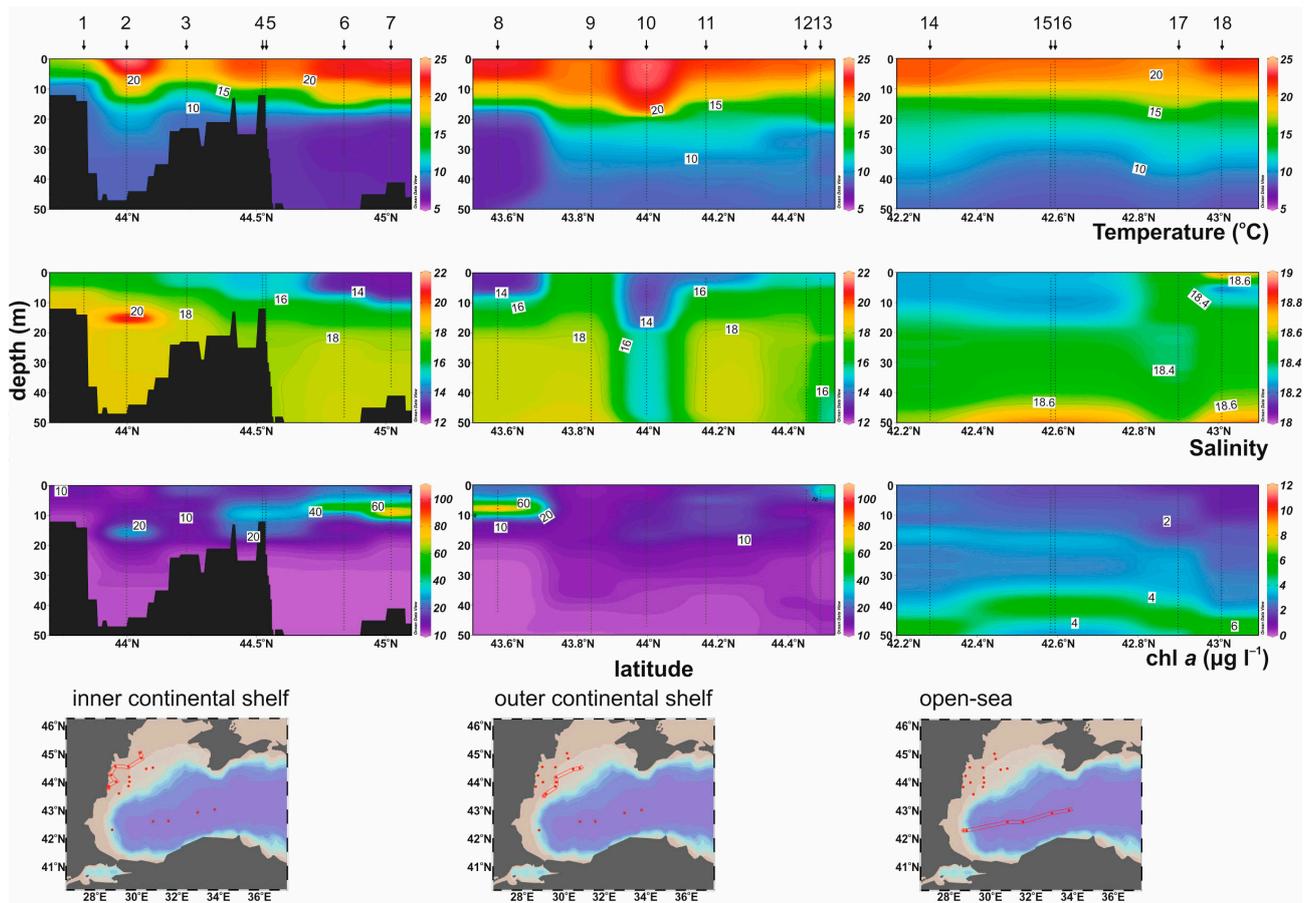
Statistical analysis was performed using the PAST (PAleontological STatistics Version 4.14) software (Natural History Museum, University of Oslo, Norway) [48]. Before analysis, the coccolithophore abundances and environmental data were logarithmically transformed to reduce the magnitude difference between the variables. Non-metric multidimensional scaling (nMDS) based on Bray–Curtis dissimilarity was used to visualize the spatial and vertical distribution patterns in the coccolithophore community structure. A stress value of less than 0.2 indicates a reasonable ordination [49]. Significant differences among the coccolithophore assemblages in different sub-regions (inner, outer continental shelf, and open sea) and sampling depths were tested using the one-way analysis of similarity (ANOSIM, 9999 permutations). In addition, the simple nonparametric Spearman's ρ correlation was carried out to determine possible relationships between the coccolithophore taxa and environmental variables (temperature, salinity, and chlorophyll fluorescence).

