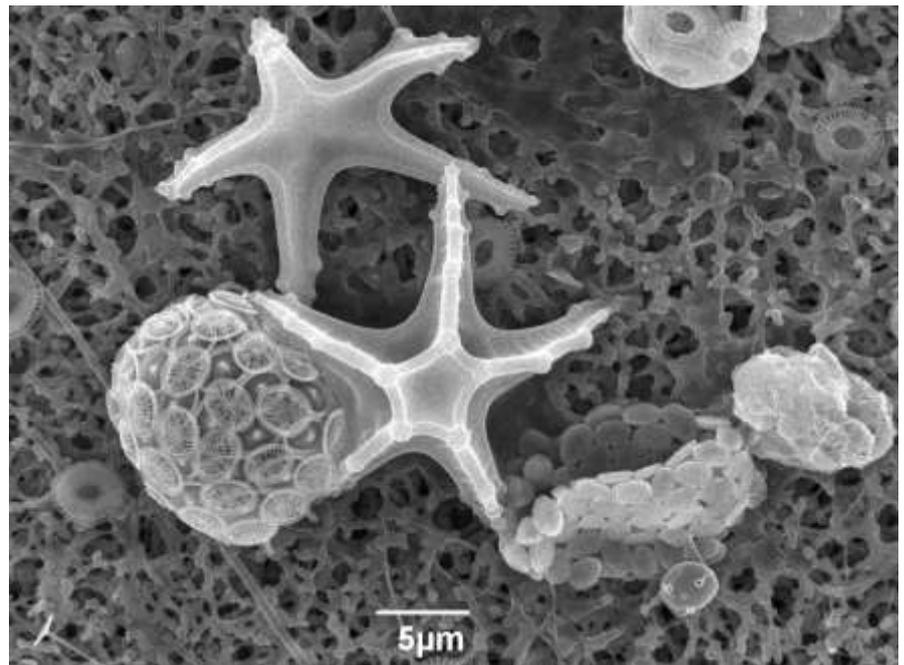




Εθνικόν και Καποδιστριακόν  
Πανεπιστήμιον Αθηνών

# Tracing the Aegean water masses using phytoplankton (coccolithophores) assemblages: modern distribution and paleoceanographic implications



**MSc Thesis**

**Boris- Theofanis Karatsolis- 214005**

**Master of Oceanography and Marine Environment Management**

**Faculty of Geology and Geoenvironment**

Thesis committee: Professor Triantaphyllou Maria, Associate Professor Nomikou Paraskevi, Research Director Psarra Stella.

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# **Topic: Tracing the Aegean water masses using phytoplankton (coccolithophores) assemblages: modern distribution and paleoceanographic implications**

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- Karatsolis, B.-Th., Triantaphyllou, M.V., Dimiza, M.D., Malinverno, E., Lagaria, A., Mara, P., Archontikis, O. , Psarra, S., 2016. Coccolithophore assemblage response to Black Sea water inflow into the North Aegean Sea (NE Mediterranean), Continental shelf research.
- Lagaria, A., Mandalakis, M., Mara, P., Frangoulis, C., Karatsolis, B., Tsiola, A., Pitta, P., Triantaphyllou, M., Psarra, S., 2016. Phytoplankton dynamics and community structure in relation to hydrographic features in the NE Aegean frontal area (NE Mediterranean). Continental shelf research.
- Triantaphyllou, M.V, Karatsolis, B., Dimiza, M.D., Malinverno, E., Cerino, F., Psarra, S., Jordan, R.W., Young, J.R., 2015. Coccolithophore combination coccospheres from the NE Mediterranean Sea: new evidence and taxonomic revisions. Micropaleontology
- Dimiza, M.D., Triantaphyllou, M.V., Malinverno, E., Psarra, S., Karatsolis, B., Mara, P., Lagaria, A., Gogou, A., 2015. The composition and distribution of living coccolithophores in the Aegean Sea (NE Mediterranean). Micropaleontology.
- Karatsolis, B., Dimiza, M.D., Triantaphyllou, M.V., 2014. Verification of the *Coronosphaera mediterranea* –“*Zygosphaera hellenica*” life-cycle association. Journal of Nannoplankton Research 34: 45-46.
- Triantaphyllou, M.V., Dimiza, M.D., Karatsolis, B., 2014. Adding proofs to *Syracosphaera histrica*-*Calyptrolithophora pappilifera* life-cycle association. Journal of Nannoplankton Research 34: 57-59.

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## **1. Introduction**

This thesis is investigating the spatial and temporal distribution of marine phytoplankton (coccolithophores and silicoflagellates) along a transect of sampling stations in the North Aegean area. The different stations are located in an area affected by the inflow of the Black Sea Water mass (BSW) at surface layer overlying the deeper Levantine Water (LW). The results of the study are compared with results from other parts of the Aegean (Kolumbo submarine volcano area, Antikithira strait and Ionian Sea) which emerge from this and older studies. Finally, the distribution of another type of marine phytoplankton organisms (silicoflagellates) is also thoroughly investigated to add information about the distinction of the water masses and about the morphotypes and species which we meet in each one of them.

The multidisciplinary approach of this thesis is aiming to improve our understanding in the relation between different water masses in the North Aegean area and how these affect the algal community variability and productivity through changes in temperature, salinity and other physico-chemical parameters. The two main masses studied are Black Sea water mass (BSW) and Levantine water mass (LW). Their distinction is further supported by the information provided from studies in the South part of Aegean and in the Ionian Sea. Another part of this work, conducted in the area of Kolumbo submarine volcano is investigating the possible effects of the submarine vent field of the Kolumbo volcano, located in Cyclades Plateau, to the coccolithophore assemblages of the area. This vent field is significantly affecting the carbon chemistry of the area by adding to the emissions of green gasses. The effects that these emissions may have to the biogenic  $\text{CaCO}_3$  producing organisms need to be further understood and are an important to help us predict what the outcome of potential ocean acidification could be.

## **2. Marine microalgae**

### **2.1 Coccolithophores**

#### **2.1.1 Biology and Taxonomy**

Coccolithophores are marine microorganisms and include all haptophyte algae possessing calcified scales (coccoliths) at some stage in their life cycle. Following the taxonomic revision of the division Haptophyta proposed by Edvardsen et al. (2000),

coccolithophores belong to the class Prymnesiophyceae which also involves non-calcifying organisms. Of the approximately 300 haptophytes in modern oceans, about 200 are in fact coccolithophores and these contribute significantly to the biodiversity of the group (Jordan and Chamberlain 1997).

One of the characteristics of the haptophytes is the flagella-like haptonema. It differs from the flagella proper in its internal structure and its basal attachment. During the non-motile phase the flagella disappear but the haptonema often remains. The algal cell contains a nucleus and two golden-brown chloroplasts which may be moved around the cell to optimise collection of available light. The cell also contains mitochondria which contain enzymes producing the energy for cell function, vacuoles which deal with waste products and the Golgi apparatus that hosts coccolith secretion in many species.

The life cycle of coccolithophores is characterized by an alternation of diploid and haploid phases. They alternate from the haploid to diploid phase through syngamy and from diploid to haploid through meiosis. In contrast with most organisms with alternating life cycles, asexual reproduction by mitosis is possible in both phases of the life cycle (Young et al.2003). The morphology and life cycle of a coccolithophore can be seen in the schematic representation from Bown et al.1998 (Fig. 1).

Table 1 presents Coccolithophores taxonomy as presented in Nannotax3: <http://ina.tmsoc.org/Nannotax3/index.php?dir=Coccolithophores>

COCCOLITHALES Mostly placolith heterococcoliths, with V-unit forming the distal shield; R-unit the proximal shield.	COCCOLITHACEAE CALCIDISCACEAE HYMENOMONADACEAE PLEUROCHRYSIDACEAE
ZYGODISCALES Heterococcoliths with V-units forming upper/outer cycle of imbricated elements and R-units forming basal plate and central mass of irregular elements	HELICOSPHAERACEAE PONTOSPHAERACEAE ZYGODISCACEAE
SYRACOSPHAERALES Coccoliths with radial lath cycle of T-units, and disjunct, often complex, axial structure, coccospheres often polymorphic	CALCIOSOLENIACEAE RHABDOSPHAERACEAE SYRACOSPHAERACEAE
COCCOLITH FAMILIES INC SED Various heterococcolith groups not obviously related to the well- defined orders	ALISPHAERACEAE PAPPOSPHAERACEAE UMBELLOSPHAERACEAE NARROW RIMMED

	PLACOLITHS COCCOLITH GENERA INC SED UNIDENTIFIED
HOLOCOCOLITHS Haploid life-cycle stages, with liths formed of numerous rhombohedral microcrystals	TUBELESS CONVEX BRIDGED FLAT-TOPPED SEPTATE OPEN-TUBE FOSSIL HOLOCOCOLITHS
DISCOASTERALES Radially symmetrical nannoliths formed from one to several separate cycles of elements that radiate from a common centre or axis.	DISCOASTERACEAE FASCICULITHACEAE HELIOLITHACEAE SPHENOLITHACEAE
NANNOLITH FAMILIES INC SED Haptophytes forming calcareous structures not obviously homologous with heterococcoliths, holococcoliths, or discoasteralids	BRAARUDOSPHAERACEAE CERATOLITHACEAE LAPIDEACASSACEAE LITHOSTROMATIONACEAE NANNOLITH GENERA INC SED RHOMBOASTERACEAE
MESOZOIC SURVIVORS Mesozoic taxa which occur in low abundances in the early Palaeogene.	BISCUTACEAE CHIASTOZYGACEAE MARKALIUS WATZNAUERACEAE

Table 1

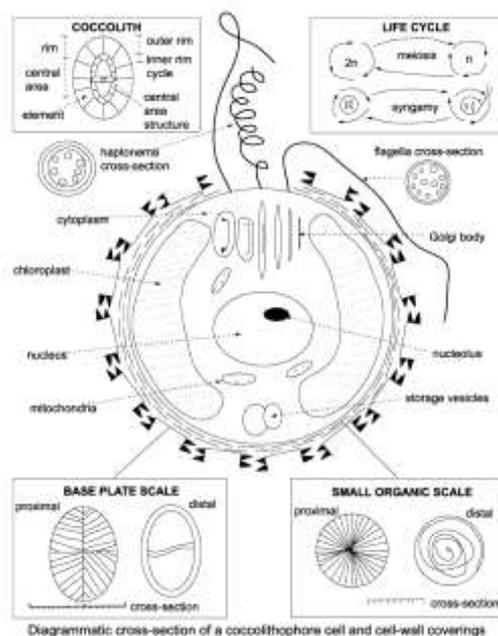


Figure 1 Diagram from Bown & Young (1998) in Bown, P. (Ed.), 1998, *Calcareous Nannofossil Biostratigraphy*. Chapman and Hall.

### **2.1.2 Distribution and Ecology**

Coccolithophores are abundant throughout the oceans today, and can be found from tropical to sub-arctic waters, even where the water temperature drops below 0°C. The coccolithophores life is related to the photic zone, the maximum abundance being recorded at 50 depth (Tappan, 1980). They form the base of the oceanic food web and hence they play an important role in marine ecosystems. Due to their capacity to control ocean- atmosphere carbon dioxide exchange, they participate actively in the formation of the climate system (Berger et al., 1989; Westbroek et al., 1993; Rost & Riebesell, 2004). Their composition and distribution are directly dependent on the climatic and oceanographic conditions of the oceans and that makes them a good indicator for studying a variety of environmental changes such as fluctuations of light, salinity, temperature, sea-level, ocean productivity, nutrients and water pollution. Additionally, coccolithophores are really important stratigraphic indicators. Their global distribution, their preservation into the sediments and their occurrence since the Late Triassic make them an important biostratigraphic tool.

#### **Light**

The coccolithophores play an important role in the carbonate chemistry of the ocean due to their ability to produce biogenic CaCO<sub>3</sub>. This deposition of calcium in coccolithophores seems to be strongly related to the photosynthesis processes. Results from cultures show that the production of coccoliths is reduced or even inhibited by lower light intensity (Young, 1994). The behavior of various nannoplankton species is different under light fluctuations. The rate of growth of *Emiliana huxleyi* looks like it is not disturbed even when the light intensity is lower than usual (e.g. 1000 µE/sm<sup>2</sup> – Nanninga & Tyrrell, 1996) or when it is exposed to long photoperiods (Young, 1994). By contrast, the growth of some species, such as *Calcidicus leptoporus*, is strongly inhibited by long photoperiods (Brand & Guillard, 1981).

#### **Salinity**

Coccolithophores can live under a big variety of salinity fluctuations. For example, *Coccolithus pelagicus* has been found in the Dead Sea, at 250 ‰ (Tappan, 1980) and *Emiliana huxleyi* lives in the Black Sea, at salinity below 20‰ (Black, 1974; Aksu et al., 2002). In general, the oceanic species tolerate narrow fluctuations of salinity, while coastal species tolerate wider salinity changes (Brand & Guillard, 1981).

## **Temperature and latitudinal distribution**

Nowadays, the calcareous nanoplankton shows a latitudinal distribution, related to their ability to survive under different physicochemical conditions. That leads to a distribution of species into different zones of the ocean. This distribution can be a characteristic for some species, where others can appear in more than one zones.

Four biogeographic zones have been identified for coccolithophore distribution: subarctic/subantarctic, temperate (transitional), subtropical (central) and tropical (equatorial) (McIntyre & Be 1967, Winter et al. 1994). Highest species diversity can be found in the tropical and subtropical zone, decreasing towards higher latitudes (McIntyre & Be 1967). From the living coccolithophores, *Syracosphaera pulchra* and *Helicosphaera carteri* are related to low-middle latitudes, *Calcidiscus leptoporus* is more common in the Temperate Zone and *Coccolithus pelagicus* is found mostly to high latitudes. The most widespread living calcareous nanoplankton species is *Emiliana huxleyi*. This cosmopolitan coccolithophore occurs today in all oceans, except the Arctic Ocean and the high-latitude Southern Ocean (Winter et al., 1994).

## **Blooms**

Under some circumstances, the development of calcareous nanoplankton species can be significantly increased leading to huge cell concentrations, called blooms. Nowadays, the most studied blooms are those of *Emiliana huxleyi*. They are common in different parts of the oceans and are important for the ecology of the area. According to Tyrrell & Merico (2004), blooms of *Emiliana huxleyi* should be considered only cell concentrations of at least 1,000,000 cells per liter. Regions with constant *E.huxleyi* blooms are the Norwegian fjords and the Black Sea. In the Black Sea, in 1992, a bloom with a maximum of 10,000,000 cells/liter (Mankovsky et al., 1996) was recorded. These blooms have significant environmental impacts, consisting of increasing water albedo, large fluxes of calcium carbonate from the surface waters to the bottom of the ocean, and a decrease in light and heat depth penetration (Tyrrell & Merico, 2004).

## **Ocean acidification**

The heating of the planet due to the anthropogenic production of CO<sub>2</sub> can severely damage the coccolithophore species. The oceans can be viewed as sink for heat, as water has much more heat inertia than air, so that more than 80% of the additional heat so far has entered the ocean (Tyrrell 2011). This heat increases the stratification of the ocean and as a result it blocks the proper mixing of the surface layers and their replenishment with nutrients. This nutrient deficiency damaging phytoplankton species which need these nutrients in all the stages of their life cycle.

In the ocean, CO<sub>2</sub> reacts with water to form H<sub>2</sub>CO<sub>3</sub>, which then dissociates into HCO<sub>3</sub><sup>-</sup> and CO<sub>3</sub><sup>2-</sup> according to equilibrium between these three compounds  $\text{CO}_2 + \text{H}_2\text{O} \rightleftharpoons \text{H}_2\text{CO}_3 \rightleftharpoons \text{H}^+ + \text{HCO}_3^- \rightleftharpoons \text{H}^+ + \text{CO}_3^{2-}$ . The current pH in the ocean is 8.1 on average (Hofmann and Schellnhuber 2010), and most of the inorganic carbon is present as HCO<sub>3</sub><sup>-</sup>. The equilibrium is strongly related to the pH since H<sup>+</sup> is produced in both dissociation steps. Although, it can be observed that the drop of the pH moves the equilibrium to the left, and affects the concentrations of CO<sub>2</sub> and CO<sub>3</sub><sup>2-</sup>, increasing the first and decreasing the second (Fig. 2). Calcifiers such as coccolithophores are affected by this change, because the calcium carbonate saturation  $\Omega$  (omega) in seawater is positively dependent on the CO<sub>3</sub><sup>2-</sup> concentration. Reducing this concentration leads to higher levels of dissolved calcium carbonate cells.

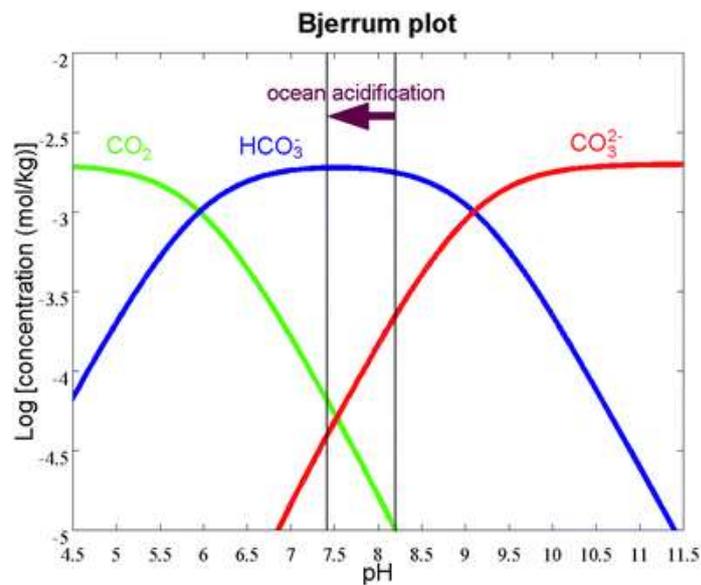


Figure 2 Concentrations of the components of the oceans carbonate buffer system versus pH for  $\text{DIC}=2000 \mu\text{mol L}^{-1}$ ,  $S=35$  and  $T=25^\circ\text{C}$ . Modified after Hofmann and Schellnhuber (2010).

## 2.2 Silicoflagellates

Silicoflagellates are a group of unicellular heterokont marine planktonic algae belonging to the class Dictyochophyceae (Silva 1982), order Dictyochales (Haeckel 1894). They represent a minor component of the siliceous plankton in the world's oceans and are most abundant in high-latitude and equatorial upwelling settings (Poelcheau, 1976), where they are usually outcompeted by diatoms. Silicoflagellate taxonomy has been developed exclusively on the morphology of their siliceous skeleton, but is also supported by biological studies on ultrastructural characters (Moerstrup and Thomsen, 1990) and recent molecular data (Chang et al., 2012). The siliceous skeleton of silicoflagellates is formed by hollow tubes that are connected to form a polygonal basal ring and an apical structure that can be shaped like a polygonal ring, a bar or a plate. Additional features include spines, developed on the basal ring (and occasionally on the apical structure) and directed outwards, and pikes, developed on the basal ring and directed abapically (see McCartney et al., 2014b for a recent review).

Silicoflagellate skeletons are recorded in marine sediments back from the late Cretaceous (McCartney 2010, 2011), and are useful biostratigraphic tools in the Cenozoic. Extant species belong to three different genera: *Dictyochoa*, characterized by a 4-sided basal ring with spines and pikes and by an apical bar; *Stephanocha*, characterized by a typically 6-sided basal ring with spines and pikes and a 6-sided

apical ring; *Octactis*, characterized by a typically 8-sided basal ring with spines but lacking pikes and an 8-sided apical ring.

Strong plasticity characterizes the skeletal morphology of silicoflagellates that changes under the influence of various environmental factors. Complicate morphologies, including aberrant forms, commonly occur in cold waters where the silicoflagellate populations are abundant, suggesting a positive forcing of environmental stress (Malinverno et al.2010). Overall, the varying abundance of silicoflagellate species and/or morphologies along sediment successions has been proved useful in paleoceanographic reconstructions of paleotemperatures (Bohaty and Harwood, 1998; Ciesielski, 1974; Ciesielski and Weaver, 1973; Whitehead and Bohaty, 2003), paleonutrients and thermocline dynamics (Barron and Bukry, 2007a, b; Barron et al., 2004; Barron et al., 2005; Barron et al., 2009; Barron et al., 2012), sea-ice development (Onodera et al., 2013; Teraishi et al., 2013).

### **3. Materials and methods**

#### **3.1 Setting**

This study involves comparison of data analysis from different study regions. The main study area of this work was the northeastern part of the Aegean Sea. Secondary, surface water samples from the Kolumbo submarine volcano region near Santorini, also contributed to the completion of this study. Data from other parts of the Aegean and the Ionian Sea played also an important role for the comparison and discussion of the results. Finally, observations from surveys conducted in several parts of the world (Southern Ocean, North Pole) contribute to the ecological study and remarks for the silicoflagellate species distribution.

##### **3.1.1 Northeastern Aegean**

The Aegean Sea (NE Mediterranean) represents a transition zone between temperate and semiarid climate conditions and is characterized by small size and complex bathymetry that includes a significant number of deep basins and trenches. The fluvial freshwater inputs are more intense in the north, due to numerous major rivers from the surrounding areas of the Balkans and Turkey, which provide 75% of the North Aegean sediment influx (e.g., Lykousis et al., 2002; Roussakis et al., 2004). These inputs constitute an important source of land-derived organic matter to the area (e.g., Gogou et al., 2007; Triantaphyllou et al., 2016). The Northeastern (NE) Aegean is comprised of a marginal sea, adjacent to the Dardanelles Straits, which connects the Marmara Sea with the Mediterranean. It presents characteristic hydrology and complex surface water circulation. Strong temperature and salinity contrasts are observed throughout the year due to the encounter and mixing of less saline, lighter Black Sea Waters (BSW) outflowing from the Dardanelles Straits with the highly saline, dense, oligotrophic Levantine waters (LW) (Zervakis et al., 2000). The differences between the physical characteristics of the two water masses result in the development of a significant thermohaline front, the position of which exhibits large temporal variation (Zervakis and Georgopoulos, 2002). Moreover, the BSW outflow and spreading influence the formation of the surface mixed layer (Androulidakis et al., 2012), which plays a vital role on the evolution of biological processes in the Aegean Sea. It should be pointed out that the NE Aegean Sea is one of the most important fishing grounds in the eastern Mediterranean (Stergiou et al., 1997) and it typically presents enhanced biomass and production, both autotrophic and

heterotrophic, compared to South Aegean (Ignatiades et al., 2002; Siokou-Frangou et al., 2002). Although these characteristics are generally attributed to the presence of BSW masses, the underlying mechanisms/pathways accounting for the productivity of the system and especially for the enhanced autotrophic activity are still not well elucidated.

