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New evidence for the ecology of *Helicosphaera carteri* in polluted coastal environments (Elefsis Bay, Saronikos Gulf, Greece)

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Abstract: The Elefsis Bay is a typical coastal setting characterized by a semi-closed shallow environment with intense anthropogenic activity. This study describes a peculiar low cell density and species poor late-winter coccolithophore assemblages from Elefsis Bay compared to those from further offshore of the Aegean Sea. *Helicosphaera carteri* contributes significantly to the assemblage inside the Elefsis Bay and together with *Emiliania huxleyi* both represent the dominant component of the calcareous nannoplankton. Water column data confirmed the opportunistic behavior of H. carteri, thus providing new evidence for the ecology of the species. It is suggested that an increase in this species can be associated with distinct pollution in neritic environments.

Keywords: living coccolithophores, eutrophication-polluted, Aegean, Ionian Seas

1. Introduction

The Aegean Sea has been described as one of the most oligotrophic areas of the eastern Mediterranean, characterized by low biological production (e.g., Ignatiades, 1998; Psarra et al., 2000). However, an established N-S trophic gradient exists from mesotrophic to ultra-oligotrophic regions. The northern Aegean Sea is relatively mesotrophic, being influenced by freshwater discharges from rivers and seasonally varying input of Black Sea surface water through the Dardanelles Straits (Ignatiades et al., 2002; Zervoudaki et al., 2011; Lagaria et al., 2013). Additional areas such as the coastal Saronikos and Evoikos gulfs and the open-ocean Rhodes cyclonic gyre display similar trophic features. In contrast, the "typical oceanic margin" of South Aegean Sea is an oligotrophic to ultra-oligotrophic environment, with very low export rates of organic carbon and organic-poor sediments (e.g., Gogou et al., 2000; Lykousis et al., 2002). In the northern Aegean Sea, diatoms are the most abundant components of the phytoplankton (Lykousis et al., 2002), whereas in the south Aegean Sea, coccolithophores are one of the major primary producers (Ignatiades et al. 2002; Triantaphyllou et al. 2004). *Emiliania huxleyi* dominates the winter coccolithophore assemblages, followed in summer by holococcolithophores and Rhabdosphaeraceae and *Syracosphaera* spp. (Dimiza et al., 2008 a,b).

Despite the oligotrophic nature of the South Aegean Sea, eutrophication problems, indicated by elevated nutrient data, have been recognized locally in a number of coastal areas as a result of increased anthropogenic activities (e.g., Sklivagou et al., 2008; Pavlidou, 2012; Kapsimalis et al., 2014). One of the most impacted coastal areas of Greece is the shallow semi-closed Elefsis Bay, located at the northern edge of the Saronikos Gulf (Fig. 1). The present study describes late-winter coccolithophore abundances and species composition in the restricted environment of Elefsis Bay, highlighting the opportunistic behavior of *Helicosphaera carteri* in neritic environments with elevated pollutant inputs.