## 4. Results

### 4.1. Environmental Conditions (CTD Measurements)

In the surface layer (≤5 m depth), the temperature varied from 15.6 °C (St 1) to 23.8 °C (St 13) with an average value of 21.5 °C (Figure 2). The vertical profiles show a

well-stratified water column with a shallow thermocline at ca. 10–25 m, increasing from the inner shelf towards the outer shelf and open sea. On the shelf, the sea surface salinity ranged from 12.76 (St 7) to 17.23 (St 1) with an average of 14.94, and 15.01 (St 10) to 18.86 (St 15) with an average of 17.79 in the deeper waters (>25 m depth) (Figure 2). In the open-sea area, the salinity was nearly constant (18.53–18.89) throughout the water column. In the shelf area, high concentrations of chl- $\alpha$  (>10  $\mu\text{g L}^{-1}$ ) were found in the subsurface layer (<20 m depth), with peak values (>60  $\mu\text{g L}^{-1}$ ) at 5–10 m depth in stations 6, 7, and 8 (Figure 2). Lower concentrations (1–7  $\mu\text{g L}^{-1}$ ) were observed in the open sea, with maximum values at 40–50 m depth.



**Figure 2.** Vertical profiles of physical parameters (temperature, salinity) and chl- $a$  in the inner, the outer continental shelf, and the open sea of the study area.

#### 4.2. Coccolithophore Abundance and Distribution

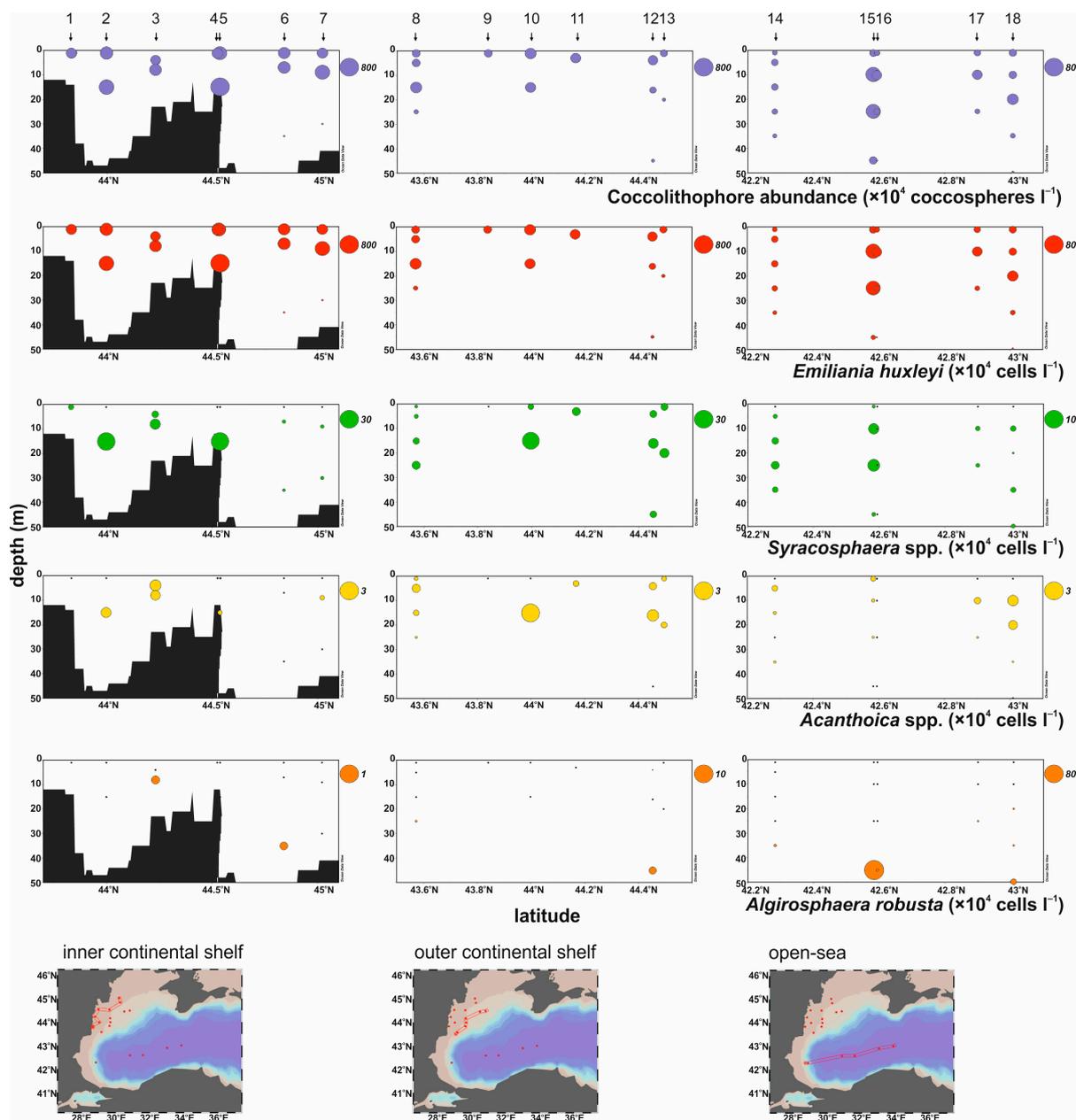
The total coccolithophore abundance varied from  $2 \times 10^4$  coccospheeres  $\text{L}^{-1}$  (St 6, 35 m depth) to  $763 \times 10^4$  coccospheeres  $\text{L}^{-1}$  (St 5, 15 m depth), with an average of  $186 \times 10^4$  coccospheeres  $\text{L}^{-1}$  (Table 1; Figure 3).