### **3.1.2 Kolumbo region**

The setting of the conducted study is the submarine volcano of Kolumbo. Kolumbo is located 7 km northeast off Santorini Island in the central part of the Hellenic Volcanic Arc (HVA). The HVA is a 500 km-long curving chain of volcanoes, Pliocene to modern in age, extending from the Methana peninsular zone on the Greek mainland, through the islands of Milos-Antimilos, Santorini, Kos-Nisyros, towards the western peninsular zone in Turkey. The HVA results from the extended and elongated subduction of the African plate beneath the Eurasian continent. In the Santorini area, volcanism extends northeastward into the submarine environment evidenced by a series of small craters and cones aligned along Christianna-Santorini-Kolumbo (CSK-Fig.1) tectonic line (Nomikou et al.2013). The largest of the CSK tectonic line craters is Kolumbo, which is 1500 m wide and has a 3 km diameter cone. This crater's floor is situated in an average depth of 505m below sea level.

Kolumbo is the most active known hydrothermal activity in the Aegean (Christopoulou et al.2016). It is the largest submarine volcano of the series of at least 19cones of the area. The rim of its crater has an inclined circular shape ranging from a depth of 18 to 150 m from sea surface (Nomikou, 2004). Near the surface region (~0–20 m) there are significant variations in temperature and salinity, while at greater depths (up to ~80 m from the surface) variations are reduced in magnitude, but are still present, resembling the typical conditions of the thermocline. The zone between 80–230 m is characterized by increased water turbidity. The uneven morphology of the rim, with a shallow peak on the SW side and a deeper level (up to 150 meters) around the rest, forces water masses to be deflected resulting in increased turbidity by turbulent entrainment of sediment (Christopoulou et al.2016). The fact that the crater walls are nearly vertical (Nomikou et al., 2013, 2014) enhances the isolation of the water masses inside the crater and reduces its interaction with surface water currents (Fig.2B).

The extensive hydrothermal vent field of the area was discovered in the northern part of the crater floor during ROV explorations in 2006 (Sigurdsson et al.2006). The gases that this field emits are dominated by CO<sub>2</sub>, with only minor amounts of hydrogen and methane. These CO<sub>2</sub> emissions are being sequestered within the crater, as a result of the formation of dense waters CO<sub>2</sub> gas is dissolved in the bottom waters of the crater (Carey et al.2013). Thus, the volcanic discharge of CO<sub>2</sub> contributes to local reductions in pH and serve as a natural experiment in ocean acidification (Hall-Spencer et al., 2008; Tunnicliffe et al., 2008).

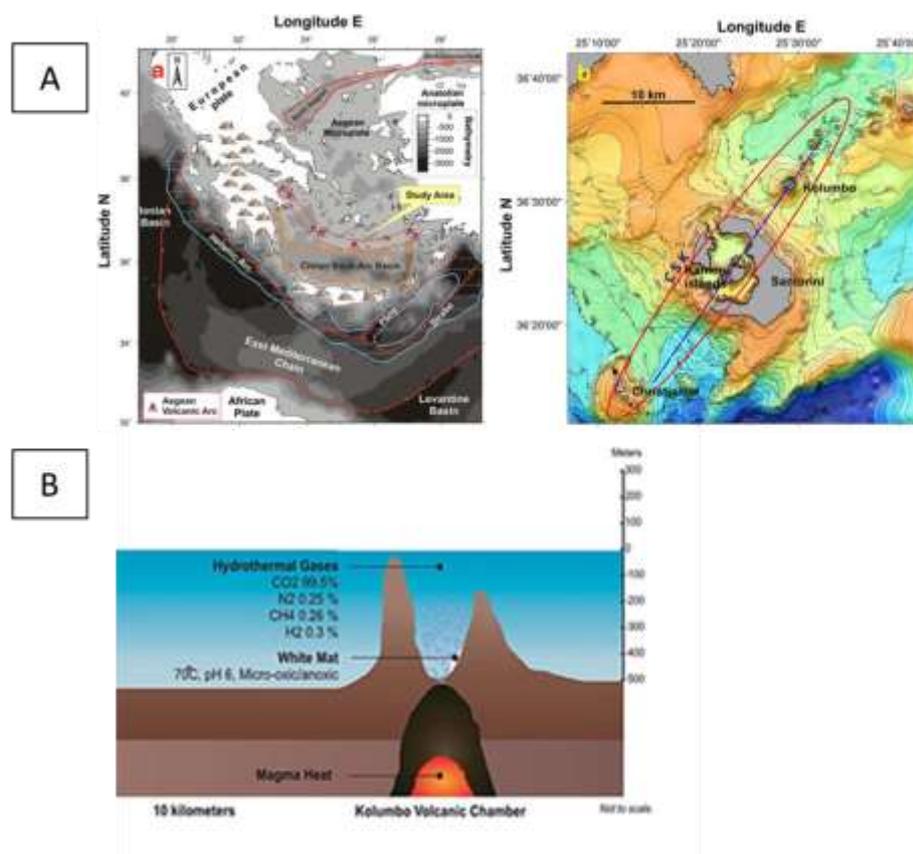


Figure 1 A1. Simplified map of the present day geodynamic structure of the HVA, showing the modern volcanic arc developed behind the Hellenic trench, the Peloponnese–Crete island arc and the Cretan back-arc basin. The study area is located in the center of the HVA (modified Nomikou et al., 2013). A2. Swath bathymetric map of Christianna-Santorini-Kolumbo (CSK) volcanic fields (modified from Nomikou et al., 2012). Figure from Rizzo et al. (2016). B. Schematic depiction of the Kolumbo volcanic chamber located in the Hellenic Volcanic arc (Figure from Oulas et al.2016).

### 3.2 Sampling

The first part of the present study focuses on 72 seawater samples from 4 stations along a transect of stations (Fig. 4) that were visited during three oceanographic cruises undertaken within the AegeanMarTech project ("Technological and oceanographic cooperation Network for the Study of mechanisms fertilizing the North-East Aegean Sea") in October 2013 (10-12/10), March 2014 (22-24/3) and July 2014 (15-18/7), on board the R/V AEGAEO. For each sampling station, a series of usually 6 standard depths were sampled within the photic layer (0-75 m). Several physico-chemical parameters including temperature, salinity, oxygen, chlorophyll-a concentrations, and mineral nutrients were measured at all stations (Lagaria et al., in press). Figure 4 shows all the sampling stations of the transect, from which AMT2, 3, 6,7 were the ones studied within this thesis. In addition, samples from previous expeditions have been used for comparison purposes (see Fig. 5A, Table 2).

For the second part of the study water samples were collected through the Seabiotech cruise from three stations CTD1, CTD2 and CTD3 in September 2013 and two stations (CTD2, CTD3) in May 2014 from 5-90 m water-depth from the site of Kolumbo hydrothermal vent field (Fig. 6A).

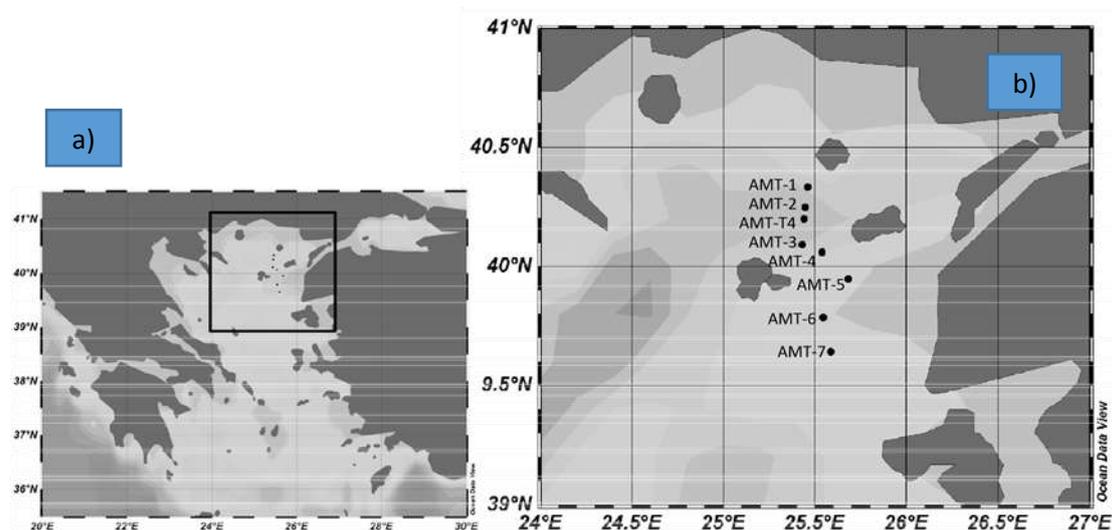


Figure 4 Sampling stations of the AegeanMarTech project: a) the general location of the study area. b) the specific transect of sampling station, from 1-7 t. The samples analyzed for the present study are derived from the stations AMT-2 AMT-3, AMT-6 and AMT-7.

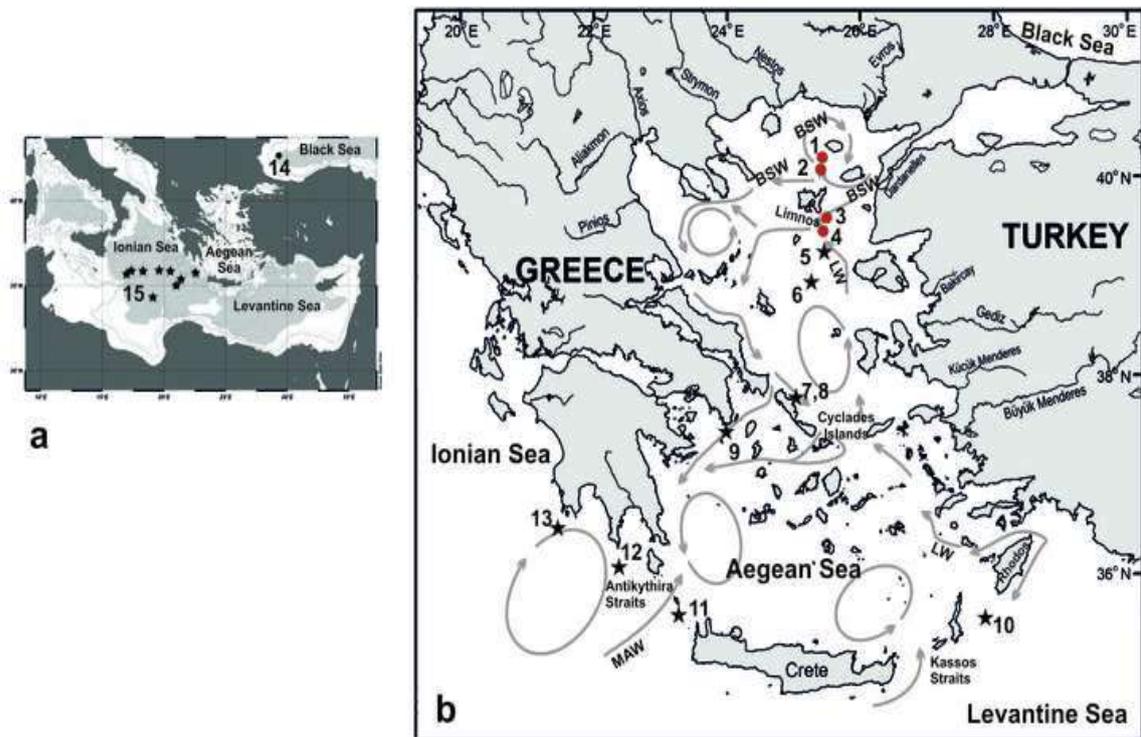


Figure 5 Geographical location of the stations discussed in the present study. A) Black Sea sediment trap site ( $42^{\circ} 58'00''N$   $29^{\circ} 29'00''E$ , 1000 m depth; Triantaphyllou et al., 2013, 2014) and sampled stations from the Ionian Sea (Malinverno et al., 2003). B) Stations in the Aegean Sea (circles: present study; stars: previous expeditions) and the main patterns of sea water surface circulation. [1] AMT2, [2] AMT3, [3] AMT6, [4] AMT7, [5] KM3NET-NSR, [6] Hermione-HAS.

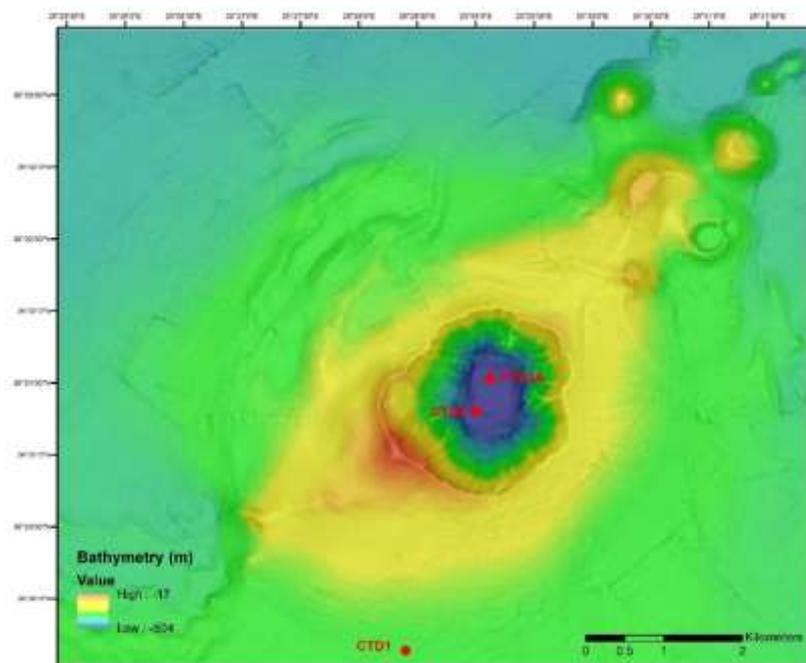


Figure 6 Sampling stations from the September 2013 and May 2014 sampling periods in the Kolumbo region (map from Nomikou et al.2012).

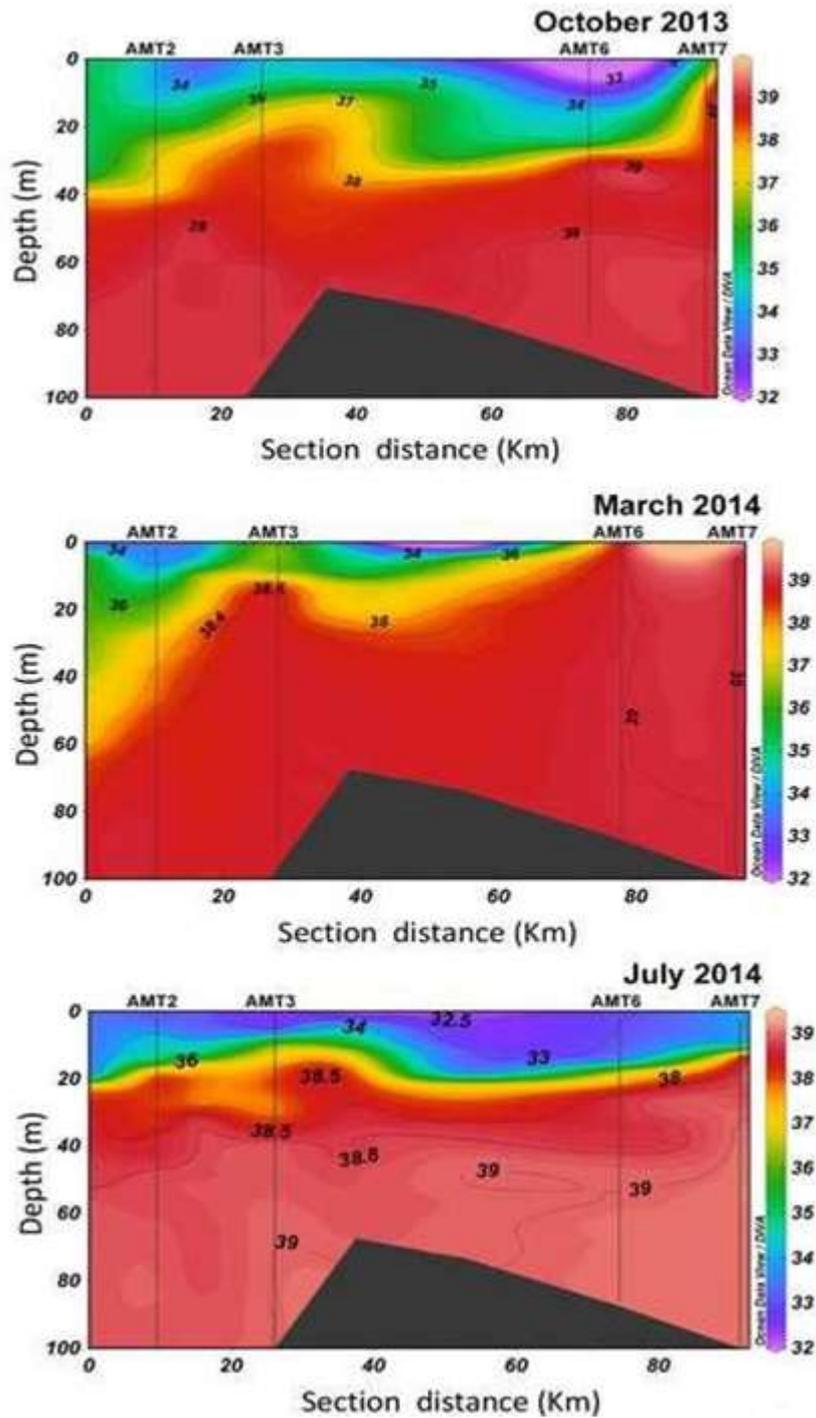


Figure 7 Salinity profiles conducted during the performed AegeanMarTech oceanographic cruises and the location of the studied stations (from Lagaria et al., 2016).

Station code number	station	latitude (°N)	longitude (°E)	ship / date	Sampling depths (m)	sampling type
1	AMT2	39°47.29'	25°32.69'	R/V Aegaeo / October 2013, March 2014, July 2014	3, 10, 20, 30, 50, 75	rosette
2	AMT3	40°06.48'	25°32.11'	R/V Aegaeo / October 2013, March 2014, July 2014	3, 10, 20, 30, 50, 75	rosette
3	AMT6	39°47.28'	25°32.70'	R/V Aegaeo / October 2013, March 2014, July 2014	3, 10, 20, 30, 50, 75	rosette
4	AMT7	39°38.75'	25°35.52'	R/V Aegaeo / October 2013, March 2014, July 2014	3, 10, 20, 30, 50, 75	rosette
5	Sesame-4	39°27.30'	25°27.72'	R/V Aegaeo / October 2007	3	ship's pump
6	Sesame-5	39°04.80'	25°12.84'	R/V Aegaeo / October 2007	3	ship's pump
7	ANDROS-AD	37°51.53'	24°57.53'	fishing boat / March 2002	5, 15, 45	single Hydrobios bottle
8	Andros T1-100	37°51.18'	24°57.42'	fishing boat / September 2004	5, 15, 60	single Hydrobios bottle
9	Sounio	37°37.28'	23°55.78'	inflatable vessel / January 2002	5, 15, 45	single Hydrobios bottle
10	M71-3-Rho 02	35°37.13'	27°42.08'	R/V Meteor / January 2007	5, 20, 50	rosette
11	Hermione-HAS	35°26.50'	23°24.66'	R/V Aegaeo / February 2012	5, 30, 60	rosette
12	Sesame-8	36°11.79'	22°29.05'	R/V Aegaeo / October 2007	3	ship's pump
13	KM3NET-NSR	36°30.24'	21°30.15'	R/V Aegaeo / February 2012	5, 30, 60, 90, 120	rosette
14	STRAP A	42°58.00'	29° 29.00'	R/V Aegaeo 16/10/2007-01/09/2008	1000	sediment trap
15	SIN97-N02-N12	35°47.85'	17°30.04'	R/V Urania / 11-12/1997	5-150	rosette

*Table 2 Location and sampling details of the NE Aegean stations analyzed in the present study (1-4), and of all Aegean, Ionian and Black Seas previous expeditions material (5-15) discussed in the present study (Karatsolis et al.2016).*

### 3.3 Sample preparation and analysis

For coccolithophore analysis of the NE Aegean samples, 2 liters of seawater per sample were filtered on Whatman cellulose nitrate filters (47 mm diameter, 0.45  $\mu\text{m}$  pore size), using a Whatman membrane filter holder and vacuum filtration system with pressure not higher than 200 mm Hg (Bollmann et al. 2002); particular caution was taken for even distribution of the filtered material. Salt was removed by washing the filters with about 2 ml of mineral water. The filters were open/oven dried and stored in plastic Petri dishes. A piece of each filter approximately 8x8mm<sup>2</sup> was attached to a copper electron microscope stub using a double sided adhesive tape and coated with gold. The filters were examined in a Jeol JSM 6360 Scanning Electron Microscope (University of Athens, Faculty of Geology & Geoenvironment) and all the individual coccospheres occurring on the studied filter area were identified and counted at 1200x; generally, at least 200 coccospheres have been determined per sample.

The absolute abundance of coccolithophore densities (number of coccospheres l<sup>-1</sup>) was calculated following the methodology of Jordan and Winter (2000), by scaling up the raw counts from a known scanned area, using the equation:  $A=NxS/V$ , where N is the number of coccospheres of a species on the scanned piece of filter, S the scaling factor (area of the whole filter/area of scanned filter piece), V the volume of the sea water filtered (l) and A the absolute abundance of the species in coccospheres l<sup>-1</sup>.

Identification and taxonomy of species is based on the taxonomic references of Kleijne (1993), Cros and Fortuño (2002), Young et al. (2003), Jordan et al. (2004), Malinverno et al. (2008) and the electronic guide to the biodiversity and taxonomy of coccolithophores Nannotax 3 (<http://ina.tmsoc.org/Nannotax3/index.html>). From the resulting data set, Shannon-Wiener diversity index (H') for each sample was calculated using the Past.exe 1.23 software package (Hammer et al. 2001).

For the measurements of the silicoflagellate abundance, a triangular piece of the filters mentioned above was mounted on a glass slide with a drop of microscope oil and examined with a Leica DMSP polarizing light microscope at 1250 x magnification for silicoflagellate count. For selected samples, similar to coccolithophore analysis, a piece of filter approximately 8x8 mm<sup>2</sup> was attached to a copper electron microscope stub using a double sided adhesive tape and coated with gold. The latter samples were examined in a Jeol JSM 6360 Scanning Electron Microscope (University of Athens, Faculty of Geology & Geoenvironment).