2. Materials and methods

Two water samples (5 and 20 m water depth) were collected

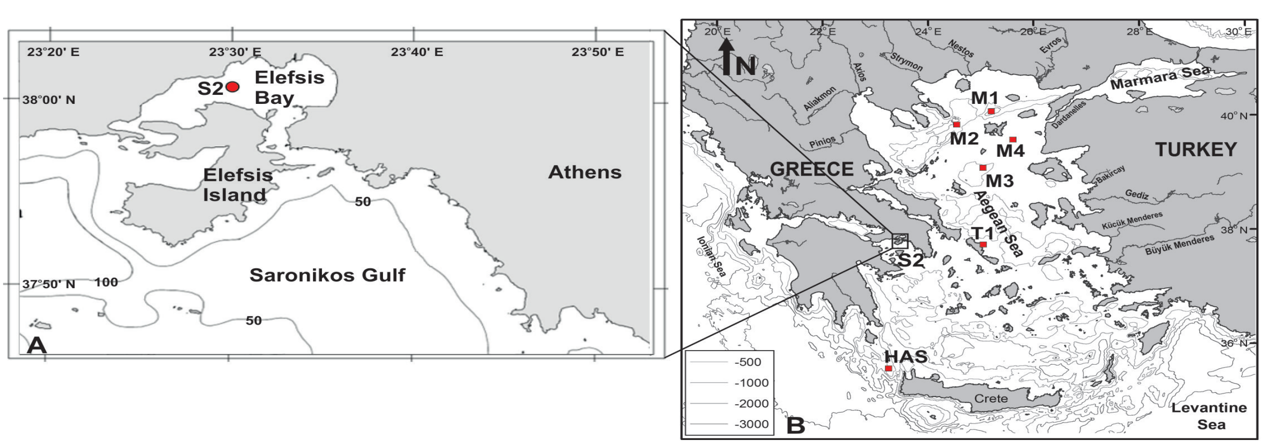


Figure 1. Bathymetric map of the study area and location of the stations sampled for coccolithophore analysis, **A**. Elefsis Bay, **B**. off-shore Aegean environments.

Station	sampling period	Water depth (m)
S2	February 2012	5, 20
M1	January 2011	3, 20
M2	January 2011	3, 20
M3	January 2011	2, 20
M4 (AMT7)	January 2011	2, 10, 20
T1-3	March 2002	5, 15
T1-4	March 2002	5, 15
T1-5	March 2002	5, 10
T1-6	March 2002	5, 10
T1-8	March 2002	0, 5
HAS	February 2012	5, 30

Table 1. Sampling period and water depth from each sampled station.

from station S2 in Elefsis Bay (38°00.00', 23°27.24'; Fig. 1A), during the R/V AEGAEO cruise (February 2012, FP7 Perseus Project). The Elefsis assemblages are compared with winter and early spring coccolithophore assemblages from several water samples in different environments, collected at depths between 0 and 30 m (Table 1). The data set comes from 10 stations (Fig. 1B) in the North Aegean (M1-4: Athos basin), central Aegean (T1: Andros Island - 5 stations), and South Aegean Sea (HAS: Antikythira straits).

For each sampling depth, 2 liters of seawater were filtered on Whatman cellulose nitrate filters (47 mm diameter, 0.45 mm pore size), using a vacuum filtration system. Salt was removed by washing the filters with about 2 ml of mineral water. The filters were open dried and stored in plastic Petri dishes. A piece of each filter approximately 8x8 mm^2 was attached to a copper electron microscope stub using a double-sided adhesive tape and coated with gold. The filter was then examined with a Jeol JSM 6360

Station	Water depth (m)	Total cocco-sphere density (x 10^3 cells/l)	Emiliania huxleyi (x 10^2 cells/l)	Heli-cosphaera carteri (x 10^2 cells/l)	Syra-cosphaera spp. (x 10^2 cells/l)	Rhab-dosphaer-aceae (x 10^2 cells/l)	Gephy-rocapsa oceanica (x 10^2 cells/l)	Holococ-colitho-phores (x 10^2 cells/l)	other species (x 10^2 cells/l)	species diversity (S)	Shannon-Wiener index (H)
semi closed Elefsis Bay											
S2	5	4.6	17	27.5			1.9			3	0.81
	20	5.1	20	27.9	0.5		2.9			4	0.90
off-shore marine environments of the Aegean Sea											
M1	3	29.5	270		16.1	9.0		0.6		16	0.50
	20	22.0	200		13.5	5.1		0.6	0.6	11	0.48
M2	3	19.3	161		14.1	9.6	2.6		6.4	15	0.69
	20	25.9	228		19.3	8.3			3.2	18	0.63
M3	2	33.7	331		33.3	1.7		1.3	0.5	22	0.76
	20	34.9	298		37.9	7.7		2.6	2.6	18	0.68
M4	2	27.7	249		20.5	5.8		0.6	0.6	17	0.53
	10	37.5	354		8.3	10.9	0.6	0.6		14	0.34
	20	33.9	318		15.4	3.2			1.9	13	0.37
T1-3	5	35.3	326	0.6	17.3	2.6		1.3	4.5	12	0.39
	15	19.8	191		1.9	0.6		0.6	4.5	7	0.22
T1-4	5	27.9	270		5.8	0.6	0.6	0.0	2.6	8	0.20
	15	25.8	244		9.0	1.9	1.3	0.0	1.3	9	0.30
T1-5	5	13.7	132		3.2	0.6		0.0	1.3	8	0.23
	10	27.6	265		7.1			1.3	2.6	8	0.23
T1-6	5	20.2	193		7.7			0.6	0.6	7	0.25
	10	21.8	213		0.6	0.6	0.6	0.6	1.9	8	0.14
T1-8	0	9.2	87	0.6	1.9		0.6	0.6	1.9	8	0.35
	5	11.4	105		7.7	0.6			0.6	7	0.40
HAS	5	18.4	132	2.6	13.5	14.8		3.8	17.3	26	1.42
	30	19.6	155		24.4	7.1		3.8	26.1	24	1.07