The dominant species, *E. huxleyi*, constituted 31% to 100% (average = 92%) of the total assemblage, reaching a maximum abundance of  $737 \times 10^4$  cells  $\text{L}^{-1}$  (St 5, 15 m depth). Higher values were observed across the inner shelf transect, with the maxima (> $400 \times 10^4$  cells  $\text{L}^{-1}$ ) recorded in the upper 15 m of the water column (Table 1; Figure 3). In the outer shelf transect, *E. huxleyi* abundances were less than  $200 \times 10^4$  cells  $\text{L}^{-1}$ , except for stations 8 and 10 that peaked at  $243 \times 10^4$  cells  $\text{L}^{-1}$  (15 m depth) and  $272 \times 10^4$  cells  $\text{L}^{-1}$  (1 m depth), respectively. Also, a notable increase in the abundance of *E. huxleyi* (> $200 \times 10^4$  cells  $\text{L}^{-1}$ ) was observed in the upper 25 m of the water column of the open-sea transect. *Syracosphaera* spp. contributed from 0% to 34% (average = 4%) of the total assemblage and reached a maximum abundance of  $26 \times 10^4$  cells  $\text{L}^{-1}$  (St 2, 15 m depth).

This taxon was more abundant in the upper 15 m of the water column of the inner shelf transect (Table 1; Figure 3). A peak value ( $23 \times 10^4$  cells  $L^{-1}$ ) was observed at 15 m depth at station 10 in the outer shelf transect, while in the open sea, the values did not exceed  $10^4$  cells  $L^{-1}$  except for stations 14 and 15 (max =  $3 \times 10^4$  cells  $L^{-1}$ , St 15, 10 m depth).