Morphometric measurements of *Stephanocha speculum* and *Dictyochoa stapedia* were manually performed with the ImageJ software on flat-lying specimens from both LM and SEM photos. For 6-sided *S. speculum*, basal and apical ring major axis lengths measures follow the concept of Tsutsui et al. (2009) and Malinverno (2010). For 7-sided *S. speculum*, basal and apical ring major axis length was measured as the distance between one corner and the mid-point of the line connecting the two opposite corners (Fig. 13 ). For *D. stapedia*, basal ring length and width were determined from the two opposed corners and apical bar length was measured from as the distance between the two triple junctions with the struts.

Finally, at least twenty *E. huxleyi* coccoliths attached on coccospheres were counted per sample in the NE Aegean, except for cases with very low densities; in total 60 coccospheres have been considered for each of the layers 0-20 m and 20-75 m. Biometric analyses of *E. huxleyi* relative tube width were performed in the NE Aegean samples (this study) and compared to available sediment trap samples from the Black Sea (42° 58'00''N 29° 29'00''E 16/10/2007-01/09/2008; Bouloubassi et al., 2010; Triantaphyllou et al., 2014) (Fig. 6a). In addition several existing *E. huxleyi* biometric data from the Aegean Sea (Triantaphyllou et al., 2010) and measurements from stations KM3NET-NSR and Hermione-HAS (February 2012; Dimiza et al., 2015) have been incorporated to our data set (Fig. 6b, Table 1); coccoliths attached on coccospheres from the Aegean Sea (in total 450) and Black Sea sediment trap (in total 120), have been photographed during the SEM analyses and analyzed through the ImageJ software following the biometric approaches of Young et al. (2014). For each coccolith image the length and width were measured by dragging an ellipse around the coccolith perimeter, on the observation that coccolith geometry is based to co-axial parallel ellipses (Young et al., 1996). In order to obtain a dimensionless and therefore size independent parameter to measure the degree of calcification variation, we used relative tube width =  $2 \times \text{tube width} / \text{coccolith width}$  (Young et al., 2014).

For the samples of the Kolumbo area, the same SEM analysis preparation was used and the cell density (cells/l) of the coccolithophore species was estimated using the methodology of Jordan & Winter (2000). Then, the diameter of 255 randomly chosen *E. huxleyi* coccospheres (approximately twenty per water sample), was calculated from SEM micrographs, using ImageJ software. In addition, the morphological parameters of the coccoliths (approximately 20 per sample) was measured.

## **Data analysis**

R-mode Hierarchical Cluster Analysis was used to determine coccolithophore species associations. Analyses (centroid linkage method; distance metric is 1-Pearson correlation coefficient) were applied to 20 taxa that exceeded 3% of the assemblage; i.e., taxa in most cases representing more than  $10^3$  coccospheres  $l^{-1}$ . The data were logarithmically transformed to reduce the score and bias of more abundant species that may have otherwise masked the effect of less abundant species. Spearman's correlation coefficient ( $r$ ) analysis was carried out to determine relationships between coccolithophore species abundance and physico-chemical parameters. All statistical analyses were performed using SPSS (version 10.1) statistical software.

## **4. Results**

### **4.1 Tracing the Black Sea water masses using coccolithophore and silicoflagellate abundances**

The results of the conducted study can be distinguished in two groups. The first group consists of the results concerning the BSW inflow and the distinction of the different water masses in the North Aegean area using the coccolithophore and silicoflagellate abundances. These results are compared with previous studies from the Antikithira Straits of the Cretan Arc and the south Ionian, areas who mark the presence of different water masses and thus contribute to this study. The second group consists of results that add information concerning the calcification rate and the possible effects of the acidification in the Kolumbo area.

Physico-chemical data for each sampled station, e.g., temperature, salinity, oxygen, concentrations of chlorophyll-a and nutrients were also available. Cell densities of heterococcolithophores and holococcolithophores recorded during all sampling periods in the studied areas are presented in Figures 7, 8, 9. A total of 101 coccolithophores have been recognized in the studied samples..

#### **4.1.1 NE Aegean sampling-Hydrography**

In October 2013 and July 2014, a strong stratification pattern was observed along the sampled transect with a sharp thermocline located at 40-45 m and 30-40 m, respectively. Low salinity (32 to <36 psu) BSW waters occupied the upper (<30 m) layer while highly saline LW (>38 psu) waters were observed underneath, with the exception of the southern station AMT-7 in October where LW occupied the entire

water column. In March 2014, the water column in the two southern stations AMT-6 and AMT-7 was well mixed and occupied entirely by LW. In stations AMT-1 to AMT-5, a thin layer of modified BSW (34-36 psu) was observed in the upper ~10 m layer, followed by an intermediate layer (36-38 psu) of varying thickness. These two upper layers were characterized by lower temperature waters (<14.4 °C) compared to the deeper and relatively warmer LW layer (14.4-15.4 °C). The explained trends can be viewed in Fig.7.

#### **4.1.2 Coccolithophores**

##### ***4.1.2.1 Total abundance and diversity of coccolithophore assemblages***

The total abundance and diversity of coccolithophore assemblages varied during the different sampling periods and in the different sampling stations.

In October 2013, the highest coccolithophore abundance was observed in the surface waters (3 m layer) at sampling stations AMT6 and AMT7 ( $13 \times 10^3$  and  $6 \times 10^3$  coccospheres  $l^{-1}$ , respectively). The rest of the studied stations displayed abundances  $< 5 \times 10^3$  coccospheres  $l^{-1}$  while total absence of coccospheres has been recorded in samples AMT2-20m and 50 m depth (Fig. 8). A total of 29 heterococcolithophores and 7 holococcolithophores were recognized, with higher values of Shannon-Wiener diversity ( $H'$ ) index (up to 2.57) occurring in surface waters (3 m) at sampling stations AMT2 and AMT3. Stations AMT6 and AMT7 displayed lower values, with the exception of AMT7-75 m (Fig. 8).

The highest coccolithophore absolute abundance was found in March 2014 (up to  $99 \times 10^3$  coccospheres  $l^{-1}$  at station AMT3-20 m depth). The coccolithophore assemblages exhibited considerably higher number of species (51 heterococcolithophores and 19 holococcolithophores) and the maximum Shannon-Wiener diversity index value was 1.61 (Fig.9).

In July 2014, the maximum abundances (up to  $5 \times 10^3$  coccospheres  $l^{-1}$ ) were recorded in the 20-50 m water layer, whereas sample AMT2-75 m was totally devoid of coccospheres. A total of 36 heterococcolithophores and 37 holococcolithophore species have been recorded and Shannon-Wiener diversity index displayed the overall highest values (up to ~3.0) in all depths.

#### 4.1.2.2. Species composition

The species composition in the area, consisted of different types of *E.huxleyi* and numerous heterococcolithophore and holococcolithophore species.

In October 2013, *E. huxleyi* type A displayed high abundances at sampling stations AMT6 and AMT7 (max=13x10<sup>3</sup> coccospheres l<sup>-1</sup> at station AMT6-3 m depth, representing ~50% of the assemblage; the species was totally absent in sample AMT2-10 m). *Syracosphaera* spp. were generally represented by low abundances (max=2x10<sup>3</sup> coccospheres l<sup>-1</sup> at station AMT6-3 m depth; Fig. 8), with *Syracosphaera pulchra* (max=22%) being the most abundant species (Fig. 10). Another important component, *Umbellosphaera tenuis* comprised about 19% of the assemblage (Fig. 10). *Emiliana huxleyi* was the most abundant species in March 2014 with absolute abundances varying between 6x10<sup>3</sup> coccospheres l<sup>-1</sup> (station AMT6-75 m; Fig. 8) and 85x10<sup>3</sup> coccospheres l<sup>-1</sup> (station AMT3-20 m; Fig. 8), constituting on average >80% of the coccolithophore assemblage (Fig. 10). *Syracosphaera* spp. were relatively abundant (min=0.2x10<sup>3</sup> coccospheres l<sup>-1</sup> at station AMT3-75 m and max=12x10<sup>3</sup> coccospheres l<sup>-1</sup> at station AMT6-20 m) and diverse (23 species); with *Syracosphaera molischii* (max=8%), *S. protrudens* (max=4%) and *S. nodosa* (max=3%) being the most frequent species. Rhabdosphaeraceae were also well represented 12 (min=0.1x10<sup>3</sup> coccospheres l<sup>-1</sup> at station AMT6-75 m and max=6x10<sup>3</sup> coccospheres l<sup>-1</sup> at station AMT6-20 m 14 depth), with *Algirosphaera robusta* (max=15%) as a significant element of the assemblage in March 2014. Between 10 and 30 m water layer, holococcolithophores were an important element comprising up to 10% of the total assemblage (station AMT3-10 m). Among them *Syracosphaera histrica* HOL (max=35%) was the most frequent species, whereas *Syracosphaera amoena* HOL (max=10%) and *Syracolithus dalmaticus* (max=7%) were also well represented.

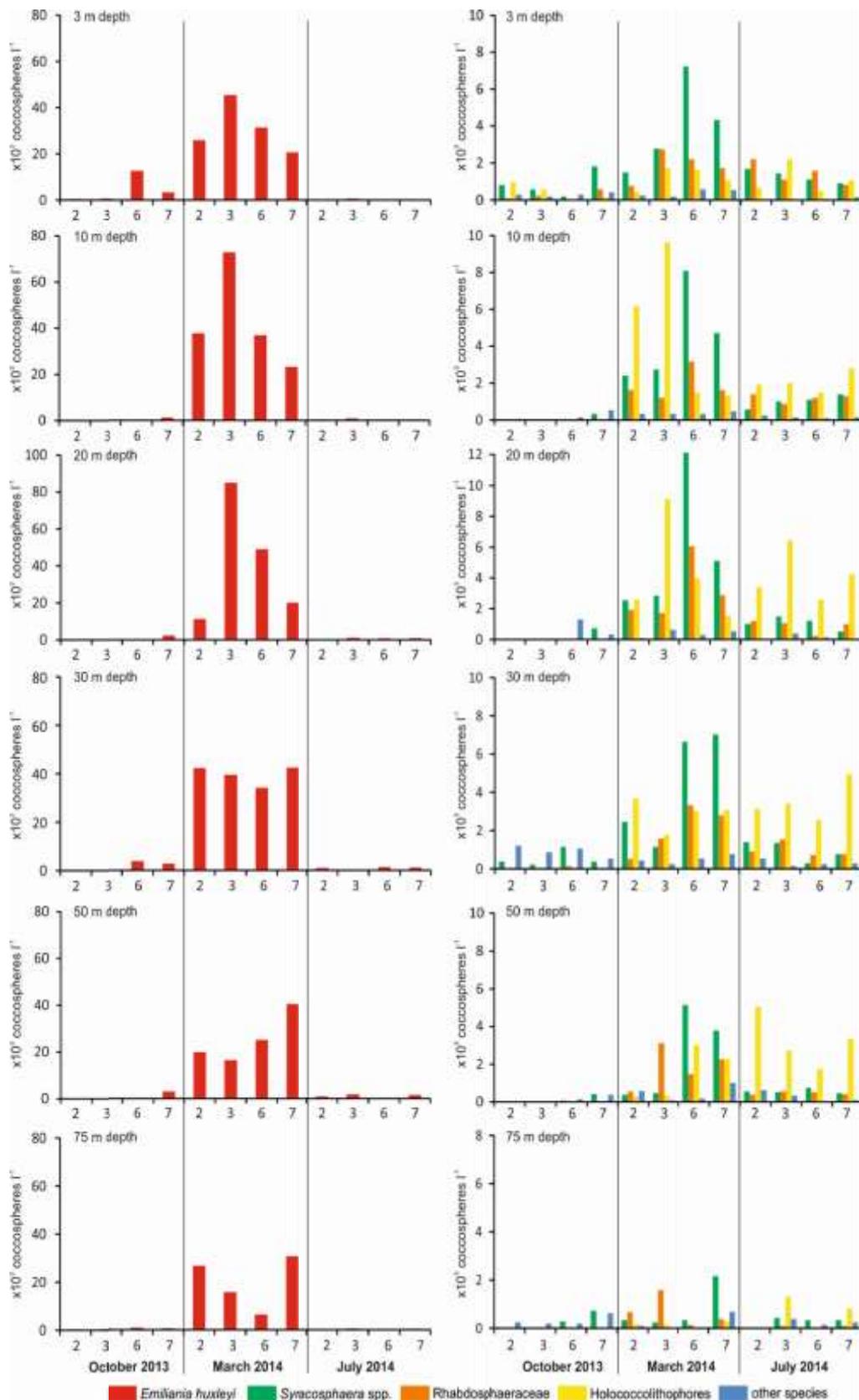


Figure 8 Absolute abundances of *Emiliana huxleyi*, *Syracosphaera* spp., *Rhabdosphaeraeaceae*, holococcolithophores and other species for the investigated stations AMT2, AMT3, AMT6, AMT7 during the different seasonal samplings (from Karatsolis et al., 2016).

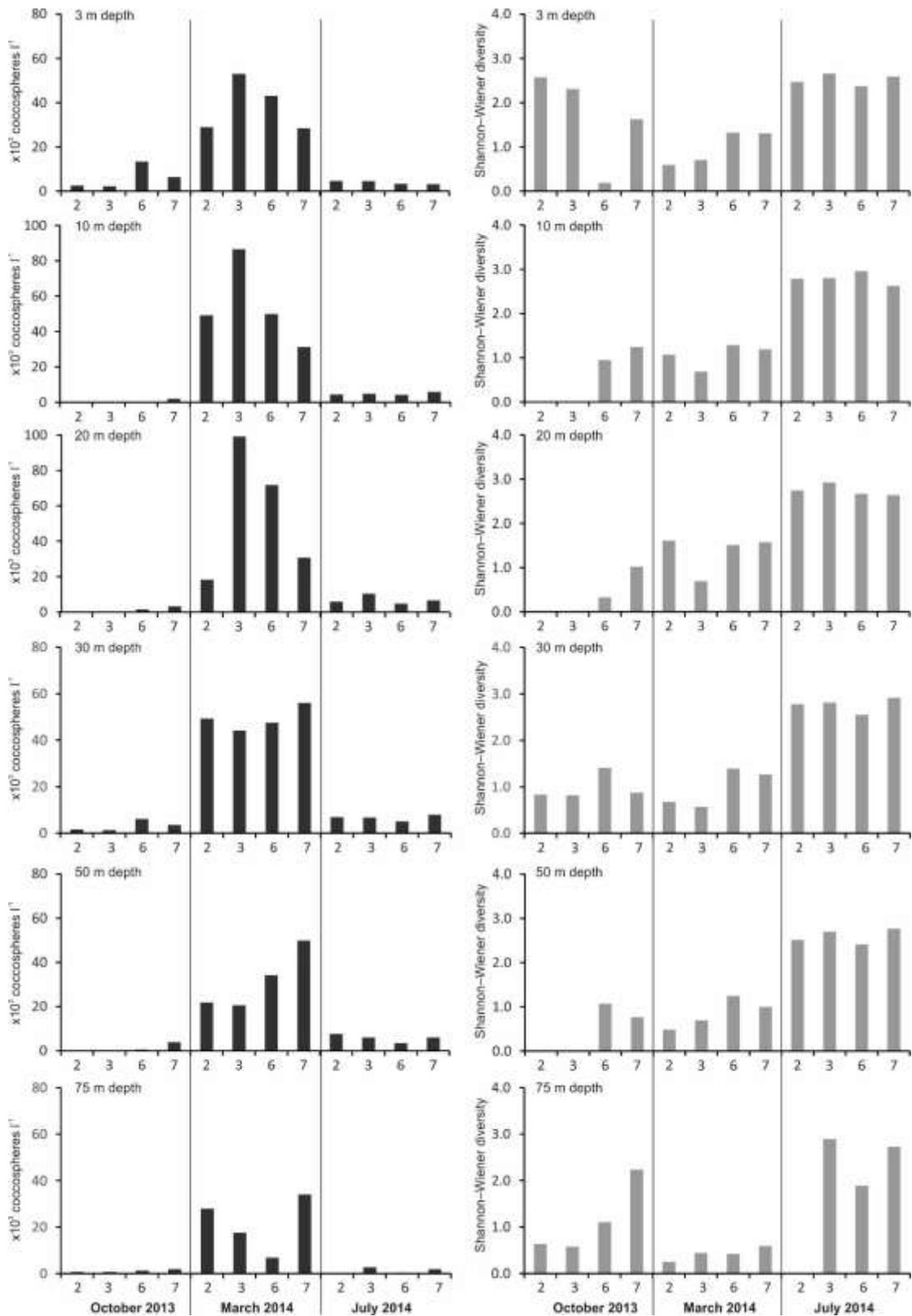


Figure 9 Total density and Shannon-Wiener diversity index ( $H'$ ) for the investigated stations AMT2, AMT3, AMT6, AMT7 during the different seasonal samplings (from Karatsolis et al., 2016).

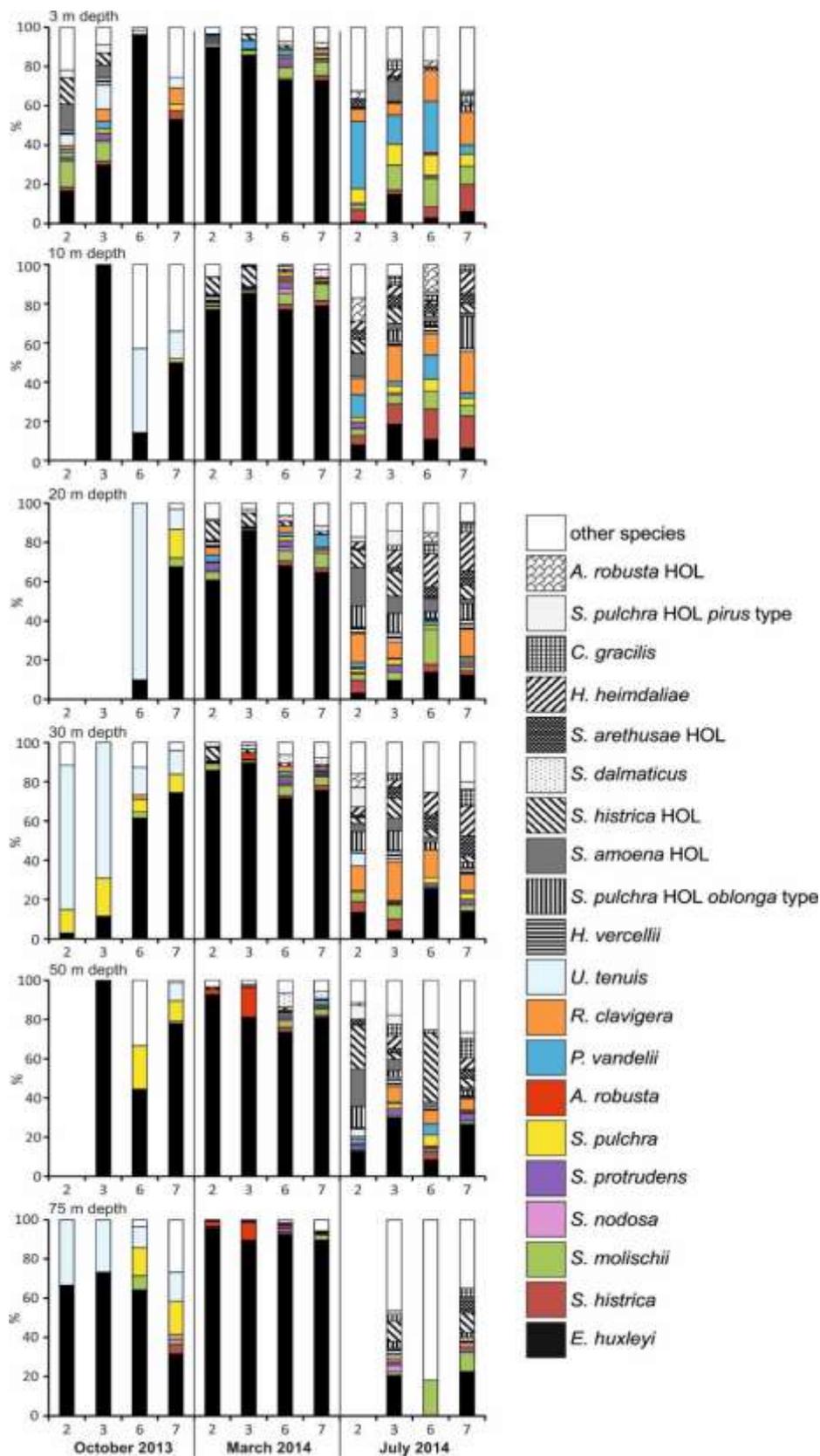


Figure 10 Total diversity (%) of the main coccolithophore species (from Karatsolis et al., 2016).

In July 2014 *E. huxleyi* was considerably lower in both absolute and relative abundance. (max=  $\sim 2 \times 10^3$  coccospheres  $l^{-1}$  at station AMT3-50 m, representing less than 30% of the assemblage; totally absent at stations AMT3-3 m and AMT6-75 m). Moreover, *Syracosphaera* spp. (mainly *S. molischii*, *S. histrica*, *S. pulchra* and *S. protrudens*) and Rhabdosphaeraceae (mainly *Rhabdosphaera clavigera* and *Palusphaera vandellii*) were present in all stations with absolute abundance values lower than  $2 \times 10^3$  coccospheres  $l^{-1}$  (Fig. 8). Holococcolithophores were relatively well represented in the summer coccolithophore assemblages with maxima of  $6 \times 10^3$  (station AMT3-20 m) coccospheres  $l^{-1}$  appearing with several species, e.g., *Holococcolithophora heimdaliae* (max=20%), *S. pulchra* HOL *oblonga* type (max=14%), *A. robusta* HOL (max=11%), *Syracosphaera arethusae* HOL (max=10%), *Corisphaera gracilis* (max=10%), *S. histrica* HOL (7%), *S. pulchra* HOL *pirus* type (max=10%), *Homozygosphaera vercellii* (5%) and *Syracosphaera amoena* HOL (5%). Eight different types of combination coccospheres involving heterococcolithophores and holococcolithophores were identified in two sampling periods of this study (March and July 2014). As a consequence a series of taxonomic revisions have been proposed, in which the names of the involved coccolithophore taxa in the same life-cycle were synonymized (Karatsolis et al., 2014; Triantaphyllou et al., 2015).