Table 2. Total coccolithophore density, species diversity, Shannon–Wiener index and abundance of the most common coccolithophore taxa.

Scanning Electron Microscope (University of Athens, Department of Historical Geology and Palaeontology). Coccolithophore densities (coccospheres/l) were calculated following the methodology of Jordan and Winter (2000).

Species diversity (S) and Shannon Shannon–Wiener diversity index (H) were calculated using the Past software package vers. 1.23 (Hammer et al. 2001). Species diversity is the total number of species observed in each sample, while the Shannon–Wiener index is a measure of diversity that have in consideration also the amount of cells.

3 Results

3.1 The semi-closed Elefsis Bay

The total coccolithophore density in the Elefsis Bay samples was relatively low - 4.6 and 5.1 x10^3 coccospheres/l (Table 2). The coccolithophore assemblage consisted only of four heterococcolithophore species, with low species diversity, but the Shannon–Wiener index was relative high (H=0.81 and 0.90), since no one species dominated the assemblages.

The most abundant species was *Helicosphaera carteri* with concentrations up to 2.7 x10^3 coccospheres/l, thus constituting more than 50% of the assemblage. No loose helicoliths have been observed in the studied samples; in general, the absence of loose helicoliths from the samples indicates the new development of the population (e.g., van der Waal et al., 1995). *Emiliania huxleyi* represents the second most abundant species that contributes with 2.0x10^3 cells/l, comprising about 40% of the assemblage. Among the minor species, *Gephyrocapsa oceanica* was a significant element with cell density up to 2.9 x10^2 coccospheres/l, whereas *Syracosphaera anthos* was present with cell densities less than 1.0 x10^2 coccospheres/l (Table 2).

3.2 Off-shore environments of the Aegean Sea

During winter and early spring the total coccosphere densities from the studied Aegean Sea off-shore environments varied between 9.2 x10^3 (central Aegean, Stn T1-8, 0 m) to 37.5 x10^3 cells/l (North Aegean, M4, 10 m) with an average of 24.5 x10^3 cells/l (Table 2). A total of 60 (48 heterococcolithophores and 12 holococcolithophores) species have been identified. The average species diversity was 13 species per sample, with highest values (26 taxa) observed in the South Aegean (Stn HAS, 5m). Shannon–Wiener index showed similar patterns with species diversity (Hmax=1.42 at South Aegean, Stn HAS, 5m).