**Table 1.** Coccolithophore species abundances at the investigated stations.

	Station	Bottom Depth (m)	Sampling Depth (m)	<i>E. huxleyi</i> ( $\times 10^4$ cells $L^{-1}$ )	<i>Syracosphaera</i> ( $\times 10^4$ cells $L^{-1}$ )	<i>Acanthoica</i> ( $\times 10^4$ cells $L^{-1}$ )	<i>A. robusta</i> ( $\times 10^4$ cells $L^{-1}$ )
inner shelf	1	33	1	257.23	2.16	0.00	0.00
	2	48	1	371.28	0.00	0.00	0.00
	2		15	474.05	26.02	0.77	0.00
	3	26	4	214.25	3.42	1.04	0.00
	3		8	282.68	8.93	0.77	0.15
	4	22	1	299.65	0.00	0.00	0.00
	5	50	1	470.21	0.00	0.00	0.00
	5		15	737.64	25.40	0.15	0.00
	6	50	1	302.24	0.00	0.00	0.00
	6		7	316.83	0.86	0.00	0.00
	6		35	1.73	0.58	0.00	0.14
	7	42	1	258.72	0.00	0.00	0.00
	7		9	442.05	1.11	0.14	0.00
7		30	1.78	0.89	0.00	0.00	
outer shelf	8	68	1	134.49	0.59	0.13	0.00
	8		5	130.30	1.21	0.57	0.00
	8		15	242.94	3.03	0.25	0.00
	8		25	31.44	4.81	0.03	0.10
	9	75	1	143.98	0.00	0.00	0.00
	10	67	1	271.95	2.61	0.00	0.00
	10		15	187.17	23.29	3.02	0.00
	11	65	3	188.95	5.01	0.28	0.00
	12	85	4	174.34	3.90	0.42	0.00
	12		16	66.82	7.70	1.08	0.00
	12		45	13.51	3.31	0.00	1.29
	13	101	1	105.68	3.83	0.17	0.00
	13		20	15.96	7.04	0.29	0.00
open-sea	14	1353	1	45.84	0.00	0.00	0.00
	14		5	87.80	0.39	0.24	0.00
	14		15	95.20	1.17	0.08	0.00
	14		25	60.10	1.62	0.00	0.02
	14		35	30.10	0.85	0.04	1.10
	15	2165	1	132.79	0.23	0.18	0.00
	15		10	445.38	2.75	0.08	0.00
	15		25	419.37	3.95	0.04	0.00
	15		45	35.90	0.35	0.00	79.45
	16	2150	1	72.51	0.00	0.00	0.00
	16		10	83.10	0.00	0.00	0.00
	16		25	68.50	0.00	0.00	0.00
	16		45	3.80	0.00	0.00	1.60
	17	2204	1	89.06	0.00	0.00	0.00
	17		10	184.00	0.54	0.31	0.04
	17		25	45.00	0.37	0.03	0.18
	18	2205	1	111.88	0.00	0.00	0.00
	18		10	110.00	0.70	0.91	0.00
18		20	241.00	0.06	0.59	0.12	
18		35	42.20	0.61	0.02	0.16	
18		50	6.82	0.63	0.00	9.91	



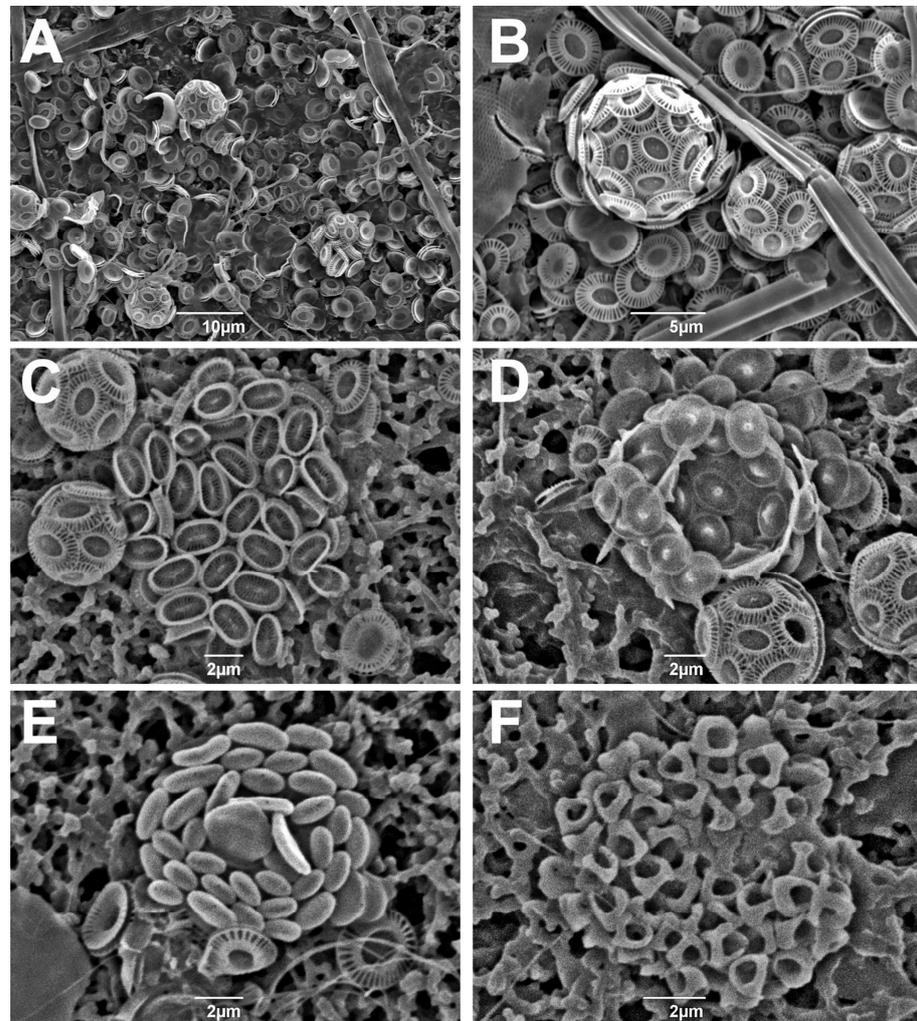
**Figure 3.** Vertical profiles of total coccolithophores, *Emiliana huxleyi*, *Syracosphaera*, *Acanthoica*, and *Algirosphaera robusta* in the inner, the outer continental shelf, and the open sea of the study area.