#### **4.1.2.3 Statistical and biometric analyses**

R-mode hierarchical cluster analysis on NE Aegean coccolithophore assemblages (Fig. 11) enabled us to recognize four of the groups described by Dimiza et al. (2015) for the Aegean Sea region. Group I includes four species, whereas Group II clusters sixteen species. Group I is characterized by *E. huxleyi*, *A. robusta*, *S. nodosa*, and *S. dalmaticus*. The second Group consists of three subgroups: Group IIa is represented by *S. molischii*, *S. histrica*, *R. clavigera*, *P. vandellii*, *A. robusta* HOL, Group IIb includes *S. protrudens*, *S. pulchra* HOL *oblonga* type, *S. arethusae* HOL, *S. pulchra* HOL *pirus* type, *S. amoena* HOL, *C. gracilis*, *H. heimdaliae*, *H. vercellii* and *S. histrica* HOL, while Group IIc is comprised by *S. pulchra* and *U. tenuis*. The percentage of the total standing stock for each of the clustered species Groups was estimated for the surface low salinity BSW layer (20 m) and the deeper LW layer (20-75 m) (Fig. 15).

Coccolithophore assemblages were compared to physico-chemical parameters using the Spearman's correlation coefficient (Table 3). *Emiliana huxleyi*, *A. robusta*, *S. nodosa* and *S. dalmaticus* (Group I) showed negative correlation with temperature and positive correlation with the concentrations of chlorophyll-*a* and all mineral nutrients ( $r > 0.2$ ). Out of Group II, only *S. molischii*, *S. protrudens* and *S. histrica* HOL are negatively correlated with temperature. In contrast, *A. robusta* HOL, *S. pulchra* HOL *oblonga* type, *S. amoena* HOL, *S. arethusae* HOL, *H. heimdaliae*, *C. gracilis* and *U. tenuis* are featured by an opposite pattern, positively loaded with temperature. The majority of coccolithophore species exhibited a direct relationship with high concentrations of oxygen. Moreover, *S. histrica*, *S. molischii*, *P. vandellii*, *R. clavigera*, *A. robusta* HOL and *S. pulchra* are negatively correlated with depth, while *A. robusta* HOL, *H. vercellii* and *S. amoena* HOL displayed significant negative values with salinity.

The biometric analysis of *E. huxleyi* coccoliths from north Aegean waters and Black Sea sediment trap samples (Fig. 12), showed that Black Sea specimens (bearing stable morphometric features all year round) and specimens from the NE Aegean station AMT6 (October 2013) presented unimodal distribution concerning low relative tube width values (peak at  $\sim 0.10$ ; Fig. 12a). Unimodal distribution but shifted to larger tube width values (peak at  $\sim 0.40$ ), was also featuring the winter-early spring *E. huxleyi* associations in stations AMT2, 3 (20-75 m depth, March 2014) and stations AMT6, 7 (0-75 m; Fig. 12b). In contrast, in March 2014, samples from stations AMT2, AMT3 (< 20 m depth) were characterized by bimodal distribution (peaks at  $\sim 0.10$  and  $\sim 0.40$ ; Fig. 12b), in respect to the larger width values dominating in deeper layer (peak at  $\sim 0.40$ ; Fig. 12b).

## HIERARCHICAL CLUSTER ANALYSIS

Dendrogram using Centroid Linkage  
Rescaled Distance Cluster Combine

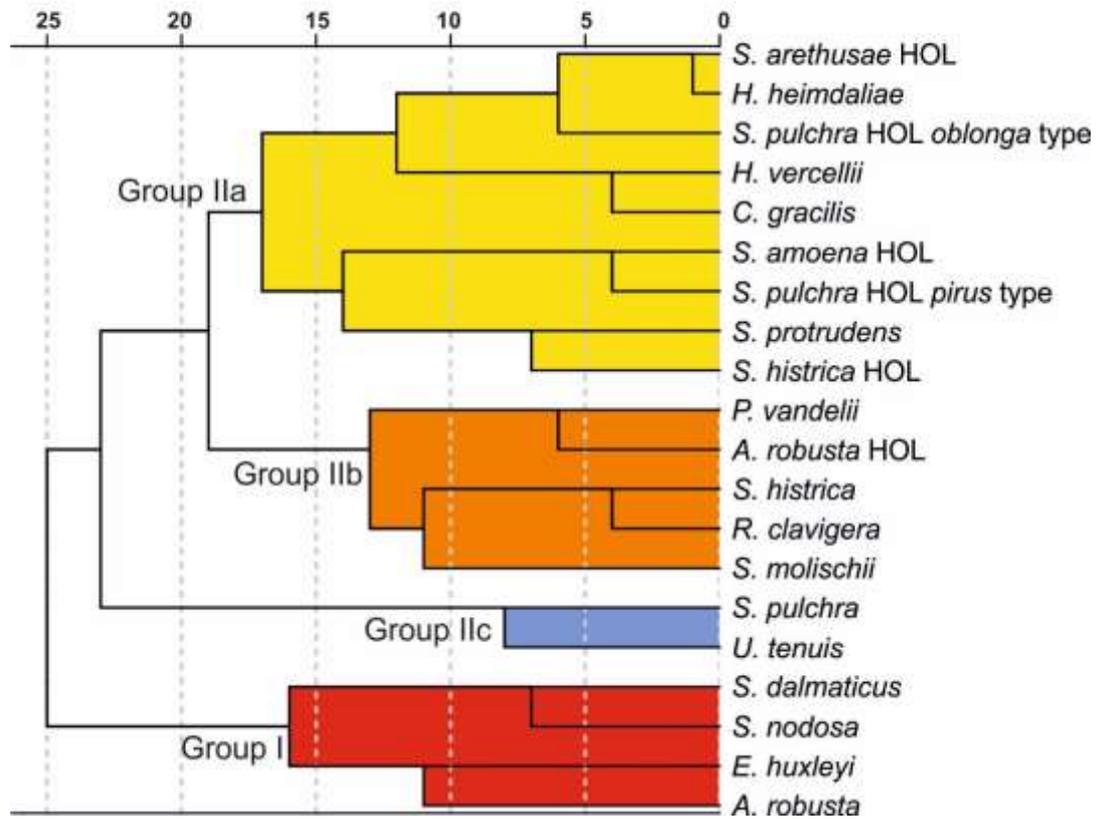


Figure 11 Hierarchical cluster analysis (centroid linkage method, distance metric is 1-Pearson correlation coefficient ) based on the abundances of the most important coccolithophore species (from Karatsolis et al., 2016).

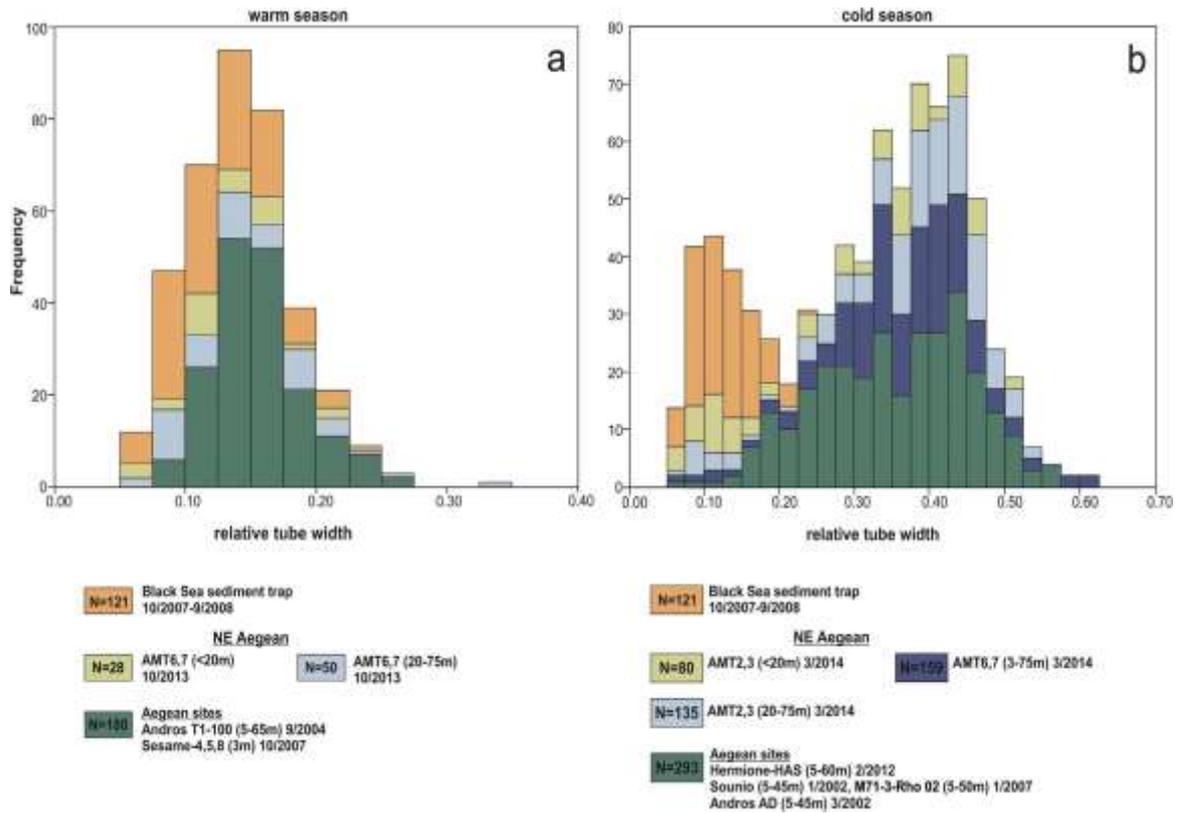


Figure 12 Biometric analysis of *E. huxleyi* coccoliths from north Aegean waters and Black Sea sediment trap samples. a) Relative tube width unimodal distribution (peak at ~0.10) in samples from stations AMT6, 7 (October 2013) in comparison with Black Sea *E. huxleyi* specimens. b) Relative tube width bimodal distribution (peaks at ~0.10 and ~0.40) in samples from stations AMT2, 3 (March 2014), AMT6, 7 (March, 2014), in comparison with Black Sea *E. huxleyi* specimens. The larger values are corresponding to *E. huxleyi* overcalcified coccoliths that are recorded all over the Aegean Sea in LW mass during the winter period. Photos of the lightly and heavily calcified morphotypes are presented in plate 10 (figure from Karatsolis et al., 2016).

	Depth	T	S	O <sub>2</sub>	PO <sub>4</sub>	NO <sub>2</sub>	NO <sub>3</sub>	Chla
<i>E. huxleyi</i>	0,046	<b>-0,668</b>	<b>0,268</b>	<b>0,440</b>	<b>0,494</b>	<b>0,308</b>	<b>0,299</b>	<b>0,546</b>
<i>A. robusta</i>	0,136	<b>-0,594</b>	0,149	<b>0,243</b>	<b>0,719</b>	<b>0,438</b>	<b>0,342</b>	<b>0,462</b>
<i>S. nodosa</i>	0,096	<b>-0,570</b>	<b>0,324</b>	<b>0,449</b>	<b>0,544</b>	<b>0,342</b>	<b>0,314</b>	<b>0,492</b>
<i>S. dalmaticus</i>	0,009	<b>-0,689</b>	<b>0,290</b>	<b>0,461</b>	<b>0,589</b>	<b>0,471</b>	<b>0,373</b>	<b>0,607</b>
<i>S. histrica</i>	<b>-0,394</b>	-0,067	-0,070	0,203	0,138	0,196	-0,030	0,077
<i>S. molischii</i>	<b>-0,269</b>	<b>-0,406</b>	0,037	<b>0,446</b>	<b>0,389</b>	0,208	-0,008	<b>0,312</b>
<i>P. vandellii</i>	<b>-0,493</b>	-0,152	-0,235	<b>0,360</b>	0,187	0,004	<b>-0,340</b>	0,131
<i>R. clavigera</i>	<b>-0,396</b>	0,111	-0,147	<b>0,370</b>	-0,139	-0,143	<b>-0,459</b>	-0,176
<i>A. robusta</i> HOL	<b>-0,329</b>	<b>0,341</b>	<b>-0,447</b>	<b>-0,251</b>	-0,059	-0,186	<b>-0,429</b>	<b>-0,430</b>
<i>S. protrudens</i>	-0,076	<b>-0,340</b>	0,192	<b>0,556</b>	0,046	0,112	-0,193	<b>0,245</b>
<i>H. vercellii</i>	-0,228	0,047	<b>-0,271</b>	<b>0,269</b>	<b>-0,306</b>	<b>-0,310</b>	<b>-0,582</b>	-0,232
<i>S. pulchra</i> HOL <i>oblonga</i> type	0,014	<b>0,370</b>	-0,079	0,076	<b>-0,475</b>	<b>-0,307</b>	<b>-0,548</b>	<b>-0,485</b>
<i>S. amoena</i> HOL	-0,220	<b>0,262</b>	<b>-0,359</b>	0,186	<b>-0,352</b>	<b>-0,402</b>	<b>-0,519</b>	<b>-0,344</b>
<i>S. histrica</i> HOL	-0,144	<b>-0,388</b>	0,012	<b>0,615</b>	0,061	-0,068	<b>-0,294</b>	0,235
<i>S. arethusae</i> HOL	-0,027	<b>0,341</b>	-0,063	0,056	<b>-0,412</b>	-0,199	<b>-0,552</b>	<b>-0,489</b>
<i>H. heimdaliae</i>	-0,074	<b>0,424</b>	-0,179	-0,026	<b>-0,367</b>	-0,225	<b>-0,482</b>	<b>-0,571</b>
<i>C. gracilis</i>	-0,045	<b>0,273</b>	-0,032	0,036	<b>-0,383</b>	<b>-0,242</b>	<b>-0,404</b>	<b>-0,389</b>
<i>S. pulchra</i> HOL <i>pirus</i> type	-0,001	0,192	-0,048	0,130	<b>-0,539</b>	<b>-0,240</b>	<b>-0,369</b>	<b>-0,353</b>
<i>S. pulchra</i>	<b>-0,305</b>	0,026	0,002	0,024	0,193	-0,054	0,040	<b>0,243</b>
<i>U. tenuis</i>	-0,019	<b>0,273</b>	-0,093	<b>-0,305</b>	<b>-0,324</b>	-0,220	0,228	0,036

Table 3 Correlation matrix (Spearman) among coccolithophore species abundance and physico- chemical parameters. *R* values greater than  $|0.238|$  are significant with 95% probability. Coccolithophore groups are shown in different colors (see Fig. 11). Figure from Karatsolis et al.2016.

### 4.1.3 Silicoflagellates

Silicoflagellates were also studied in the area during the March sampling period and their distribution and species composition helped significantly the aim of this thesis.

Total silicoflagellate abundances ranged from <100 to 2000 skeletons/L at the different sampling stations and depths. *Dictyocha stapedia* (Haeckel 1887) was the most abundant species, making up >50 to 100 % of the silicoflagellate assemblage (excluding the two surface samples at station 2AMT-2 and 2AMT3, where it had a lower relative abundance). *Stephanocha speculum* (Ehrenberg) (McCartney and Jordan 2015) was the second most abundant species, while *Dictyocha aculeata* (Lemmermann) Dumitrica, 1973a and *Octactis pulchra* Schiller 1925 occurred sporadically.

Looking at the vertical distribution of the two major species at the three stations studied (Fig. 14c, d), *D. stapedia* displayed maximum concentration at 20 m water depth, with maximum abundance at station 2AMT-3, intermediate values at station 2AMT-2 and much lower values at 2AMT-6; *S. speculum* presented maximum concentration at 10-20 m depth, showing a pattern of decreasing abundance from station 2AMT-2 to 2AMT-3 and very low values at station 2AMT-6.

Specimens of *D. stapedia* were square-rhombic: basal ring measured  $19.4 \pm 0.9 \mu\text{m}$  along the major axis,  $18 \pm 0.6 \mu\text{m}$  along the minor axis, with an average major/minor axis ratio of  $1.08 \pm 0.03$  ( $n = 20$ ). Basal ring spines, as observed under the SEM, typically had a double-pointed termination. Basal ring pikes were short and had a “spiny-bulb” termination.

In the observed populations of *S. speculum*, 7-sided specimens dominated (average 72%) over 6-sided ones. Basal ring length was  $30.4 \pm 1.3 \mu\text{m}$  for 6-sided specimens ( $n = 13$ ), excluding 2 specimens that measured  $25 \mu\text{m}$ ;  $28 \pm 1.4 \mu\text{m}$  for 7-sided specimens ( $n = 38$ ). The sides of the basal ring were straight or concave, and these two configurations could occur on the same specimen (e.g. Plate 3 fig. 5).

A typical feature of the observed specimens was the presence of apical spines, which formed a more or less complete crown. Apical ring spines were pointed outwards and upwards. Specimens without apical spines represented <10% of the observed

populations: the measured specimens lacking apical spines were overall smaller (basal ring length 25  $\mu\text{m}$ ), although the number of observations is limited.

Aberrant forms were observed. They included forms with open basal ring (Plate 3 fig. 4, Plate 4 fig. 7, 8) or with one additional spine projecting from the basal ring (Plate 3 fig. 5, 11; Plate 4 fig. 9), presence of an additional strut (Plate 3 fig. 7), spines projecting from the strut (Plate 3 fig. 9; Plate 4 fig. 2, 9), very small rounded apical ring (Plate 3 fig. 9), apical ring with a different number of sides from that of the basal ring (not figured), bipartite to tripartite apical ring (Plate 3 fig. 8; Plate 4 fig. 11, 12), deformed apical ring (Plate 3 fig. 11a) and apical ring transformed into an apical plate (Plate 3 fig. 10).

Double skeletons of *D. stapedia* were rarely observed. In contrast, double skeletons of *S. speculum* were abundant in all samples where the species was present, representing on average 40% of the observed specimens. Rare dinoflagellate endoskeletons of genus *Actiniscus* were also recovered (Plate 2 fig. 12).

Very few specimens of *O. pulchra* were recovered in the samples and some of them were incomplete, with broken apical ring. The few observed specimens were 8-sided and measured 33-38  $\mu\text{m}$  across the basal ring. Small spines or protrusions could be present on the apical ring (Plate 2, fig. 5).

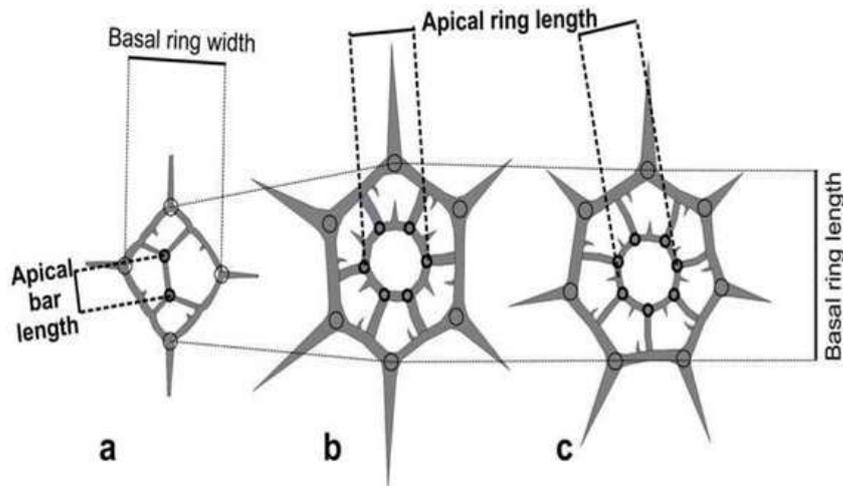


Figure 13 – Schematic morphologies of a) *D. stapedia* and b) hexagonal and c) heptagonal *S. speculum* with details of morphometric measurements. Circles indicate the nodes at corners and triple element junctures, following fig. 2 of Tsutsui et al. (2009) and fig. 3 of Tsutsui and Takahashi (2009): thin lines, measurements of the basal ring; bold lines: measurements of the apical bar / ring (from Malinverno et al., 2016).

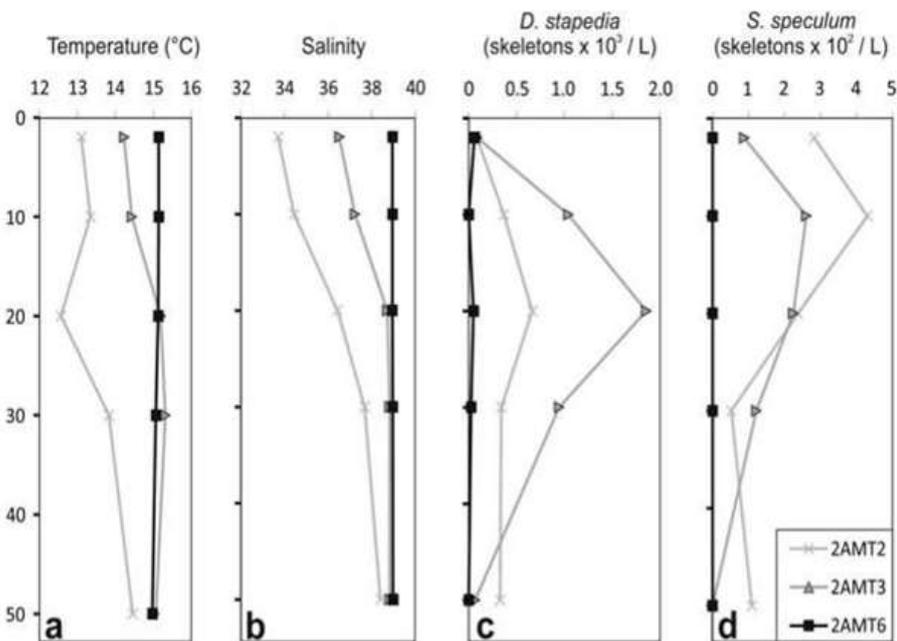


Figure 14 – Depth profiles of a) temperature, b) salinity and c) abundance of *D. stapedia*, d) abundance of *S. speculum* skeletons at the three sampling stations during March 2014 (from Malinverno et al., 2016).

#### 4.1.4 Combination coccospheres

During the conducted study, an important number of combination coccospheres were identified, adding valuable information to the life-cycle of coccolithophore species.

This resulted to the proposal of some taxonomic remarks for a series of coccolithophore species (Karatsolis et al., 2014; Triantaphyllou et al., 2015).

All the combination coccospheres were identified in the NE Aegean sampling transect, except for the *Syracosphaera mediterranea* HOL (“*Calyptrolithophora gracillima*”) combination that was found off Panarea, Aeolian Islands in the Tyrrhenian Sea during the ECO2 project cruise, May 2014.