Emiliania huxleyi was the most abundant species with concentrations up to 35.4 x10^3 cells/l in the North Aegean (Stn M4, 10m), typically constituting >70% of the coccolithophore assemblages. *Syracosphaera* spp. (up to 3.8 x10^3 coccospheres/l at Stn M3) were generally represented by more than 15 species, among which the most abundant are *Syracosphaera molischii*, *Syracosphaera pulchra* and *Syracosphaera ossa*. Rhabdosphaeraceae were well represented with concentrations up to 1.5 x10^3 cells/l at Stn HAS, 5m). Among them *Algirosphaera robusta* and *Rhabdosphaera clavigera* were the most prevalent. *Helicosphaera carteri* and *Gephyrocapsa oceanica* showed a maximum abundance 2.6 x10^2 cells/l in stations HAS, 5m and M2, 3m respectively.

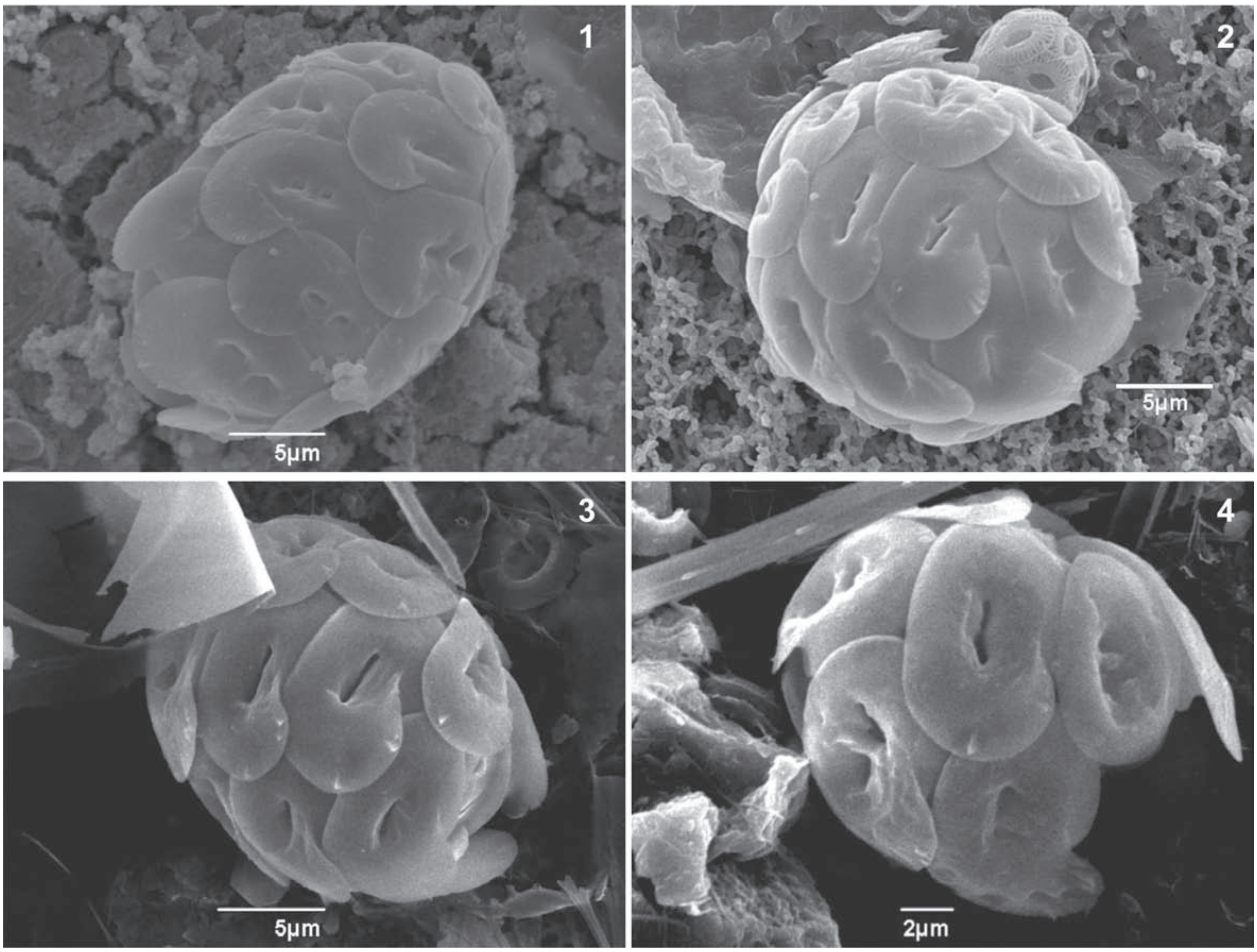
4. Discussion and conclusions

Elefsis Bay is a shallow semi-closed embayment (maximum depth 33 m) that presents a typically seasonal variation of the hydrological parameters. The annual maximum average temperature occurs around July/August (25^o C); minimum values (10^o C) are reached in February/March. Salinity shows an almost homogeneous vertical profile, with seasonal variation ranging from 37.5 in March to 39.0 in summer (e.g., Friligos, 1983; Siokou-Frangou et al., 1998). During summer, the bay is influenced by a persistent thermocline which develops at 15 m leading to water column stratification and consequent bottom-water anoxia, while intense vertical mixing of the water column occurs during winter (e.g., Scoullos and Riley, 1978; Zarkanellas, 1979; Papathanasiou and Zenetos, 2005). In addition to water mass circulation, the increased levels of domestic, industrial and shipping activities are continuously degrading the environmental quality, especially associated with eutrophic conditions (e.g., Friligos, 1981; Pagou, 1995; Pavlidou et al., 2010; Pavlidou, 2012), high values of hydrocarbons and trace elements (e.g., Scoullos et al., 2007; Sklivagou et al., 2008; Valavanidis et al., 2008). Particularly, the trophic status is characterized as eutrophic