*Acanthoica* spp. were presented mostly in the surface layer (up to 30 m depth) in all three transects (Table 1; Figure 3), with maximum frequencies below 3% and abundances up to  $3 \times 10^4$  cells  $L^{-1}$  (St 10, 15 m depth). *Algirosphaera robusta* represented 0% to 69% (average = 4%) of the total assemblage and reached a maximum abundance of  $79 \times 10^4$  cells  $L^{-1}$  (St 15, 45 m depth). This species was rare in shallow waters and was mainly restricted to deeper layers below 30 m depth (Table 1; Figure 3).

### 4.3. Coccolithophore Taxonomy

According to the SEM observations, *E. huxleyi* type A (Figure 4A,B) was the only form of *E. huxleyi* found in all the examined samples. *Syracosphaera* spp. were identified as *Syracosphaera dilatata* (Figure 4C) and *Syracosphaera molischii*, while *Acanthoica* spp. as *Acanthoica acanthifera* and *Acanthoica quattospina* (Figure 4D). The only documented representative of *Algirosphaera* was *Algirosphaera robusta* (Figure 4E). Although the holococcolithophore

abundance was not counted in this study, two forms, *Syracosphaera arethusae* HOL and *Helladosphaera cornifera* (Figure 4F), were observed in the open-sea assemblages during the SEM analysis. It is interesting to notice that there were no differences in species composition between coccospheres and loose coccoliths. The loose coccoliths observed in the studied samples comprised mainly of *E. huxleyi* placoliths and, to a much lesser degree, *S. dilatata* cancoliths.



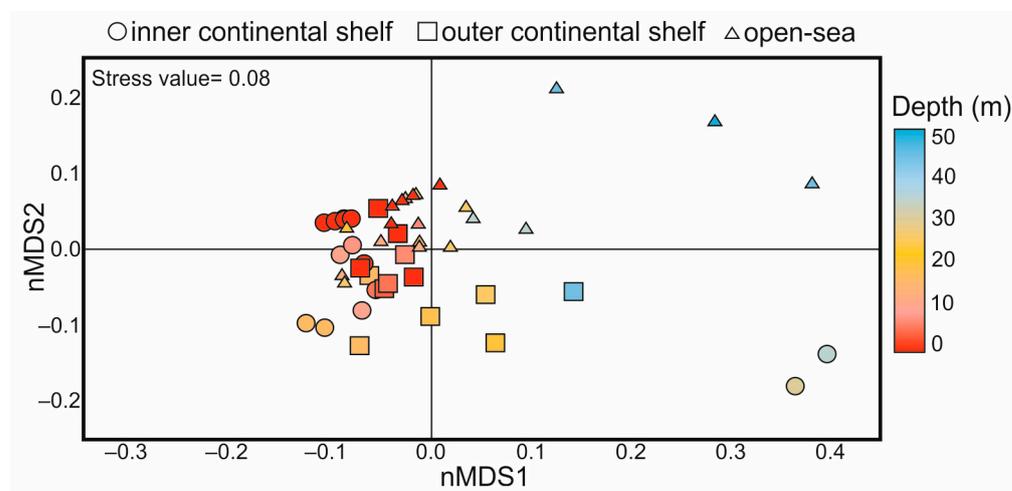
**Figure 4.** SEM micrographs of selected coccolithophore specimens identified in the studied samples. (A) General sample view, station 8, 5 m; (B) *Emiliana huxleyi*, station 8, 5 m; (C) *Syracosphaera dilatata*, station 17, 25 m; (D) *Acanthoica acanthifera*, sample 15, 1 m; (E) *Algirosphaera robusta*, station 16, 45 m; (F) *Helladosphaera cornifera*, station 17, 25 m.

#### 4.4. Statistical Analyses

The nMDS results based on the coccolithophore composition data are represented in Figure 5. The plot shows a degree of separation between the samples collected from the inner shelf, outer shelf, and open sea, respectively. A pattern of variation between the samples from the different sampling depths was also observed. ANOSIM analysis revealed that the significant dissimilarity in the composition and abundance of coccolithophore taxa throughout the water column (ANOSIM  $R = 0.50$ ,  $p = 0.0001$ ) was more important than the spatial difference between the sub-regions (ANOSIM  $R = 0.18$ ,  $p = 0.0006$ ).