The study of these combination coccospheres documented numerous heterococcolithophore-holococcolithophore combinations that involve different life-cycle phases of the same coccolithophore species. Their systematic taxonomy after the remarks made by Triantaphyllou et al. (2015) is emended as follows: *Coronosphaera mediterranea* – *Zygosphaera hellenica*, *Coronosphaera binodata* – *Calyptrolithophora gracillima*: combined now as *Syracosphaera mediterranea*, *Syracosphaera histrica* – *Calyptrolithophora papillifera*: combined now as *Syracosphaera histrica*, *Syracosphaera didyma* – *Homozygosphaera arethusae*: combined now as *Syracosphaera arethusae*, *Syracosphaera molischii*- *Anthosphaera fragaria*: combined now as *Syracosphaera molischii*.

Photos of these coccospheres, taken by SEM, are presented at plates 5-9.

Date	Station / Depth (m)	Latitude	Longitude	Temperature (°C)	Salinity (psu)	Chl-a (µg/L)
22/03/2014	2AMT3-10m	40,10805	25,53516	14.42	37.23	0.251
	2AMT3-20m			15.18	38.71	0.475
23/03/2014	2AMT6-2m	39,78814	25,54481	15.13	38.95	0.280
	2AMT6-10m					0.238
	2AMT6-20m					0.283
24/03/2014	2AMT7-2m	39,64586	25,59194	15.40	39.00	0.225
	2AMT7-10m					0.084
	2AMT7-20m					0.262
	2AMT7-30m					0.293
	2AMT7-50m					0.251
13/01/2011	M1-100m	40° 15.018' N	25° 12.013' E			
09/05/2014	LB7-11 m	38,63743	15,1131	17.08	37.70	

Table 3 Geographical location, depth and physicochemical parameters of the samples containing the combination coccospheres presented in this thesis.

## 5. Discussion

### 5.1 Tracing the BSW mass using coccolithophore assemblages

#### 5.1.1 *Emiliana huxleyi* in different Aegean water masses: evidence from biometric analyses

It is well known that *E. huxleyi*, the most abundant cosmopolitan coccolithophore species in the world ocean, forms massive blooms, when water conditions are favorable (e.g., Young, 1994; Tyrell and Merico, 2004).

Young and Westbroek (1991) demonstrated that the size of the different *E. huxleyi* morphotypes is influenced by ecophenotypic factors, usually associated with temperature, salinity and available nutrients (e.g., Watabe and Wilbur, 1966; Paasche, 1998, 2002; Bollmann and Herrle, 2007), and/or genotypic variations (e.g., Findlay and Giraudeau, 2000; Paasche, 2002; Iglesias-Rodriguez et al., 2002; Young et al., 2003).

Increase in atmospheric  $p\text{CO}_2$  and the consequent changes in the seawater carbonate chemistry may reduce *E. huxleyi* cellular PIC/POC ratio and cause malformations of the coccoliths (Riebesell et al., 2000), although further experimental work indicated the importance of species-specific response to changes in the seawater carbonate chemistry (Langer et al., 2006).

In the Aegean Sea, Triantaphyllou et al. (2010) documented a tendency for *E. huxleyi* specimens to express larger coccospheres and bigger coccoliths with heavier calcification in the central area when sea surface temperatures are lower, i.e during winter- early spring period; The size increase coccospheres/coccoliths is observed during chlorophyll maxima, reflecting highest coccolithophore productivity. This pattern is not associated with a phosphate limitation effect; neither with salinity gradient (Triantaphyllou et al., 2010), in contrast the relatively increased  $[\text{HCO}_3^-]$  content observed during spring samplings may be related to the size increase of *E. huxleyi* coccoliths, known as a major parameter in *E. huxleyi* coccolith formation (e.g., Paasche, 2002; Berry et al., 2002).

Further biometric analyses performed on *E. huxleyi* coccoliths within the present study, revealed the unimodal distribution concerning the coccolith relative tube width for NE Aegean samples with the maximum BSW influence similar to those provided by the Black Sea *E. huxleyi* specimens, as well as other Aegean sites sampled in the

warm season, therefore dominated by the LW mass (Fig. 12a). Both in October and July, the NE Aegean assemblage was dominated by lightly calcified morphotypes that is rather expected for the warm season Aegean Sea *E. huxleyi* populations (Triantaphyllou et al., 2010), whereas they are the dominant element of Black Sea coccolithophore assemblages all year round (e.g., Triantaphyllou et al., 2014). In contrast, the relative tube width from both Aegean winter waters and Black Sea sediment trap samples, showed that stations with the maximum BSW influence were characterized by bimodal distribution; the distribution became unimodal and was dominated by larger values in the underlying LW water layers (Fig. 12b). Apparently the larger relative tube widths correspond to *E. huxleyi* overcalcified coccoliths that are recorded all over the Aegean Sea in LW mass during the winter period (Triantaphyllou et al., 2010).

Evidently, the presented data on *E. huxleyi* morphology in the Aegean Sea further complicate the so far documented seasonal variation. As already stated by Triantaphyllou et al. (2010), this may result from ecophenotypic or genotypic variation, now involving Black Sea morphotypes in addition to Aegean specimens. Culture experiments and genetic analysis are needed to test the reliability of both hypotheses and associate the different morphotypes with the carbonate chemistry of the Aegean Sea water masses.

### **5.1.2 Coccolithophore assemblages in the different NE Aegean water masses**

In October and July, *E. huxleyi* abundances decreased with increasing depth (Fig. 8), implying the presence of the low cell density summer-autumn LW mass (e.g., Malinverno et al., 2009; Triantaphyllou et al., 2004, 2010; Dimiza et al., 2015). During our autumn sampling (October 2013), BSW waters of 34-36 psu salinity occupied the upper (<30 m) layer while highly saline (>38) LW were observed below it, with the exception of the southern station AMT7, where LW occupied the entire water column (Fig. 7), associated with the dominance of Group I (Fig. 15a). Notably, a 'fresher' BSW mass of even lower salinity (32 psu) was recorded in the first few meters of the water column around station AMT6 (Fig. 7), with the highest coccolithophore total standing stock ( $13 \times 10^3$  coccospheres  $l^{-1}$ ; sample AMT6-3 m; Fig. 8), dominated by *E. huxleyi* (Fig. 8) lightly calcified morphotypes similar to those of Black Sea waters (Fig. 12). Elements of Group II, especially *U. tenuis* and *S.*

*pulchra* in Group IIc, were marking all sampled stations. Both species have preference for warm temperate waters with low nutrients (e.g., Kleijne 1993; Malinverno et al. 2003), and reflect the influence of LW masses (Dimiza et al., 2015). In the sampling of July 2014, holococcolithophores (Group IIa, IIb) formed a low abundance-high diversity assemblage (Figs. 8-10) that inhabited the BSW-influenced upper 20 m of the water column (Figs. 7, 15a). However, both Groups IIa and IIb showed significantly higher absolute abundances below 20 m depth (Fig. 8). This distribution pattern is unusual compared with the high holococcolithophore standing stocks in the Aegean, which typically were found in the summer surface layers (Triantaphyllou et al., 2002; Dimiza et al., 2008, 2015). In particular, Group IIa has been so far recorded mostly in the thermally stratified south Aegean oligotrophic LW surface waters (Dimiza et al., 2015). As the warm and saline LW flows from the oligotrophic south Aegean northwards (e.g., Zervakis et al., 2002, 2004), it obviously carries the characteristic Group II coccolithophore assemblage along the eastern Aegean coast to the Dardanelles Straits and Limnos basin. In this area it is forced to flow below the less saline BSW lid (e.g., Zervakis et al., 2002, 2004; Velaoras et al., 2014), and as a result holococcolithophores and other typical surface water species (Groups IIa, IIb; Fig. 11), were found increased deeper in the water column as remnants of the highly diversified and oligotrophic LW assemblage of south Aegean-origin. The lack of any significant correlation of Groups IIa, IIb with salinity (Table 3) rather results from the characteristic water column conditions in the NE Aegean, with the associated LW-origin K-selected holococcolithophore species peculiarly thriving in the deeper layers, but also struggling to get adapted to the low salinity BSW lid, in an attempt of the motile cells to move upwards to their normal surface waters ecological niche.

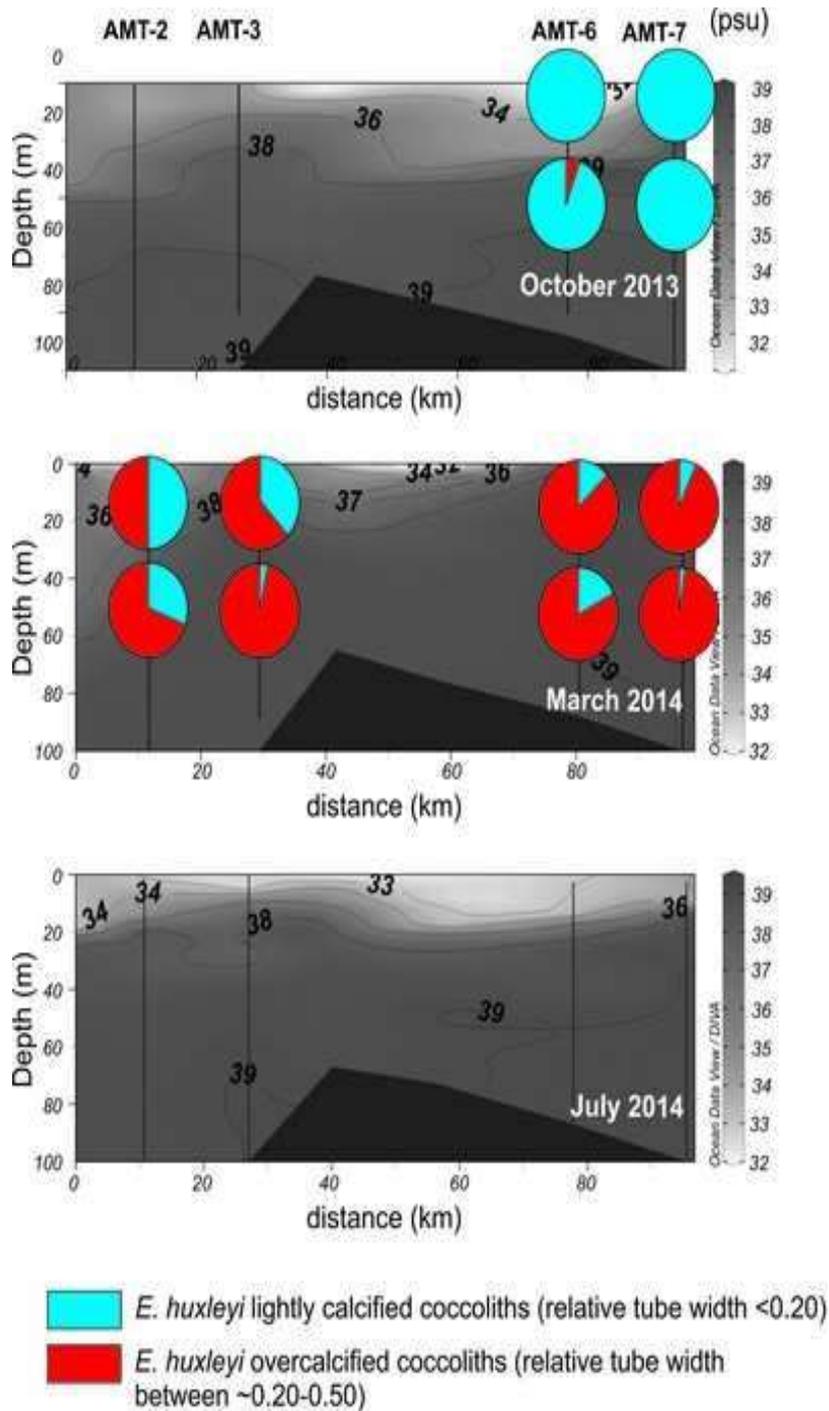
To our surprise, *E. huxleyi* was practically absent in the summer NE Aegean samples (Fig. 8) however, the few specimens recorded in sporadic samples belonged to lightly calcified morphotypes. Oguz & Merico (2006) have shown that the most important limiting nutrient for the interior Black Sea ecosystem is nitrogen rather than silicate and phosphate, implying that the preferential growth of *E. huxleyi* in the Black Sea takes place under nitrogen limited conditions, with N:P ratio typically less than 8. In the NE Aegean, the mean N:P ratio ranged from 11-20 in October, 2-12 in March and 5-15 in July and besides the latter phase all other samplings revealed *E. huxleyi* as an

important component of the coccolithophore assemblage. However, this species plays a leading role in summer assemblages, as shown by sediment trap data from north Aegean Athos basin (Triantaphyllou et al., 2014). Therefore, its absence during July 2014 NE Aegean sampling needs to be explored in greater detail. It may be assumed that the strong stratification of the NE Aegean summer water-column resulting in low mineral nutrients concentrations and phytoplankton biomass (Ignatiades et al., 2002; Siokou-Frangou et al., 2010; Lagaria et al., 2013, 2016), seriously affected the summer *E. huxleyi* assemblages. The contemporaneous but peculiarly elevated diatom production rates and the increased contribution of nano-and microphytoplankton biomass and production in the surface BSW, further support the idea of quick consumption of available nutrients by a metabolically active community in the BSW masses (Lagaria et al., 2016). Definitely further research is needed before getting to any solid conclusion on the absence of *E. huxleyi* in the NE Aegean summer assemblages, as it is also possible that different than usual environmental factors, namely a potential coccolithovirus attack resulting to cell lysis (e.g., Wilson et al., 2002), might have impacted on *E. huxleyi* assemblage during the certain sampling period.

During the spring sampling period (March 2014; Fig. 7), all studied stations in NE Aegean displayed elevated concentrations of *E. huxleyi* (Fig. 8), along with positive correlations with nutrients (Fig. 10; Table 3). Indeed, *E. huxleyi* that is well known for its quick response to nutrient enrichment, is typically prevailing all over the Aegean Sea under LW mixing conditions during the late winter-spring (Triantaphyllou et al., 2004, 2010; Dimiza et al., 2008, 2015; Malinverno et al., 2009). Salinity profiles showed that the water column in the two southern stations AMT6 and AMT7 was totally mixed and occupied entirely by LW (Fig. 7; Lagaria et al., submitted this issue). The slight increase of species of Groups IIa, IIb, mostly holococcolithophores that thrive in the summer assemblages of the oligotrophic south Aegean LW surface layers (e.g., Triantaphyllou et al., 2002; Dimiza et al. 2008, 2015), complies with the presence of LW mass in stations AMT6, AMT-7 during the spring sampling campaign (Fig. 7). In contrast, stations AMT2 and AMT3 north of Limnos Island, exhibited a surficial thin (~10 m) BSW influenced layer (34-36 psu) (Fig. 7). The smaller relative tube width values found in the low salinity surface lid of stations AMT2, 3 are well comparable to the Black Sea *E. huxleyi* measurements (peak at

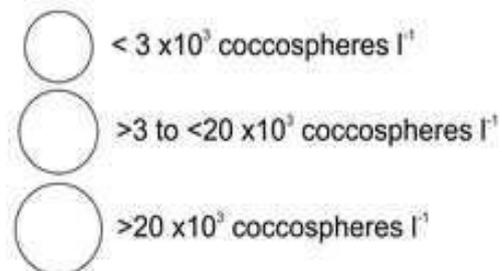
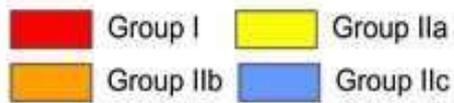
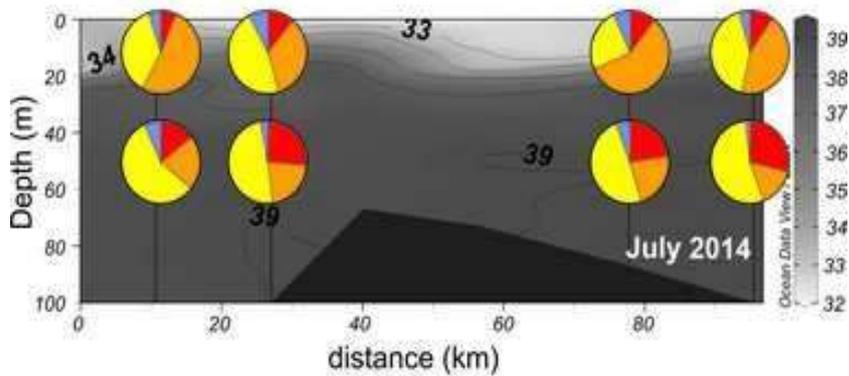
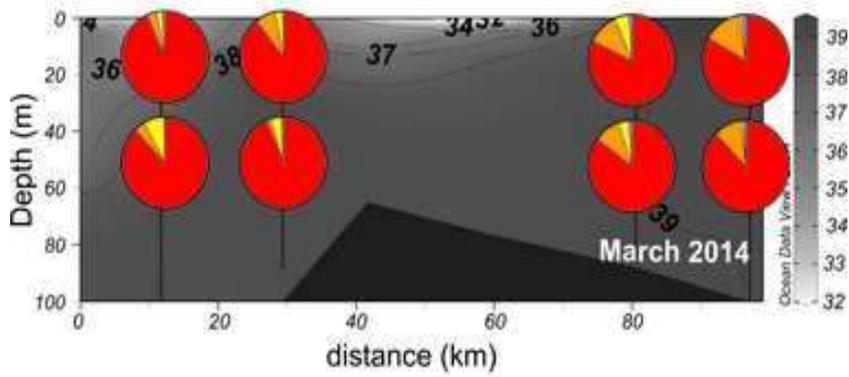
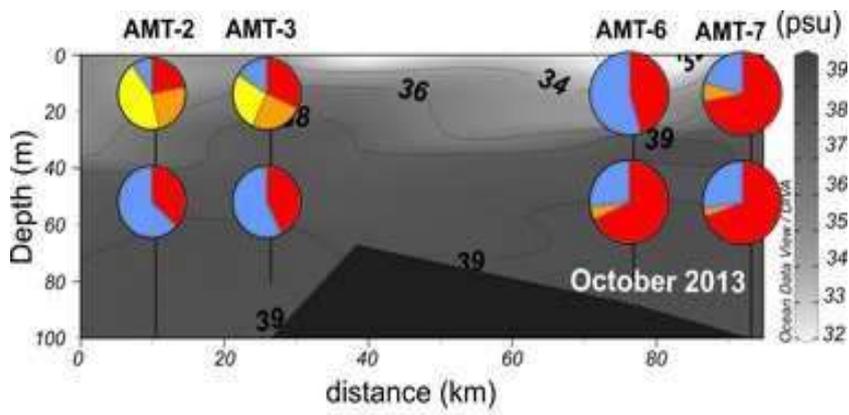
~0.10; Fig. 15b), providing additional proof for the layer's Black Sea origin. The surface BSW outflow and interchange of the water masses directly influence autotrophic activity of the underlying water layers (Ignatiades et al. 2002; Siokou et al., 2002), accounting for significant export from the surface BSW layer and 'fertilize' the underlying water masses with organic matter (Frangoulis et al., 2010; Lagaria et al., submitted this issue).

Coccolithophore data from stations KM3NET-NSR and Hermione-HAS (Dimiza et al., 2015) in south Aegean have been used to compare the NE Aegean spring coccolithophore assemblages in the frontal area of Dardanelles Straits with available winter-early spring data from other eastern Mediterranean straits; namely the Antikithira Straits that represent the main entrance of the less saline MAW in the Aegean Sea (Fig. 6). Coccolithophore assemblages affected by the Atlantic Waters have been previously reported for western Mediterranean consisting of *Gephyrocapsa* species (*G. oceanica*, *G. muellerae*, *G. ericsonii*; e.g., Knappertsbusch, 1993; Cros, 2001). Recently, Oviedo et al. (2015) proposed the tracers for Atlantic Water influx, being mostly present until  $\sim 10^{\circ}$  E after the Sardinia Channel and in the Tyrrhenian Sea in early spring. Notably, data from southern Ionian Sea and the Antikithira Straits evidenced the presence of *G. ericsonii* in the surface waters, although in very low abundance (Malinverno et al., 2003; Dimiza et al., 2015). We therefore consider this faint presence of *G. ericsonii*, as a trace of less saline Atlantic Waters towards eastern Mediterranean, though reduced salinity does not represent the controlling factor for its distributions, as it is practically absent from both the front of BSW flow (e.g., Dimiza et al., 2015; this study) and the hyposaline Black Sea waters (Triantaphyllou et al., 2014).



a

Fig. 15 a. Spatial and temporal distribution of *E. huxleyi* morphotypes along the salinity profiles of the investigated NE Aegean transect. b. Spatial and temporal distribution of coccolithophore groups in the NE Aegean Sea during the different seasonal samplings. The percentage of the total absolute abundance for each of the clustered species Groups is shown in two major depth bands (<20 m, 20-75 m). Note circle size for abundance index.



b

## 5.2 Taxonomic remarks and ecology of *D. stapedia* and *Stephanocha speculum* and their importance as tracers of the Levantine and the Black Sea Water mass

The study of the silicoflagellate abundances led to taxonomic remarks and ecology considerations of two species: *D. stapedia* and *S. speculum*.

*Dictyocha stapedia*, as illustrated in the present study, includes the morphologies identified as *D. messanensis* in the wide geologic literature (McCartney et al., 2014b; Murray and Schrader, 1983; Onodera and Takahashi, 2005; Poelcheau, 1976; Stradner and Bachmann, 1978), and as *D. fibula* in most recent studies on extant phytoplankton. *Dictyocha stapedia* is the most common *Dictyocha* species in waters of the Mediterranean (= *D. fibula* of Rigual-Hernández et al., 2010, their plate 2 fig. a,b,d,e) and nearby Black Sea (*D. messanensis* of Osawa et al., 2005).

The dominance of *D. stapedia* over *S. speculum* in the present samples is consistent with the temperate setting of the study area, although the morphotypes of *S. speculum* are indicative of peculiar water masses. Thin skeletons of *D. stapedia* occasionally observed in the present material are similar to those illustrated by Abe et al. (2015) in the Kuroshio, which are observed to increase in abundance, along with the thin skeletons of *O. pulchra* var. *takahashii*, at higher temperature and lower silicate concentration. Similar observations were done in sediments from the North Aegean, where an increase of delicate skeletons of *D. stapedia* (Dumitrica, 1973a = *D. messanensis*) occurred in samples where the assemblage of siliceous microfossils was poor, suggesting a correspondence with low silicate concentrations.

*Stephanocha speculum* has been recently transferred from the illegitimate genus *Distephanus* to the new genus *Stephanocha* (McCartney and Jordan in Jordan and McCartney, 2015). *Stephanocha speculum* is often indicated as a species typical of cool nutrient-rich oceanographic settings, characterized by shallow thermocline and/or coastal upwelling (Barron and Bukry, 2007a; Barron et al., 2009; Poelcheau, 1976; Takahashi et al., 1989).