for nitrate: ~ 1.88 μM and higher mesotrophic for phosphate: ~ 0.38 μM and ammonium: ~ 1.12 (Pavlidou, 2012) have a prevailing control on the seasonal succession of phytoplankton (e.g., Ignatiades, 1983; Gotsis-Skretas, 1995; Pagou, 1995) and significantly decrease the zooplankton richness (e.g., Simboura et al., 1995; Siokou-Frangou et al., 1995, 1998).

In the present study, the late-winter coccolithophore assemblages in the restricted environment of Elefsis Bay show significantly lower abundance and species diversity in comparison with off-shore assemblages of the Aegean Sea. This is consistent with the results by Gotsis-Skretas (1995) who reported the lowest phytoplankton densities in Elefsis Bay in February, in contrast to the more open marine environments of Saronikos Gulf that present maxima in March. Furthermore, the low coccolithophore species diversity (Table 2) may reflect the unstable environmental conditions in the coastal environments. Indeed, the assemblage consists mainly of the two species *H. carteri* and *E. huxleyi*. Interestingly, the coccolithophore Shannon-Wiener index in the area is higher than in the off-shore environments of the Aegean Sea during the same season (Table 2). From the statistical point of view, the high Shannon-Wiener index values are due to both *H. carteri* and *E. huxleyi* high concentrations, while in the off

Plate 1

Morphological variability in the coccoliths of *Helicosphaera carteri* (Wallich 1877) Kamptner 1954. **fig. 1.** Coccosphere with helicoliths having one longitudinal slit or two small pores in the central area, arranged along the long axis of the coccolith, Stn HAS 5m, **fig. 2.** Coccosphere with helicoliths having one or two longitudinal slits in the central area, arranged along the long axis of the coccolith; one coccolith having two oblique slits (arrow), Stn T1-3-5m, **figs 3, 4.** Coccosphere with helicoliths having one or two longitudinal slits in the central area, Stn S 5 m.



shore Aegean coccolithophore Shannon-Wiener index is lower due to the dominance of *E. huxleyi*.