The Spearman correlation matrix between the coccolithophore taxa and environmental variables is presented in Table 2. *Emiliana huxleyi* showed strong positive correlations with the temperature and chl- $\alpha$  and negative correlations with the salinity. In contrast, *A. robusta* was positively correlated with the salinity, and negatively with the temperature.

*Acanthoica* spp. and *Syracosphaera* spp. did not reveal any significant correlation with the environmental variables.



**Figure 5.** Grouping of coccolithophore communities based on non-metric multidimensional scaling (nMDS), differentiated by water depth (color), and sub-regions (shape).

**Table 2.** Matrix of Spearman’s rho correlation coefficients for coccolithophore taxa and environmental parameters.

	<i>E. huxleyi</i>	<i>Syracosphaera</i>	<i>Acanthoica</i>	<i>A. robusta</i>
temperature	<b>0.46</b>	−0.15	0.11	−0.65
salinity	−0.43	−0.17	−0.12	<b>0.53</b>
chl- <i>a</i>	<b>0.57</b>	0.25	0.19	−0.26

Values in bold: correlation is significant at the 0.05 level (two-tailed); values in bold and italic: correlation is significant at the 0.01 level (two-tailed).

## 5. Discussion

A shallow mixed layer with low salinity was observed during the sampling time, typical of summer stratification in the Black Sea. The water stratification has a significant impact on the distribution of coccolithophores, as the presence of the seasonal thermocline restricts their mixing depth, thus increasing their chance of remaining near the surface where high levels of irradiance and temperature occur. These favorable conditions for coccolithophore growth seem to prevail in the uppermost 20 m depth, within the thermocline, where they showed the highest concentrations, up to  $763 \times 10^4$  coccospheres  $L^{-1}$ . The trend in vertical distribution is consistent with previous studies of phytoplankton in the Black Sea [19,22,30], which have shown that the early summer coccolithophore bloom mainly develops in the upper mixed layer and then extends into the thermocline and sub-thermocline part. In general, the biomass of coccolithophores reaches the maximum at depths ranging from 5% to 25% of the surface Photosynthetically Available Radiation (PAR), regardless of the season [30]. It is worth noting that the bottom of the photic zone ( $\sim 1\%$  PAR) in the Black Sea increases from about 20 m in winter to 40 m in summer [30,50].

The significant amount of freshwater inflow from the Danube to the western shelf of the Black Sea is expected to have an impact on the coccolithophore assemblages. These inputs enhance pycnocline stratification due to salinity differences and increase nutrient supply, leading to an increase in phytoplankton biomass [51], as evidenced by the high chl- $\alpha$  levels in the studied area. The coccosphere abundance also showed a decrease from the inner shelf area (average of  $375 \times 10^4$  coccospheres  $L^{-1}$ ), near the Danube river mouth, towards the outer shelf and open sea (average of  $145 \times 10^4$  coccospheres  $L^{-1}$ ). These results are consistent with biogeochemical Argo data [25] and satellite observations [26] that detected a weak coccolithophore bloom ( $N = 1.5 \times 10^6$  cells  $L^{-1}$ ) in June 2016, in the

central basin of the Black Sea, and moderate or extensive on the continental shelf mainly near the shore.