The *S. speculum* populations described in the present samples from the Northeast Aegean displayed a combination of peculiar features as compared to the most typical morphotype: specimens were large in size, predominantly 7-sided, with an intermediate to small apical ring and apical ring spines. Other peculiar characters

included the slight rotation of the apical structure and concave basal ring sides. The possible ecological forcing of each different morphological character will be discussed below.

Morphometric work on 7-sided populations from the Arctic (Tsutsui and Takahashi, 2009) provided size data based on basal side length. The specimens described here for the Northeast Aegean fell in the range of large forms (basal side on average 15  $\mu\text{m}$ ), with average basal ring axis of 30  $\mu\text{m}$  for 6-sided forms, 28  $\mu\text{m}$  for 7-sided forms. It is worth to note that large forms (apical ring axis  $>30$   $\mu\text{m}$ ) have only been observed from around the Polar Front, corresponding to temperatures around 2°C, that are thus much colder than the temperatures measured in the Northeast Aegean Sea.

In the Arctic, the abundance of 7-sided specimens is associated with low temperature in the range -1.2 to -1.8°C, low light and sea-ice cover (Takahashi et al., 2009) and is used as a proxy for cold, ice-covered water conditions along sedimentary successions from the North Pacific (Onodera et al., 2013; Teraishi et al., 2013).

In the Southern Ocean, maximum abundances of 7-sided specimens occur in correspondence with peak concentration of normal 6-sided ones, thus pointing to optimal growth conditions for the species (Malinverno, 2010).

In the fossil record, 7-sided specimens are reported in association with 5-sided and aberrant ones (McCartney and Wise, 1990) and their abundance is thus considered as linked to some environmental stress.

The high occurrence of 7-sided specimens in the temperate Northeast Aegean is thus unexpected given the higher temperature of these water masses as compared with sub-polar settings. We can therefore imply an influence of salinity on such morphologies, as related to the influx of relatively cold, low-salinity BSW. In fact higher relative abundances of 7-sided specimens (2AMT-2, 75% > 2AMT-3, 69%) can be correlated with stronger contribution of BSW (Fig. 14) and coincide with higher total *S. speculum* populations and proportion of double skeletons. Only a few published illustrations of Black Sea populations of *S. speculum* exist. However, observations from one sediment trap mooring deployed for one year at 1000 m water depth in the Western Black Sea (Triantaphyllou et al., 2013) showed that most *S. speculum* recorded there were 7-sided (Plate 4, fig. 12).

Basal ring spine length is another parameter that has been proved to be climatically controlled: specimens with short spines are described as typical of warmer conditions (Stradner and Bachmann, 1978) while specimens with long spines are typically described from high latitudes (Malinverno, 2010) and cold time intervals (Ciesielski, 1975). Our specimens show an intermediate spine length, as typical of temperate settings.

Apical ring spines are shown to be associated, in extant forms, with waters poleward of the winter-sea-ice limit in the Southern Ocean (Malinverno, 2010; Malinverno et al., 2016; van der Spoel and Hallegraeff, 1973). Illustrations of extant specimens from the Arctic confirm that apical ring spines are a bipolar high-latitude character (Onodera and Takahashi, 2012).

Interestingly, apical ring spines in *S. speculum* appear in the sedimentary record of the North Atlantic since the late Miocene, coinciding with an overall climatic cooling and thus possibly representing an ecologically-induced evolutionary trait (Locker and Martini, 1989). The presence of apical ring spines in the Northeast Aegean *S. speculum*, such as in the present extant material and recent fossil populations (Stradner and Bachmann, 1978), implies that apical ring spines are not necessarily an indication of sea-ice. Black Sea populations of *S. speculum* have not been adequately illustrated and the only specimen shown by Osawa et al. (2005) do not seem to possess spines. However, our observations from sediment traps deployed in the Western Black Sea (Triantaphyllou et al., 2013) allowed to define that apical ring spines are a characteristic trait of Black Sea populations (one specimen is shown in our Plate 4, fig. 12). Therefore the 7-sided coronatid specimens described here were likely transferred to the Northeast Aegean along with the surface BSW. As mentioned above, apical ring spines are described as typically associated with a large apical ring and with *S. minutus* in high latitudes (Malinverno, 2010). The illustrations of *S. speculum* possessing apical spines are in fact always characterized by a large apical ring (Barron et al., 2009; Frydas, 2006; Locker, 1974; McQuoid and Hobson, 2001). In this sense, our specimens from the Northeast Aegean and Black Sea are an exception within the known variability of morphological characters of *S. speculum*. Finally, aberrant forms are often an accompanying component of extant *S. speculum* populations and usually show maximum concentrations in correspondence with maximum total abundance of the most common non-aberrant forms (Malinverno,

2010; van der Spoel and Hallegraeff, 1973), but also coincide with low salinity and high nutrient concentration (McCartney and Wise, 1990), thus suggesting the response to some ecological stress and/or extremely variable conditions. Aberrant forms were not common in the present material, although the peculiar 7-sided specimens with concave sides represent unusual morphologies.

## **6. Possible effects of Kolumbo submarine volcano emissions (Cyclades, Aegean Sea) to plankton assemblages**

### **Results and Discussion**

#### **6.1 Results**

The results from the samples collected in this study can be grouped in results for nutrients in the study area ([PO<sub>4</sub>], [NH<sub>4</sub>], [NO<sub>3</sub>], [NO<sub>2</sub>] and [SiO<sub>2</sub>]) and results for the coccolithophore abundances, with a focus on the *Emiliania huxleyi* coccoliths and the presence of malformed specimens.

#### **Nutrients**

The conditions observed in the study area were oligotrophic. Nutrient values were generally higher during May sampling period with some high values observed in station CTD3, where the nitrates [NO<sub>3</sub>] showed peaks in 20 and 90m depths, reaching concentrations higher than 14 and 18μm respectively (Fig.17A and B ).

During September 2013 sampling period, [NO<sub>3</sub>] concentrations ranged from 0,22 to 0,8μm while [NO<sub>3</sub>] and [SiO<sub>2</sub>] from 0,01 to 0,06μm. [PO<sub>4</sub>] ranged from 0,04 to 0,12μm and [NH<sub>4</sub>] ranged from 0,02 to 0,12μm (Fig.17A ).

In May sampling period, [NO<sub>3</sub>] concentrations showed high values ranging from 2 to 18μm, [NO<sub>2</sub>] showed values from 0 to 0,32μm and [SiO<sub>2</sub>] from 0,51 to 3,1 μm. [PO<sub>4</sub>] and [NH<sub>4</sub>] both showed oligotrophic values ranging from 0,02 to 0,11μm and 0,02 to 0,4μm respectively (Fig.17B ).

The total pigments showed similar distribution during both sampling periods in the sampled stations, with peaks reaching higher concentrations in station CTD3 (around 300 and 800 ng/L in September and May respectively- Fig.19 ).

The pH was also consistent in the first 100m of the water column for the two sampling periods and stations (around 8.1), with an exception of slightly higher pH observed in station CTD2 during May sampling period (Fig.18). In the deeper parts of the water column, below the first 150m, the pH shifted to lower values, especially during the September sampling. There, values lower than 7 were observed below 250m depth.

## Coccolithophores

The total abundances were low, reaching typical oligotrophic values for the summer period in the Aegean and ranging from 481 to 5154 coccospheres  $l^{-1}$ . Maximum values were observed in station CTD3 during September 2013 in 5m depth and during May in 90m depth.

The most abundant species in the studied area were *Emiliana huxleyi* during both sampling periods, *Syracosphaera pulchra* during May and *Rhabdosphaera clavigera* and *Umbellosphaera tenuis* during September (Figs.20,22, Table 4).

*Emiliana huxleyi* was more abundant during May in the sampling depths of 5, 20 and 90 m of both stations (CTD2, CTD3), where there were observed values higher than 2000 coccospheres  $l^{-1}$  for this species (Figs.21,23 Table5).

During September, coccolithophore groups of Syracosphaeracea, Rhabdosphaeracea and holococcolithophores showed low abundances ranging from 0 to almost 2000 coccospheres  $l^{-1}$  (Fig.22). Syracosphaeracea reached values of 1972 coccospheres  $l^{-1}$  in station CTD3 and in 5m depths. Holococcolithophores were almost absent in stations CTD1 and CTD3, whereas in station CTD2 (5m and 45m depth) their cell densities were 722 coccospheres  $l^{-1}$  and 2000 coccospheres  $l^{-1}$  respectively. Rhabdosphaeracea were more abundant during this period in comparison to May and appeared in maximum abundance in station CTD1, 45m depth, where they exceeded 1000 coccospheres  $l^{-1}$  (Fig.22).

During May, the abundances of these groups continued to be low, lower than September, and holococcolithophores were almost absent (Fig.23). Syracosphaeracea reached cell densities of  $>1000$  coccospheres  $l^{-1}$  in the lower parts of the water column (CTD2-60m and CTD3-90m).

Malformed coccoliths were observed in sampling stations CTD2 and CTD3 and were missing from station CTD1 during September sampling period (Fig.20). In sampling station CTD1, malformed specimens have not been observed. The highest abundances in malformed coccoliths were observed in station CTD2 during May sampling period, in all sampling depths (Fig.21). In 5m depth, the malformed coccoliths counted reached 86% of the total sampled *Emiliana huxleyi* coccoliths (Table 5- Fig.21) and in 45m depth they were 100% of the total *Emiliana huxleyi* cell density. High

concentrations of malformed coccoliths were also observed in the same station and period in CTD3, in depths from 0-60m, where they consisted nearly 50% of the *Emiliana huxleyi* coccoliths(Fig.21). In some depths the malformed cells exceeded 30% of the total abundance (Fig. 20-21, Table 5). In September, our data were limited but we can observe that *Emiliana huxleyi* reached similar cell densities in both sampled stations in the first 5m of the water column, but in station CTD3 the cells were malformed (Fig.20). In the deeper parts of the water column, in station CTD3, the coccolithophore abundance was significantly reduced, along with the percentage of malformed coccoliths (Fig.20). On the other hand, in station CTD1, *Emiliana huxleyi* abundances were higher especially in the deeper part of the column; malformed coccoliths have not been observed (Fig.20).

## 6.2 Discussion

Coccolith malformations, disturbance of the coccolith symmetry, occur in both field and culture samples, but usually more so in the latter known (Ziveri et al., 2014). In the field malformations do occur, and have been observed in the Indian, Pacific, Atlantic ocean and the Mediterranean Sea (Okada & Honjo 1975, Woellner et al. 1988, Kleijne 1990, Young & Westbroek 1991, Girardeau et al. 1993, Riebesell et al. 2000, Triantaphyllou et al. 2002, Dimiza et al. 2003, Rost & Riebesell 2004, Yang et al. 2004, Kahn & Aubry 2006, Langer et al. 2006).

In the Aegean, malformed coccoliths of the species that showed similar features with this study have also been previously observed (Dimiza et al.2012). These phenomena are mainly typical of coastal environments and the upper photic zone and were initially linked to lack of nutrients. After studying cultures of coccolithophores (Riebesell et al., 2000) and (Rost & Riebesell et al., 2004) observed increasing number of malformed coccoliths with elevation of the CO<sub>2</sub> concentrations. Other studies have also showed that perturbations in the pH levels of the sea water create malfunctions in unicellular organisms and that moving away from the optimal growth temperature increases the malformations in coccoliths (Watabe and Wilbur, 1966, Nimer et al. 1994, Thoresen et al. 1984, Felle et al. 1994).

Our preliminary water column data from the Kolumbo site have indicated decreased calcification and coccolithophore malformation in a dinoflagellate-abundant assemblage. Malformation was observed in three coccolithophore species (*Emiliana*

*huxleyi*, *Rhabdosphaera clavigera* and *Syracosphaera pulchra*). For *Emiliana huxleyi*, the phenomenon was stronger with stations where the malformed coccoliths reached values bigger than 70% of the total assemblage.

The coccolithophore species observed were typical of the Aegean Sea and the Levantine water mass, marked with the intense sea water warming and oligotrophic conditions during the summer season (September sampling). Also, the species abundances were also typical for the sampling period, with low cell densities not greater than  $6 \times 10^3$  cells l<sup>-1</sup>. Holococcolithophores were not a significant part of the total abundances, phenomenon that is not usual, especially for the summer period samplings in the Aegean. However, it has been repeatedly observed that holococcolithophores mostly thrive in coastal environments of the Aegean during the early autumn- late spring period (Dimiza et al., 2015), whereas our area can be described as pelagic.

During the surface water samplings in our area, nitrates showed typical oligotrophic values during September but were distinctively increased in March. Measured pH values did not evidence signs of acidification, ranging within normal values in the first 100 m of the studied area.

During September, malformation was observed in coccoliths, within the optimal growth temperature of the species in the warm season. In station CTD3, 5m depth, they consisted the whole *Emiliana huxleyi* abundance, whereas they were absent from the other stations. During May, in station CTD2, the percentage of the malformed coccoliths was high in all the sampled depths, and their abundances showed a consistency as we were going from the surface to the deeper parts of the water column. On the other hand, in station CTD3, the malformations showed a decreasing tendency in abundances with increasing depth following the cell densities of *Emiliana huxleyi*.

The above mentioned malformations cannot be directly linked to significant changes in the pH values. It cannot also be attributed to limitation in the main nutrients, since the typical oligotrophic values observed in this study area are also observed in many parts of the Aegean where malformation phenomena in coccoliths are lacking. Interestingly, the malformed specimens are also increased in May sampling, following the nutrient peaks. During this period, the malformation is more evident in the

samples coming from the closest part of the volcanic emissions, but they are not related positively related to the increasing of the depth. So, since no perturbation in the pH is observed in this part, no direct linkage to any acidification process can be made. During September, the malformed coccoliths are actually present only in the closest station to the emissions and absent from the other two. However, the environment still doesn't show acidification signs.

The malformed *Emiliana huxleyi* coccoliths displayed similar size parameters to normal ones (Fig. 24), thus suggesting that the malformation was mainly restricted to disturbed coccolith shape and symmetry and not to significant calcite loss. They also showed typical size parameters, if compared with the typical summer morphotype A of *Emiliana huxleyi* in the Aegean (Triantaphyllou et al.2009).

It is important to note, that the CO<sub>2</sub> gas produced by the submarine hydrothermal vents, as observed by the pH values, doesn't seem to influence the upper photic zone during the time of the conducted study, phenomenon that can be attributed to the earlier mentioned water circulation in the area of the hydrothermal field (Christopoulou et al., 2016). The hydrothermal emissions, which consist of more than 97% of CO<sub>2</sub>, are trapped in a dense lake inside the area of the crater, in depths greater than 350 m (Carey et al., 2013). This makes impossible the direct observations to coccolith calcification under high CO<sub>2</sub> levels, since this phenomenon does not influence the upper 100 m of the water column. Also, taking into consideration that the study area was cited in a pelagic environment with currents having strong influence in the upper part of the water column, thus continuously drifting away the phytoplanktonic mass from the area where the hydrothermal vent gas productions are influencing the water column.

The constant appearance of malformed coccoliths in the Aegean area has yet to be explained. It is possible that other parameters of the water are playing the controlling role for this phenomenon. However, malformed coccoliths displayed, in this study, similar size parameters to normal ones, suggesting that malformation was mainly restricted to disturbed coccolith shape and symmetry and not to significant calcite loss. That raises further questions about this phenomenon, since we observe the same *Emiliana huxleyi* type, with the same size parameters but with malformations that we cannot directly attribute to calcification loss or pH drop. There is the possibility that

except the physical and chemical parameters, there might be even a potential coccolithovirus attack resulting to cell lysis (e.g., Wilson et al., 2002).

In a related article (Oviedo et al. 2016), while studying coccolithophore communities in the oligotrophic Mediterranean area during two mesocosm experiments, it was also showed that higher pCO<sub>2</sub> did not cause any significant effect on *E. huxleyi* (type A) coccolith morphology or on the degree of coccolith calcification. She thus suggested that it is possible that in oligotrophic regions other parameters such as nutrient availability, temperature or intrinsic phenological changes might play a bigger role on the coccolithophore community structure than the elevation of pCO<sub>2</sub>. This suggestion seems logic if we also consider our data. Interestingly, in our study the temperature and nutrient availability showed typical values for the study period in the Aegean, especially in the upper water column where significant malformations were observed. Obviously, further investigation about the above described phenomenon must be done in areas where we can make direct observations of low pH values, caused from volcanic activity and thus study the coccolithophore response in a more stable environment.

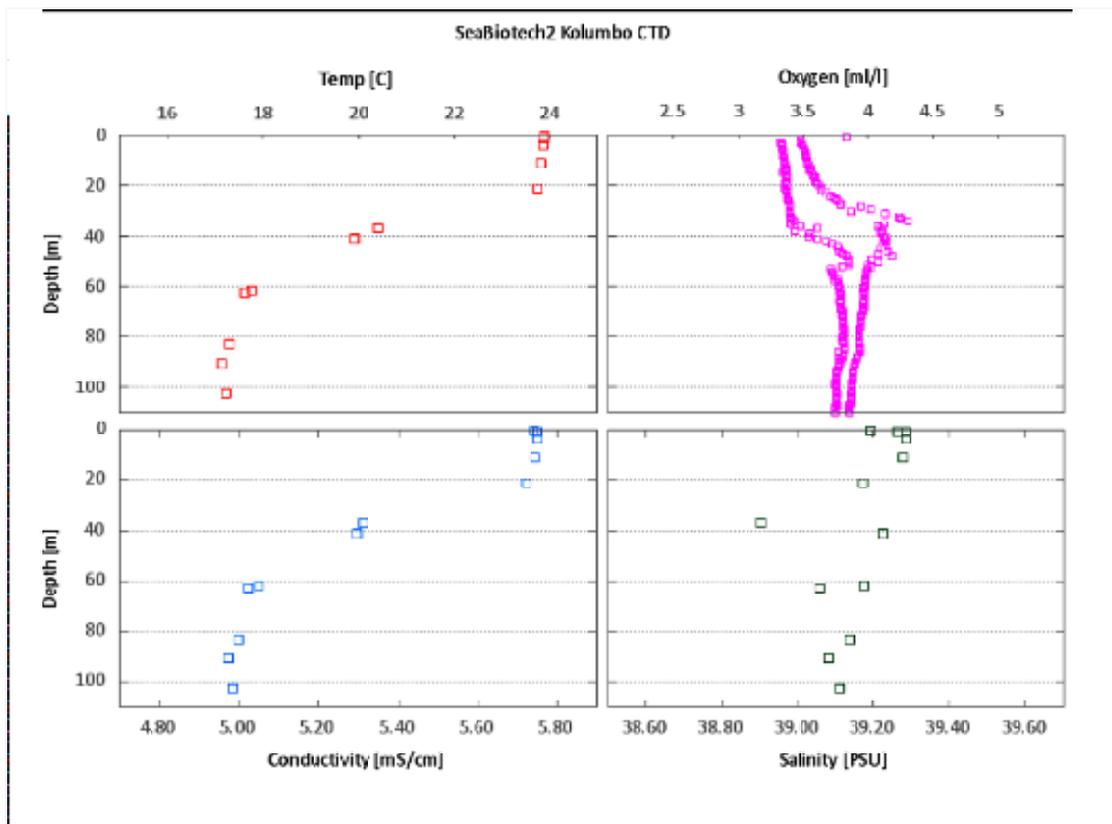
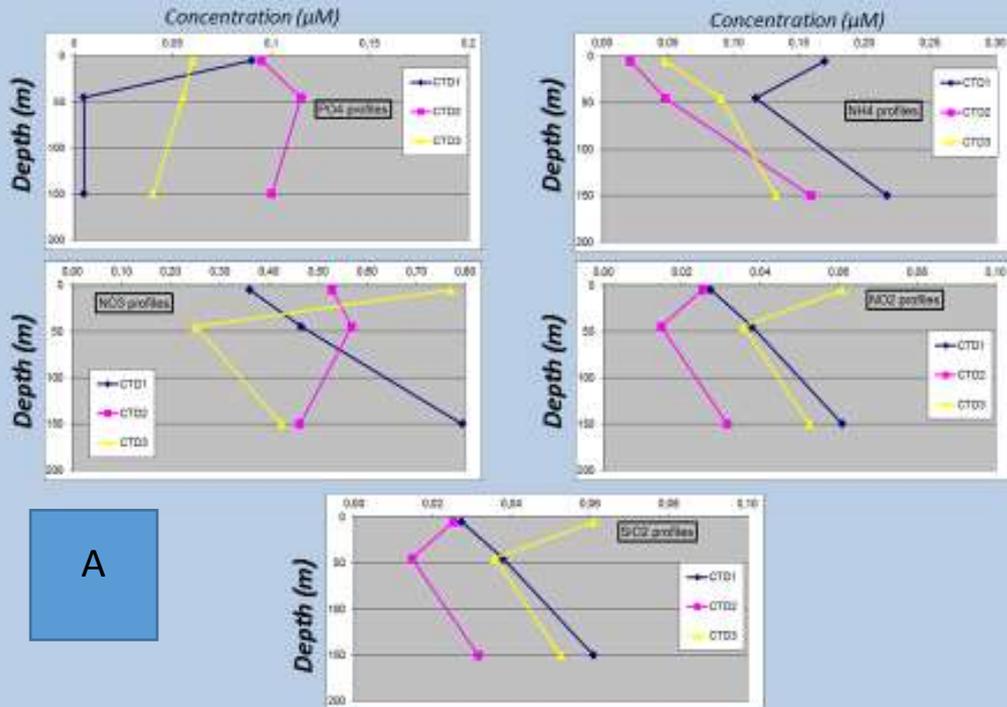


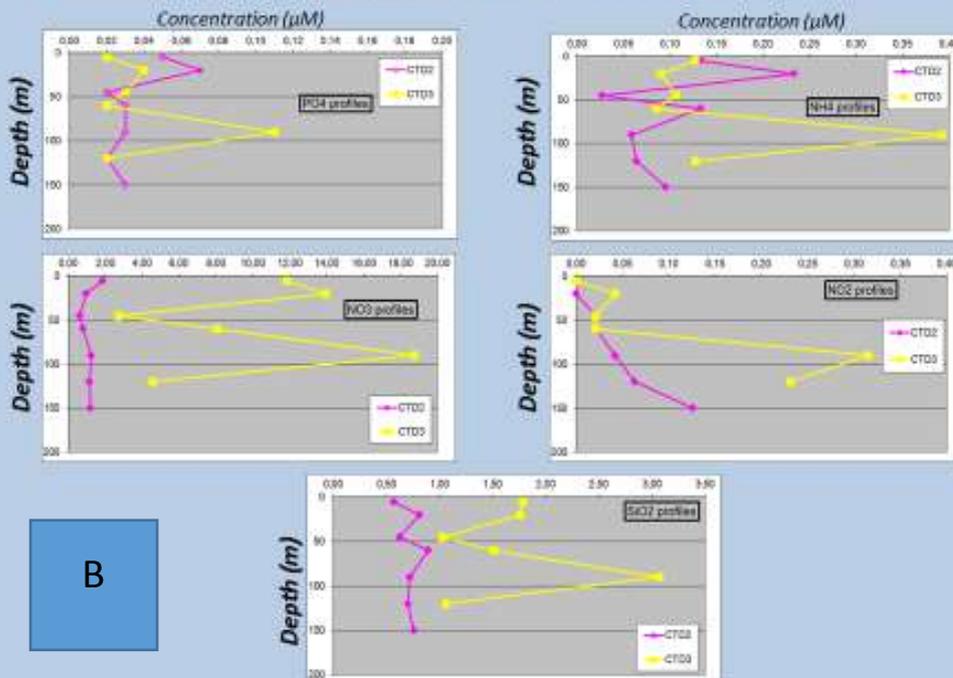
Figure 16 CTD temperature, oxygen, conductivity and salinity related to the sampling depth during September 2013 sampling. Figure from Karatsolis et al.2016 (poster presentation).