The opportunistic species *E. huxleyi* is by far the most abundant coccolithophore on a global basis and has a wide ecological distribution (e.g., Young, 1994). In the Mediterranean Sea waters, this species prevails throughout the year in the living coccolithophore assemblages (e.g., Knappertsbusch, 1993; Kleijne, 1993; Cros, 2001; Malinverno et al., 2003; Triantaphyllou et al., 2004, 2010), and predominates during winter in the Aegean Sea (Dimiza et al., 2008a, b). In Elefsis Bay, it has been reported as one of the most abundant species in the July peak of microplankton abundance, with concentrations up to 3.1 x10^6 cells/l, constituting more than 70% of the population (Gotsis-Skretas, 1995; microplankton countings with inverted microscope).

Helicosphaera carteri has been rarely reported from the North Aegean, whereas it is a common minor component of the assemblages of the South Aegean Sea (e.g., Triantaphyllou et al., 2004). However, its abundance in the restricted environment of Elefsis Bay is unusually high, replacing in dominance *E. huxleyi*. In addition , *H. carteri* coccospheres display notable morphological variability. Most helicoliths have two longitudinal slits in the central area, arranged along the long axis of the coccolith, however helicoliths with two clockwise oblique slits or one longitudinal slit are also observed (Plate 1, figs. 1-4)**.** Traditionally, *H. carteri, H. wallichii* and *H. hyalina* have been considered as varieties, since different coccoliths mostly based on the slits type in the central area, have been recovered in association on the same coccosphere (Jordan and Young, 1990; Kleijne, 1993; Jordan and Green, 1994; Cros and Fortuño, 2002; Malinverno et al., 2008; present study). However, culture studies and molecular analysis showed that coccolith morphology is stable in culture and that strains of the different morphotypes are genetically distinct, strongly suggesting that they represent separate species (Sáez et al., 2003; Geisen et al., 2004). An intriguing possibility, although it needs to be tested, is that the high morphological variation seen here is a result of malformation in response to the environmental stress/pollution and so there is potential to use *H. carteri* morphology for biomonitoring.

Helicosphaera carteri is commonly found in warm waters (e.g., Brand, 1994; Baumann et al., 2005), associated with moderately elevated nutrient levels (e.g., Ziveri et al., 1995; Findlay and Giraudeau, 2000, 2002; Andruleit and Rogalla, 2002, Ziveri et al. 2004); in the western Mediterranean Sea waters this species has been reported to live close to the chlorophyll maximum (Cros, 2001). In the Ionian Sea, *Helicosphaera carteri* is scarce to nearly absent in the late fall to early winter coccolithophore assemblages (Malinverno et al., 2003) and represents <1% of the yearly coccolith flux (Malinverno et al., 2014), while its contribution to the yearly coccolith flux is higher (2.6%) in the more coastal Ionian Sea offshore Crete (Malinverno et al., 2009) It is commonly considered as a species tolerant of low salinity and terrigenous input (Giraudeau, 1992; Flores et al., 1997; Colmenero-Hidalgo et al., 2004; Triantaphyllou et al., 2009), with high frequencies in regions influenced by riverine discharge (Cros, 2001; Colmenero-Hidalgo et al., 2004), as a coastal water taxon (e.g., Giraudeau, 1992; Ziveri et al., 1995). Hints for an opportunistic behavior, triggered by moderately elevated nutrients in estuarine environments, have been already given by Cachão et al. (2002) and Guerreiro et al. (2005), when studying Holocene sediment records. Our results reinforce these findings, verifying the opportunistic behavior of *H. carteri* in water samples, thus providing new evidence for the ecology of the species, suggesting that its increase may also be observed in distinctly polluted neritic environments. However, the correlation between *H. carteri* distribution and chemical data, abnormal concentrations in nutrients and/or other trace elements, measured in the same water samples is essential to confirm these results. Detailed future research of coccolithophore distribution on a seasonal basis and its relationships with the environmental factors, will monitor the role of *H. carteri* in the semi-closed Elefsis Bay ecosystem.

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