In general, the coccolithophore distribution and abundance patterns closely resembled those of *E. huxleyi*, which accounted for an average of 92% of the total assemblage. This species has a wide biogeographical distribution and exhibits high morphological, genetic, and physiological variability [52,53]. Environmental parameters such as temperature (e.g., [54]), light (e.g., [55]), salinity (e.g., [56–58]), nutrients (e.g., [59]), and carbonate chemistry (e.g., [11]) are known to influence the distribution of *E. huxleyi* eco-morphotypes. *Emiliana huxleyi* type A observed in this study is widespread, is the most common bloom-forming morphotype [53], and shows a significant variation in the degree of calcification (e.g., [32,45,60,61]). Based on the morphological observations, the *E. huxleyi* population in the Black Sea is characterized by lightly calcified delicate specimens that have been previously documented in sediment trap data from the study area [32,62]. Specimens of this form produce coccoliths with delicate and well-separated distal shield elements, a relatively narrow tube, and broad central area covered by lath-like elements [60]. Data from the Mediterranean Sea indicate that lightly calcified *E. huxleyi* type A dominates in the summer assemblages of the Aegean Sea associated with high temperature–low productivity conditions and lower bicarbonate content [60], but has also been found in the low salinity environments of the shallow environment of Thessaloniki bay [63], the upper photic zone of the northeastern Aegean Sea [62], and the Atlantic–Gibraltar Strait and southwestern Mediterranean region [61]. The *E. huxleyi* lightly calcified morphotype in the present study inhabited the salinity range of around 13 to 19, indicating good adaptation and growth in low salinity environments. Furthermore, the higher abundances (max.  $737 \times 10^4$  cells  $L^{-1}$ ) in the inner shelf area near the Danube river mouth suggest the response of the certain morphotype to freshwater and nutrient inputs. This is also supported by the significant negative correlation with salinity ( $\rho = -0.43$ ,  $p < 0.05$ ). Additionally, the strong correlation observed between *E. huxleyi* and chl- $\alpha$  ( $\rho = 0.57$ ,  $p < 0.01$ ) indicates a direct relationship between this species and primary productivity. Despite being the most frequent and abundant coccolithophore species, *E. huxleyi* contributes less to the total biomass compared to other phytoplankton (e.g., diatoms and dinoflagellates) in oligotrophic regions, as its cells are relatively small ( $\sim 5 \mu m$ ) [5] and contain a low amount of chlorophyll *a* ( $\sim 0.3$  pg Chl cell $^{-1}$ ; [64]). However, studies have shown that the blooms of coccolithophores in the Black Sea produce high shares of phytoplankton biomass, often exceeding that of diatoms or dinoflagellates, depending on seasons (see e.g., [27]).

In addition to *E. huxleyi*, seven species contributed in the coccolithophore assemblages of the western Black Sea: five species belonging to the genera *Syracosphaera*, *Acanthoica*, *Algirosphaera*, and two holococcolithophore forms. Among them, *Syracosphaera* is the most abundant taxon, followed by *Acanthoica* in the upper 20 m of the water column, while *A. robusta* seems to prefer the lower water layers, below 30 m depth.

*Syracosphaera* is the most diverse genus in the extant coccolithophore assemblages [46], including species with different ecological tolerances (e.g., [65–68]). In this study, *Syracosphaera* was represented by *S. dilatata* and *S. molischii*. *Syracosphaera dilatata* has also been documented in the coccolithophore fluxes of the western Black Sea during the warm period (May–September) [32]. Both species along with *E. huxleyi* exhibit a preference for the high nutrient concentrations of the cold period in Mediterranean environments [67–69]. *Syracosphaera* spp. were mostly found in shelf assemblages in Danube-influenced waters, with particularly high concentrations of up to  $26 \times 10^4$  cells  $L^{-1}$ . These values are close to the summer concentrations of *Coronosphaera mediterranea* (syn. *Syracosphaera mediterranea*) (max =  $0.13 \times 10^6$  cells  $L^{-1}$ ) observed by Mikaelyan et al. [22] in the northeastern Black Sea. Despite that *Syracosphaera* generally represents a typical portion of the mesotrophic to oligotrophic coccolithophore assemblages (e.g., [32,66–72]), the extremely high concentrations exceeding  $10^5$  cells  $L^{-1}$ , recorded in the Black Sea, have also been reported in coastal waters (Southern Benguela Upwelling System: [73]; Bay of Biscay: [74]; Krka estuary of the Eastern Adriatic Sea: [75]).

*Acanthoica* spp. (*A. acanthifera* and *A. quattrosipina*) were uniformly distributed in both shelf and open-sea assemblages, with somewhat higher values at station 10, where the lowest salinity was observed. *Acanthoica acanthifera* is known to occur sporadically with low concentrations in living assemblages [76], while *A. quattrosipina* is more commonly found in marine waters [77] as well as estuarine environments [78], indicating that it can adapt to a wide salinity range. The latter has already been described in phytoplankton assemblages of the Black Sea and is considered a cold-water dweller with the maximum biomass found in the upper mixed layer during the winter period [30].

*Algirosphaera robusta*, a common component of the Mediterranean coccolithophore assemblages [32,66–70,72,77], is reported in the Black Sea for the first time, in the present study. This species is considered a deep-water taxon [65], it prefers relatively low light and temperature conditions, and responds well to eutrophic conditions [76]. Although it has been documented to be seasonally important in oligotrophic settings, mainly restricted to the low SST and high productivity season [66], it was also found in shallower depths [67,70,79], showing an affinity for near-coastal settings. Interestingly, in the open-sea domain of the Black Sea, *A. robusta* showed an increase in absolute and relative abundances below the thermocline, opposite to the significant decrease in the total coccolithophore abundance. The highest concentrations of this species coincided with the deep chlorophyll maximum, which was located below 40 m depth during the sampling period, highlighting the species' ability to thrive in low light and temperature conditions.