**Cruise 2-BIOTECH (SEABIOTECH) – SEPTEMBER 2013**



A

**Cruise 4-BIOTECH (SEABIOTECH) – MAY 2014**



B

Figure 17 Nutrient concentrations as measured in the different depths, sampling stations and periods. A) September sampling period. B) May sampling period. Figure from Karatsolis et al.2016 (poster presentation).

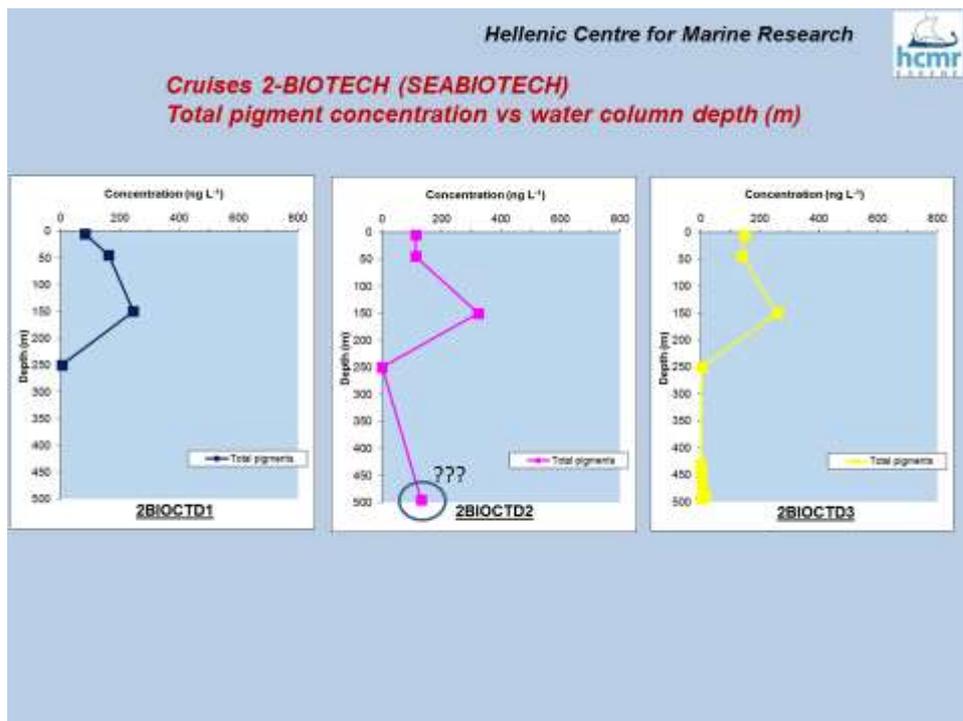
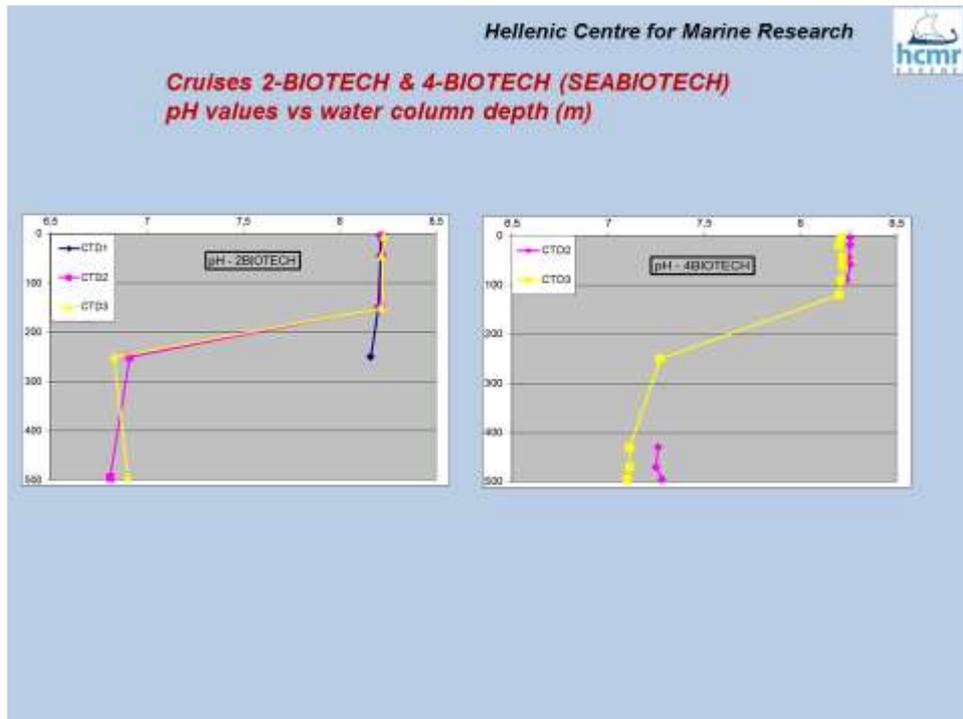


Figure 18 pH and total pigment concentration values for the different depths of the sampling stations during the September sampling period. Figure from Karatsolis et al.2016 (poster presentation).

**Cruises 4-BIOTECH (SEABIOTECH)**  
**Total pigment concentration vs water column depth (m)**

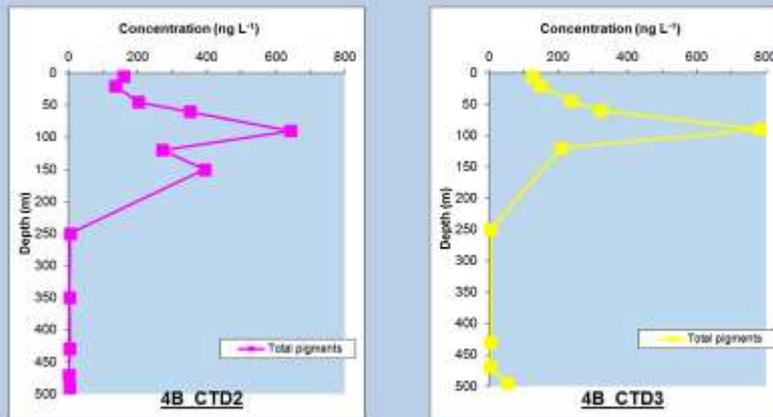


Figure 19 Total pigment concentration values for the different depths in the sampling stations during the May sampling period. Figure from Karatsolis et al.2016 (poster presentation).

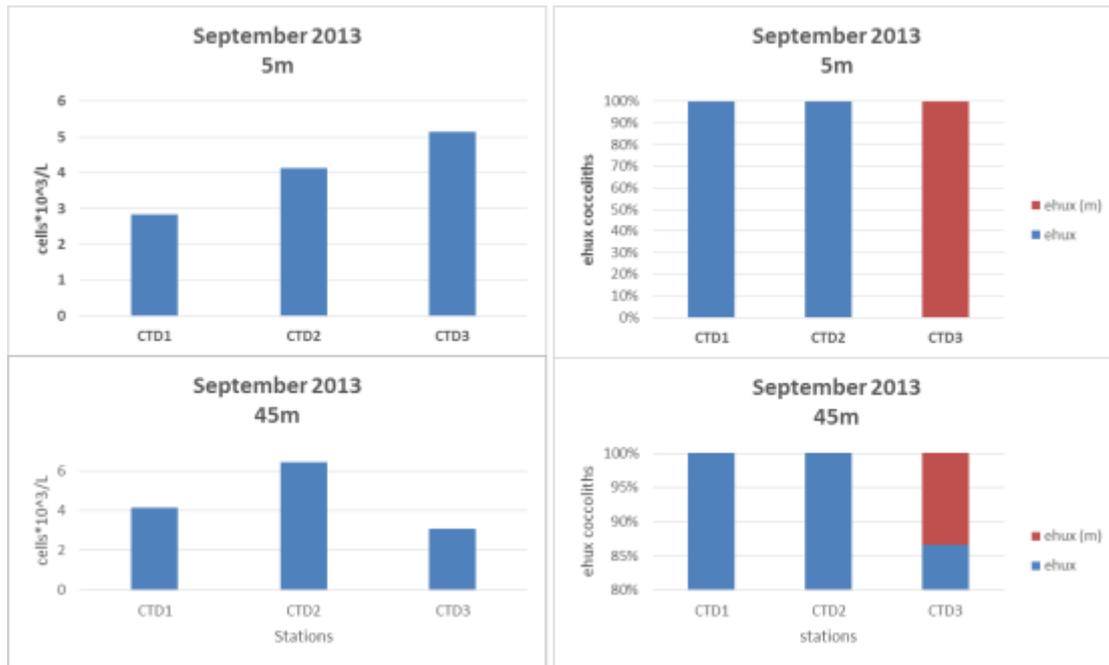


Figure 20 Diagrams showing the total coccolithophore abundances and the percentages of the normal and malformed *Emiliana huxleyi* coccoliths during the September 2013 sampling period in the different sampling stations and depths.

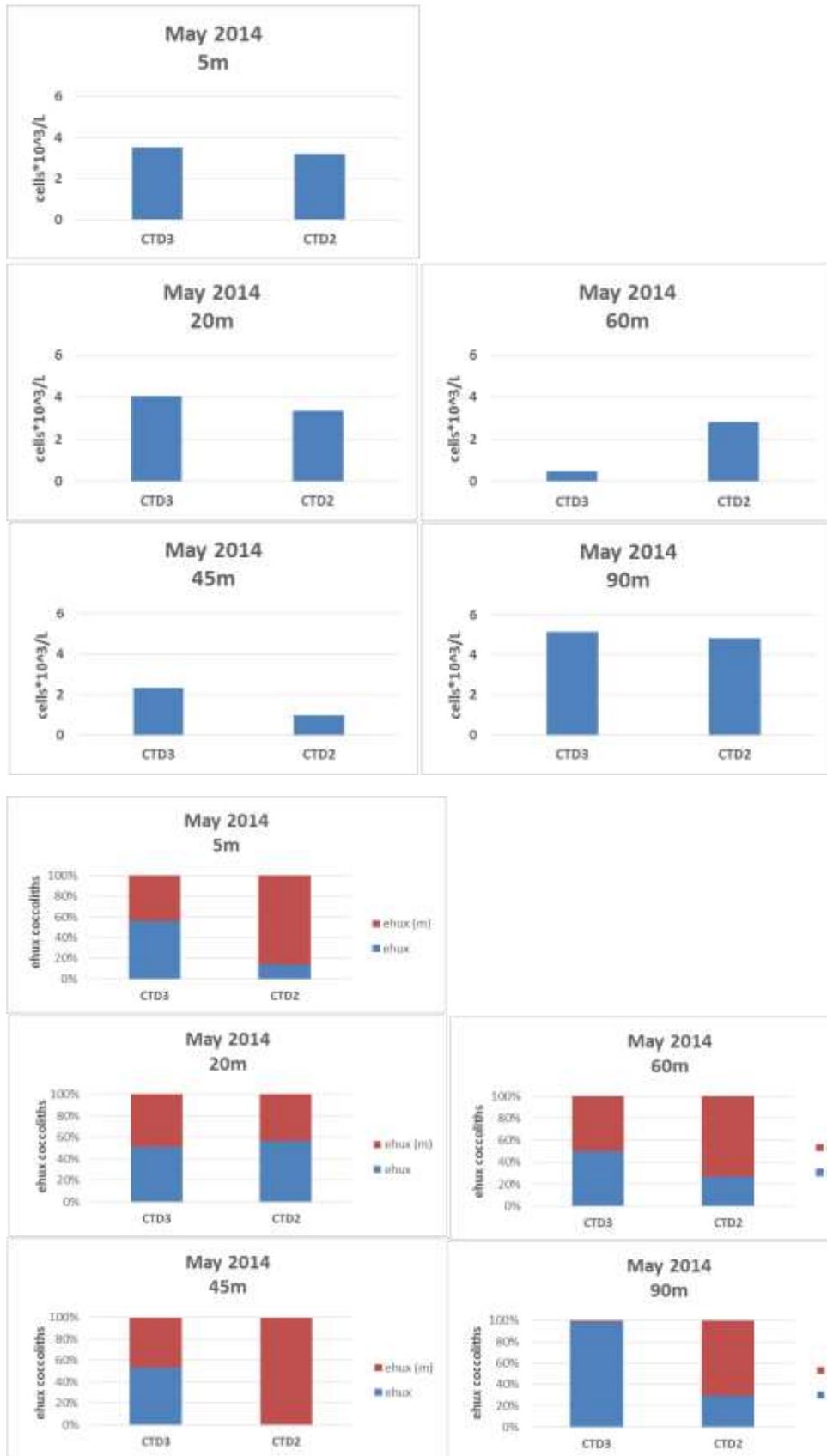


Figure 21 Diagrams showing the total coccolithophore abundances and the percentages of the normal and malformed *Emiliana huxleyi* coccoliths during the May 2014 sampling period in the different sampling stations and depths.

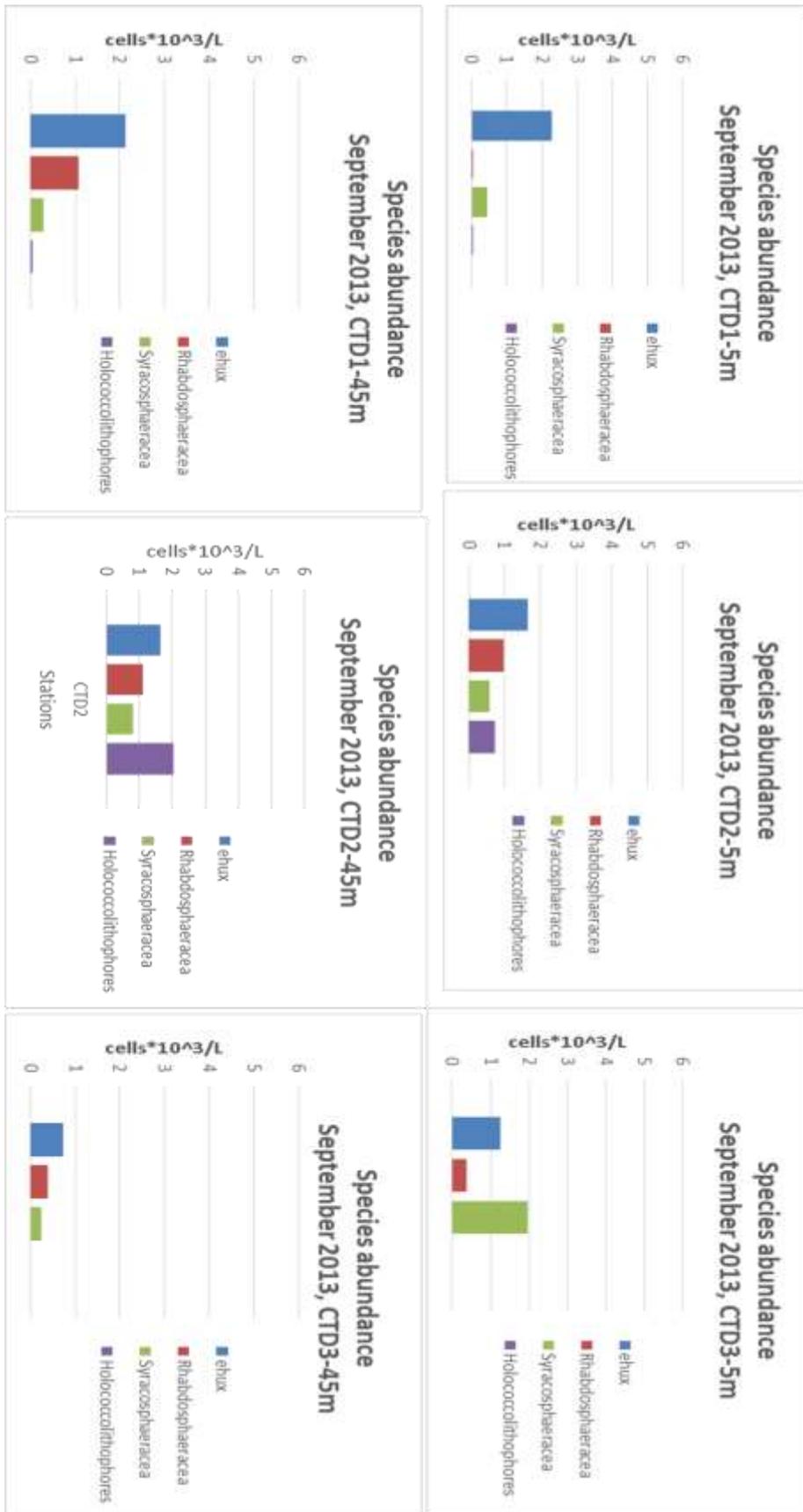


Figure 22 Diagrams showing the species abundances of important coccolithophore groups during the September 2013 sampling period in different sampling stations and depths.

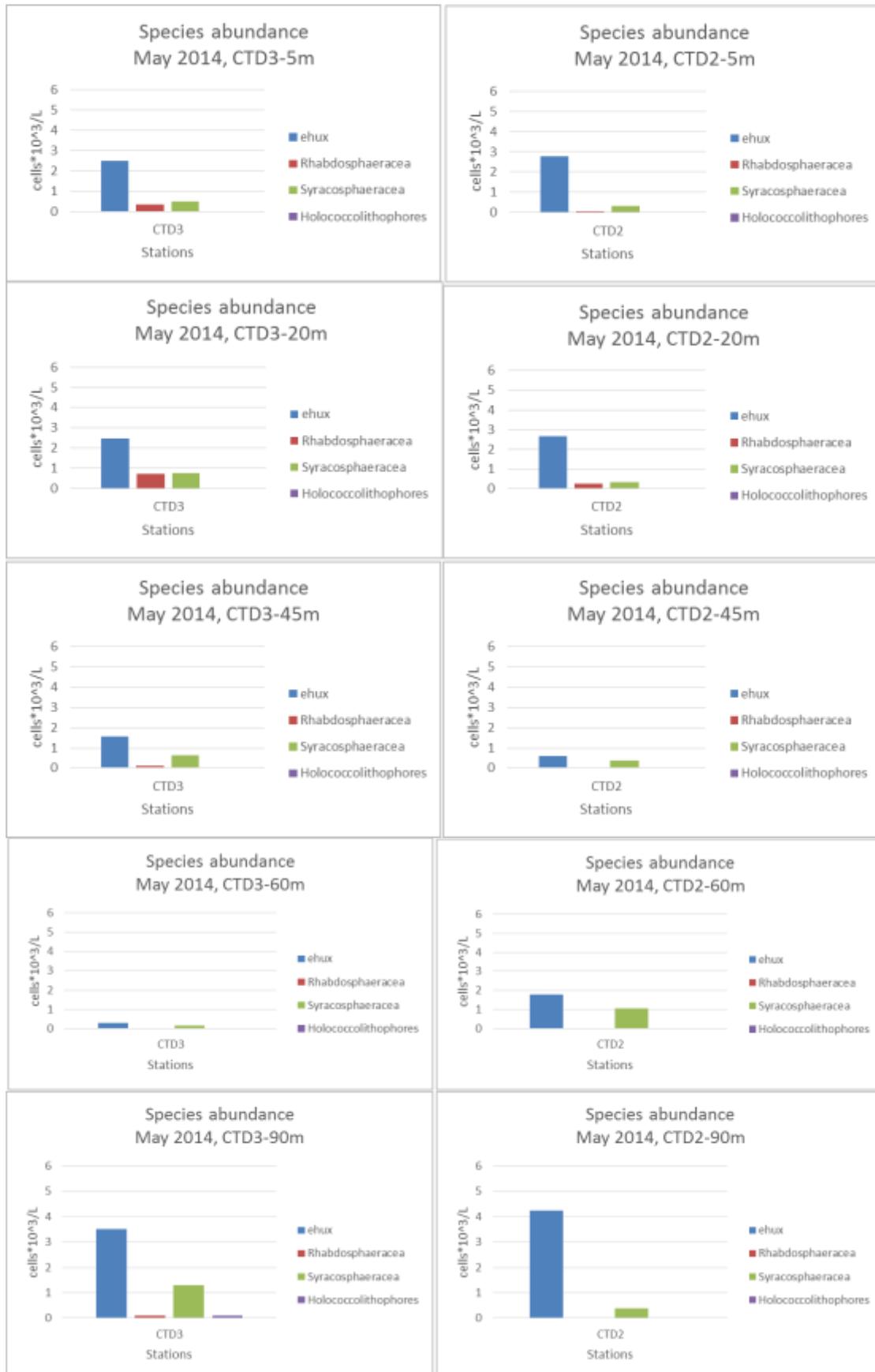


Figure 23 Diagrams showing the species abundances of important coccolithophore groups during the May 2014 sampling period in different sampling stations and depths.