Holococcolithophores were not included in the counting results, but their presence in the open-sea assemblages was documented via SEM. Some holococcolithophore forms have previously been observed in living assemblages [22] and sinking particles [31] in the Black Sea. This group is a significant component of the Mediterranean coccolithophore assemblages and usually displays the highest species diversity and abundance in warm, oligotrophic, and stratified conditions [62,65–70,77,78,80–82]. Two forms, *S. arethusae* HOL and *H. cornifera*, were identified in this study, suggesting their ability to adapt to the low salinity conditions of the Black Sea.

Overall, the vertical and spatial distribution of the coccolithophore assemblages showed significant differences, which were also confirmed by the nMDS plot. The vertical distribution appears to be related to the formation of the seasonal thermocline. *Emiliana huxleyi* along with a low number of *Syracosphaera* and *Acanthoica* species were more abundant in the upper water layers within the thermocline. In contrast, *A. robusta* had higher abundances in the deeper water layers below the thermocline. Although the coccolithophore species composition did not reveal spatial differences between the shelf and open-sea assemblages, there were clear variations in the total coccosphere abundances. The highest concentrations, mainly consisting of *E. huxleyi* and *Syracosphaera*, were recorded in the inner shelf area near the Danube river mouth, associated with the response of these taxa to freshwater and nutrient inputs. Nevertheless, the ANOSIM results demonstrated that the vertical variation (ANOSIM  $R = 0.50$ ,  $p = 0.0001$ ) is more significant than the spatial variation (ANOSIM  $R = 0.18$ ,  $p = 0.0006$ ), suggesting that the formation of the seasonal thermocline has a greater impact on the coccolithophore distribution compared to the influence of freshwater input.

The coccolithophore assemblages in the western Black Sea consisted of only eight species that exhibited significantly high abundances. Similar low diversity assemblages have been recorded in restricted coastal environments in the Eastern Mediterranean (Thermaikos Gulf [63] and Elefsis–Saronikos Gulf [68]). The recorded differences in the species composition between these assemblages could be attributed to variations in the temperature and salinity, which have been documented to influence the taxonomic composition of phytoplankton between the Black Sea and Mediterranean coastal ecosystems [83]. Further research on the seasonal distribution of coccolithophores as well as their relationship with different physical and chemical parameters will provide a comprehensive picture of species diversity in the Black Sea.

## 6. Conclusions

The spatial and vertical variations of living coccolithophores were analyzed from 48 samples taken from the oxygen-rich surface waters (<50 m depth) at 18 stations in the northwestern continental shelf and from the open sea of the western Black Sea during a coccolithophore bloom in June 2016. The results of this study can be summarized as follows:

1. Living coccolithophores showed high abundances, reaching a maximum of  $763 \times 10^4$  coccospheres  $L^{-1}$  with the lightly calcified morphotype of *E. huxleyi* type A being the dominant species accounting for an average of 92%, confirming its typical dominance in summer coccolithophore assemblages of the Black Sea;
2. Apart from *E. huxleyi*, and the other heterococcolithophores *S. dilatata*, *S. molischii*, *A. acanthifera*, and *A. quattrosquina*, *Algirosphaera robusta*, and two holococcolithophore forms *S. arethusae* HOL and *H. cornifera*, contributed to the coccolithophore assemblages, indicating their ability to thrive in the low salinity conditions of the Black Sea;
3. The vertical distribution of coccolithophore species appears to depend on the temperature and irradiance levels. *Emiliania huxleyi*, *Syracosphaera* spp., and *Acanthoica* spp. were mainly distributed in the upper water layers, within the thermocline. High abundances of *A. robusta* occurred below the thermocline, indicating its preference for low light and temperature conditions;
4. The highest abundances of *E. huxleyi* (max.  $737 \times 10^4$  cells  $L^{-1}$ ) and *Syracosphaera* (max.  $26 \times 10^4$  cells  $L^{-1}$ ) were found over the northwestern inner shelf region in Danube-influenced waters, associated with increased freshwater and nutrient inputs;
5. Our results indicate that the seasonal thermocline is the main factor regulating the distribution of living coccolithophores, whereas significant spatial variations in the assemblages are related to the influence of freshwater input.

**Supplementary Materials:** The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/d15121194/s1>, Table S1: location data, environmental parameters measured, and filtered seawater volume for coccolithophores at the investigated stations.

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