September (%cells*10 <sup>3</sup> /l)	CTD1-5m	CTD1-45m	CTD2-5m	CTD3-5m	CTD3-45m
Ehux	79,66101695	51,1627907	39,534884	0	20,3125
Ehux(malformed)	0	0	0	24,29906542	3,125
Rhabdosphaera	1,694915254	24,41860465	23,255814	6,542056075	9,375
Coronosphaera	3,389830508	0	0	0,934579439	0
Syracosphaera	10,16949153	6,976744186	0	10,28037383	7,8125
Umbellosphaera tenuis	1,694915254	13,95348837	2,3255814	28,03738318	56,25
Syracosphaera pulchra	1,694915254	0	0	0	0
Periphylophora	1,694915254	0	5,8139535	0	0
Syracosphaera (malformed)	0	0	0	28,03738318	0
Calciosolenia	0	0	0	1,869158879	0
Gephyrocapsa	0	1,162790698	0	0	0
Gliscolithus	0	1,162790698	1,1627907	0	0
Discosphaera	0	1,162790698	0	0	3,125
Helladosphaera	0	0	8,1395349	0	0
Syracosphaera ossa	0	0	1,1627907	0	0
Syracolithus confusus	0	0	11,627907	0	0
Syracosphaera haldalii	0	0	1,1627907	0	0
Syracosphaera pulchra oblong	0	0	1,1627907	0	0
Alisphaera	0	0	1,1627907	0	0
Sphaerocalyptra	0	0	2,3255814	0	0
Neosphaera	0	0	1,1627907	0	0

*Table 4 Percentage values of the cell densities during September sampling period and in all sampled depths.*

May (%cells*10 <sup>3</sup> /l)	CTD2-5m	CTD2-20m	CTD2-45m	CTD2-60m	CTD2-90m	CTD3-5m	CTD3-20m	CTD3-45m	CTD3-60m	CTD3-90m
Ehux	11,9403	44,28571	0	16,94915	26	39,72603	30,95238	34,69388	30	67,28972
Ehux(malformed)	74,62687	34,28571	0,578022	45,76271	62	31,50685	29,7619	30,61224	30	0,934579
Rhabdosphaera clavigera	1,492537	5,714286	0	0	0	8,219178	17,85714	4,081633	0	0
Coronosphaera sp	1,492537	4,285714	0	0	0	1,369863	0	0	0	1,869159
Syracosphaera sp	0	0	0	0	0	0	0	0	0	0
Umbellosphaera tenuis	0	1,428571	0	0	0	5,479452	2,380952	0	0	0
Syracosphaera pulchra	8,955224	0	0,385348	37,28814	8	12,32877	19,04762	26,53061	30	18,69159
Discosphaera	0	0	0	0	0	1,369863	0	0	0	0
Syracosphaera pulchra oblor	0	8,571429	0	0	0	0	0	0	0	0
Alisphaera	0	0	0	0	0	0	0	0	0	0,934579
Sphaerocalyptra	0	0	0	0	0	0	0	0	0	0
Neosphaera	0	0	0	0	0	0	0	0	0	0
Calcidiscus leptoporus	1,492537	0	0	0	0	0	0	0	0	0
Palusphaera vandellii		1,428571	0	0	0	0	0	0	0	0
Umbilicosphaera	0	0	0	0	4	0	0	4,081633	10	0,934579
Corisphaera gracilis	0	0	0	0	0	0	0	0	0	0,934579
Syracosphaera amoena	0	0	0	0	0	0	0	0	0	0,934579
Algirosphaera robusta	0	0	0	0	0	0	0	0	0	1,869159
Scyphosphaera	0	0	0	0	0	0	0	0	0	0,934579
Syracosphaera molischii	0	0	0	0	0	0	0	0	0	2,803738
Hellad. Cornifera	0	0	0	0	0	0	0	0	0	0,934579
Syr. Histrica	0	0	0	0	0	0	0	0	0	0,934579

Table 5 Percentage values of the cell densities during May sampling period and in all sampled depths.

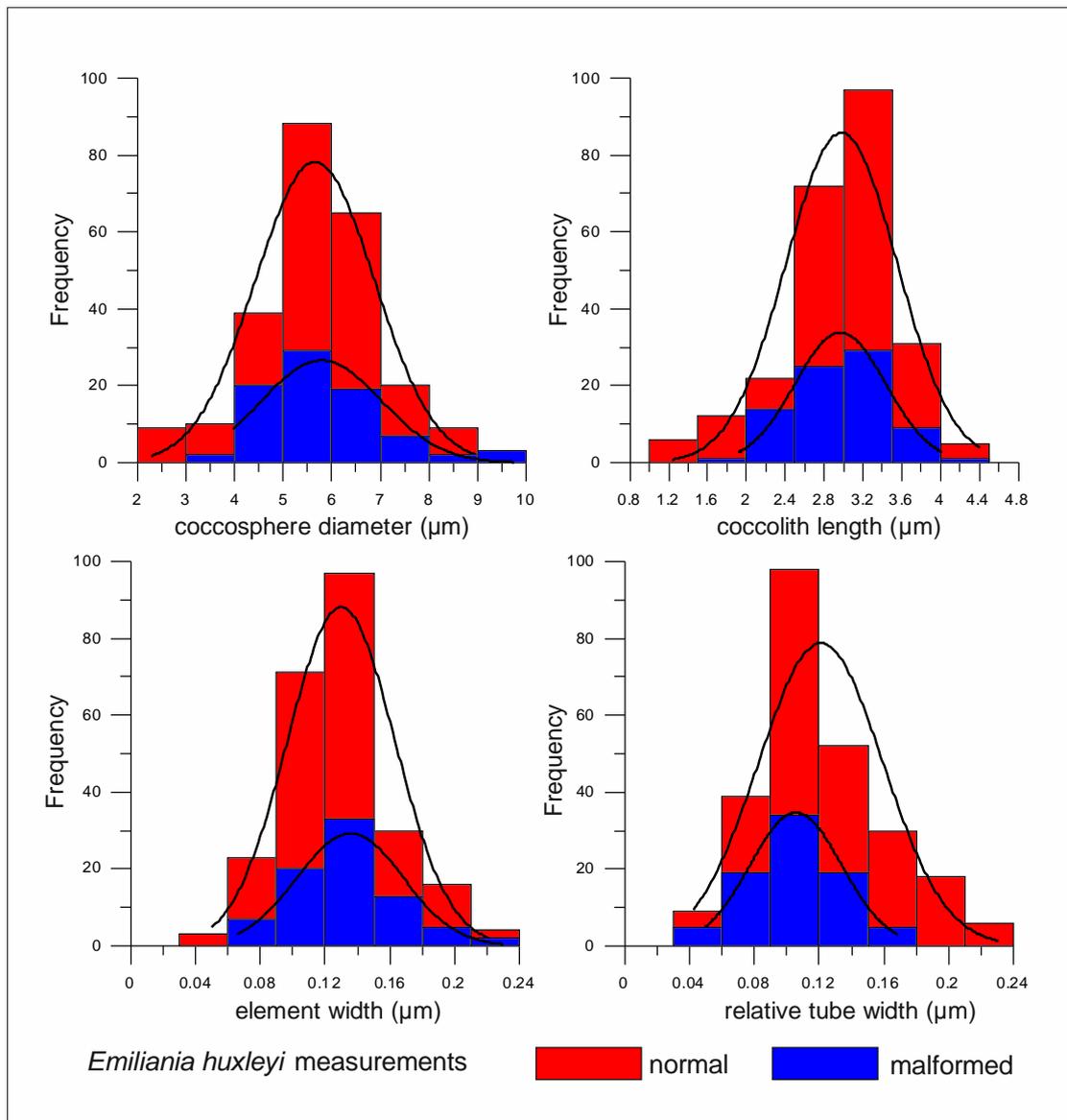


Figure 24 Frequency diagrams showing the coccosphere diameter, the coccolith length, the element width and the relative tube width values of the normal and the malformed coccoliths. Figure from Karatsolis et al.2016 (poster presentation).

## 7. Paleoceanographic implications

The differences between the growth and calcification level and the various morphotypes that coccolithophore species present is a strong tool for distinguishing different water masses. One of the most used species for this purpose is *Emiliana huxleyi*, which shows a high diversity in morphotypes and also in parameters such as size, tube width, calcification rate, central area length etc. This species has been continuously used as a proxy for paleoenvironmental reconstructions and the information that offers, along with some other species are really valuable. It is well known that physical parameters of the water have varying effects in coccolithophores and thus these organisms can give us important information about the geochemistry, temperature, salinity and generally the conditions that occurred in the past. Finally, when different masses occur in the study area, the paleoenvironmental reconstruction using these organisms and their size parameters and morphotypes, can be a proxy for studying the influence rate of these masses in the area.

In our study, the previously discussed distribution and presence of *Emiliana huxleyi* coccoliths and their differences in size, calcification rate and relative tube width were related to the different water masses that appear in the Aegean (Karatsolis et al. 2016). This information can serve as an opportunity to use coccolith parameters for finding what was the influence of these same masses in the past. For example, the presence of heavily calcified coccoliths in the Aegean is strongly related to low temperatures in the winter period (Triantaphyllou et al. 2010, Karatsolis et al. 2016) and to the LW mass, whereas the BSW is characterized by coccoliths with smaller relative tube width and more lightly calcified, something that is also typical for the LW coccoliths in the summer period. Thus, the use of the fluctuations of these morphotypes could help further studies in terms of paleocirculation. Generally, coccolithophore species and their changes in abundance, frequency peaks and/or local occurrences or temporary disappearances of specific taxa allows to reconstruct variations in primary productivity, the depth of the nutricline, stratification of the water column and fresh water input in eastern Mediterranean paleoceanographic records (e.g., Negri et al., 1999; Negri and Giunta, 2001; Giunta et al., 2003; Principato et al., 2003, 2006; Triantaphyllou et al., 2009a, b, 2010; 2013, 2014) and can thus be combined with other morphological aspects for a more completed paleoreconstruction of the

circulation state in the area. Other species with well known ecological niches such *Florispahera profunda*, *Braarudosphaera bigelowii* and *H.carteri* can also help in that direction (Triantaphyllou et al. 2014).

It is with great interest if the same seasonal and water mass variations can be spotted also in sediment samples. In the North Aegean area, proved existence of different periods with distinct paleotemperatures (Triantaplyllou et al.2013) serve as an opportunity to try to distinguish between different coccolithophore morphotypes and their significance to the circulation of the area.

Culture studies have shown that salinity is one of the parameters that has an influence in the morphology of *Emiliana huxleyi* coccoliths. Additionally, other studies have showed that this species can be used as a proxy to study paleosalinities, especially if they are between 33 to 38 (Bollmann and Herrle et al. 2007). Comparing results from sediments in the area, along with other data such as alkenones, can give us a clearer view about the past of the area and about the influence of the BS in the North Aegean. Although, it is very important that we analyze the signals we are getting from each proxy. The alkenones, are used to depict the SST, but the origin of the temperature we are getting is not always clear. In our area, alkenone data and coccolithophore morphometry studies can state low temperature conditions without clearly stating if this low temperature is a result of the seasonal variation or BSW inflow phenomena. Moreover, the presence of different *Emiliana huxleyi* coccolith parameters in the past, can mean different things from the ones we believe in the present. It is proved, that adaptation and variability in the strains of these species react distinctively to changes of the environmental conditions. Evidence for this with respect to *E. huxleyi* comes from culture experiments in which three different clones of *E. huxleyi* (all type A of (Young et al. 1991), isolated from widely distributed areas, were cultured under different salinities (Green et al. 1998). In these culture experiments the size of the distal shield of two clones decreased significantly with decreasing salinity, whereas the third strain did not show any significant changes. Other studies also show that the decrease of the mean amount of calcium deposited in each coccolith declines with a decrease of the salinity (Paasche et al.1996) and there also data that support that the the existence of different morphotypes within this species are ecologically controlled (Poulton et al.2011). It is although possible, that when moving forward to the past, the

possibility of the existence of genotypic differences with distinct reaction to physicochemical parameters is possible inside the same species. Although, if significant similarities between the morphological parameters and the distribution of the species are found, then information of great importance can be added about the circulation of the water masses in the past and about extreme events that may have occurred in the area. For this to happen, we need additional data from plankton samples so that the data of this study are further supported and also monitoring so that the seasonal variations and their effect to *E.huxleyi*'s morphology are understood in a more complete way. Finally, genotypic experiments can shed light to the question if the *E.huxleyi* morphotypes we are observing have genetical or ecophysiological roots.

## 8. Conclusions

The main conclusions of the study conducted can be summarized as follows:

- R-mode hierarchical cluster analysis on NE Aegean coccolithophore assemblages defined four groups. Group I consists mostly of *E. huxleyi*, followed by *A. robusta*, *S. nodosa*, and *S. dalmaticus*. Group IIa is represented by Rhabdosphaeraceae, *Syracosphaera* spp. and *A. robusta* HOL, Group IIb includes holococcolithophore species, while Group IIc is comprised by *S. pulchra* and *U. tenuis*.
- A biometric analysis of *E. huxleyi* coccoliths from north Aegean waters and Black Sea year-round sediment trap samples, showed that the sampled Aegean waters with the maximum BSW influence, although in different seasonal samplings, were carrying specimens with the characteristic morphometry of Black Sea *E. huxleyi* (i.e., peak relative tube size at  $\sim 0.10$ ); overcalcified morphotypes (i.e., peak relative tube size at  $\sim 0.40$ ) were also present, implying winter-early spring LW mass.
- During autumn 2013, BSW was mainly present as a surface less saline and cooler lid at station AMT6 (south of Limnos Island). The high concentrations of Group I coccolithophore assemblage, were dominated by *E. huxleyi* that presented similar biometry to Black Sea specimens, at  $< 20$  m depth.
- In early spring 2014, BSW was influencing mostly stations AMT2 and AMT3  $< 20$  m depth, north of Limnos Island. All stations were featured by increased concentrations of *E. huxleyi* monopolizing Group I that showed positive correlation with nutrients in most of the high abundance-low diversity spring water column. The biometric analysis of *E. huxleyi* coccoliths proved that the stations with the highest BSW influx displayed a bimodal pattern of relative tube width, revealing the presence of both Black Sea and LW winter overcalcified morphotypes.
- Surprisingly, *E. huxleyi* was practically absent during the summer sampling when BSW presented maximum inflow, implying the impact of low mineral nutrients related to a metabolically active community in the BSW surface

masses. Notably, the highly diversified holococcolithophore assemblages were observed to be more abundant at depth than in their normal summer surface waters ecological niche, therefore marking LW mass flowing beneath the less saline BSW surface lid.

- Silicoflagellates served as a good indicator for different water masses. The existence of a small number of species makes it easier to make direct observations for the species distribution in relevance with the water masses.
- The silicoflagellate assemblages were dominated by *D. stapedia* followed by *S. speculum*, with rare occurrences of *D. aculeata* and *O. pulchra*.
- Both major species displayed higher overall abundances at stations 2AMT-2, 3, that were characterized by the presence of a lid of cold low-salinity water from the Black Sea, while they were scarce at station 2AMT-6 which was homothermal and occupied by warmer and saltier LIW.
- Peculiar morphologies of *S. speculum* characterized the observed populations: specimens were predominantly 7-sided and large (28-30  $\mu\text{m}$ ), corresponding to the size range of the large *S. speculum* morphotype described by Tsutsui et al. (2009) and reported in Malinverno (2010) in the Southern Ocean around the Polar Front. Their apical ring was intermediate to small, on average 42% of the basal ring; apical ring spines characterized >90% of the observed specimens. The skeleton of *S. speculum* is known to be extremely plastic in both extant and fossil populations, showing gradual changes in one or more of the morphological characters as a response to environmental parameters and with teratoid morphologies usually associated with environmental stress.
- The morphologies that we observed in the Northeast Aegean Sea, and in particular the association of apical spines on a rather small apical ring, were not previously described in other oceanographic settings. Populations dominated by 7-sided specimens have been reported only from the cold high-latitudes in both hemispheres and were associated with the presence of sea-ice. The peculiar combination of the observed morphological characters of *S. speculum* in the Northeast Aegean represent thus a novelty for temperate

settings and can be explained as related to the inflow of cold low-salinity water from the Black Sea.

- During our conducted study in the Kolumbo area, a significant number of malformed coccoliths was observed.
- The malformations were observed in *Emiliana huxleyi*, *Syracosphaera pulchra* and *Rhabdosphaera clavigera* coccoliths but the mainly malformed species was *Emiliana huxleyi*.
- The conditions were oligotrophic, typical for the Aegean area with characteristic species and low cell densities.
- Syracosphaeracea, Rhabdosphaeracea and holococcolithophores were more abundant during September, with the last ones almost absent in May.
- The pH did not show any significant divergence from normal values.
- The malformations followed the nutrient peaks during the May sampling period and they cannot be attributed to low pH.
- The malformed coccoliths displayed similar size parameters to normal ones , thus suggesting that the malformation was mainly restricted to disturbed coccolith shape and symmetry and not to significant calcite loss.
- More data about the carbonate chemistry of the water are needed, in order to further understand the acidification processes linked to malformation phenomena.
- The link between volcanic emissions, high CO<sub>2</sub> levels and the response of coccolithophore species to that, is easier to be studied where the currents are not strong and the volcanic emissions create consistent changes in the water column.
- Further study of the area can add information that can be used as paleoceanographic tools and give handy indicators of differences in physical parameters of the water. That way, these information can help in the paleoreconstruction of the circulation of the water masses in the area.

## 9. Future Plans

This thesis presents data for the species distribution of coccolithophore and silicoflagellate species in the Northern Aegean area and the Kolumbo area. Although, it raises many questions as far as it concerns the ecophysiology of these organisms. Different types of *E.hux*, the high biodiversity, the malformed coccospheres and their different distributions need to be further studied. The reasons why different types of *E.huxleyi* do occur, what their phylogeny is and how they can be linked to the environmental conditions are still poorly understood. Additionally, their life-cycle and their molecular functions under stressed environmental conditions are a bet for the future. Finally, further investigation is also needed so in order to be able to understand the coccolithophore species response to low pH environments. Several parts of the Aegean Volcanic Arc serve as natural laboratories for this kind of studies.

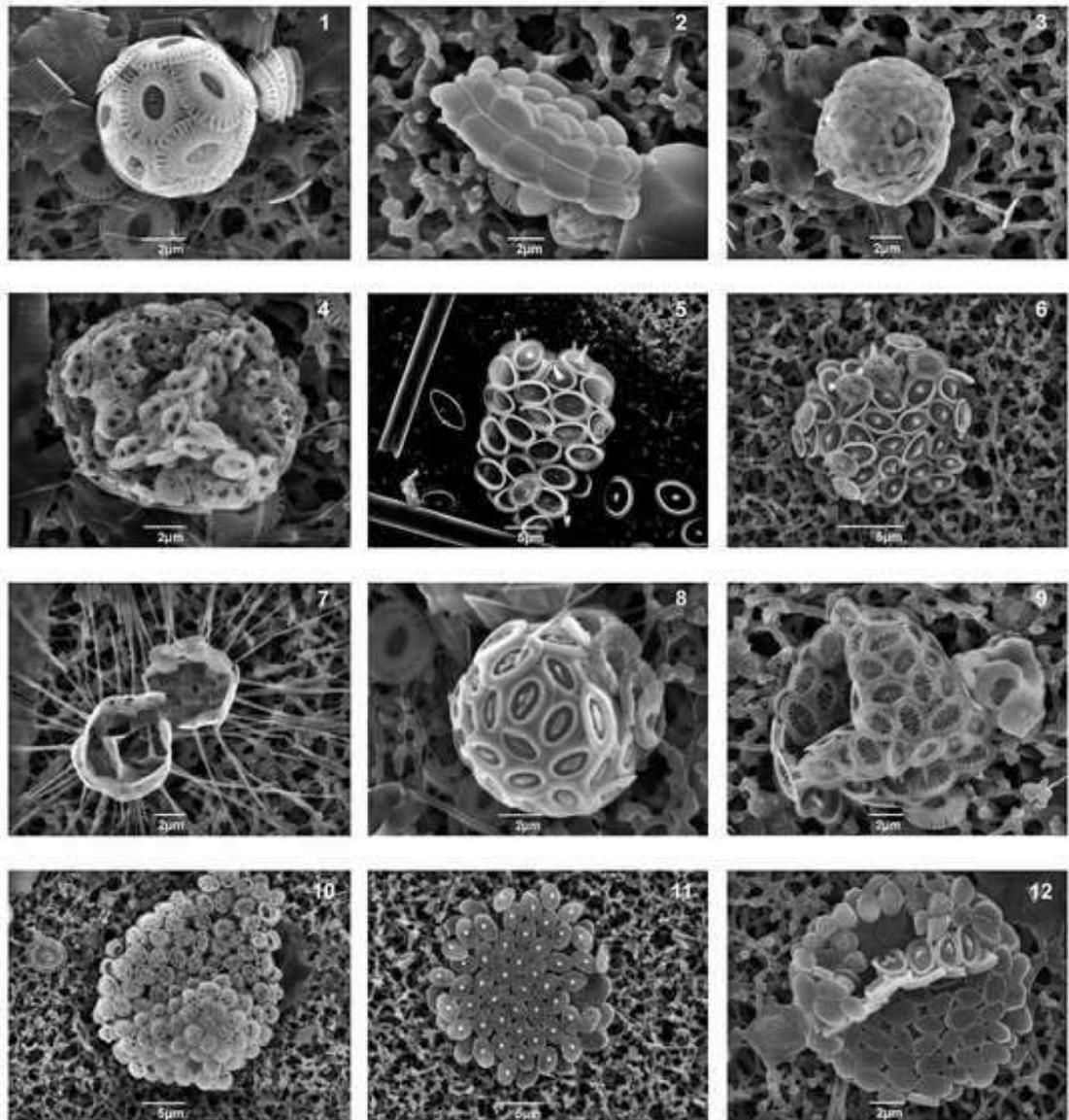


Plate 1 *Emiliana huxleyi* (Lohmann) Hay and Mohler var. *huxleyi*, AMT3, 3 m, March 2. *Algirosphaera robusta* (Lohmann) Norris, AMT3, 50 m, March 2014. 3. *Syracosphaera nodosa* Kamptner, AMT6, 3 m, March 2014. 4. *Syracolithus dalmaticus*

**Group I.** 1. (Kamptner) Loeblich and Tappan, AMT6, 10 m, March 2014. **Group IIa.** 5. *Syracosphaera pulchra* Lohmann, AMT6, 3 m, October. **Group IIb** 6. *Syracosphaera histrica* Kamptner, AMT6, 50 m, July 2014. 7. *Palusphaera vandellii* Lecal; emend. Norris, AMT6, 50 m, July 2014. 8. *Syracosphaera molischii* Schiller, AMT7, 10 m, March 2014. 9. *Syracosphaera halldalii* Gaarder ex Jordan and Green, AMT6, 3 m, March 2014. 10. *Syracosphaera pulchra* Lohmann HOL *pirus* type, AMT2, 20 m, July 2014. 11. *Helicosphaera carteri* (Wallich) Kamptner HOL solid, AMT7, 10 m, July 2014. 12. *Syracosphaera histrica* Kamptner HET and HOL, AMT6, 50 m, July 2014.

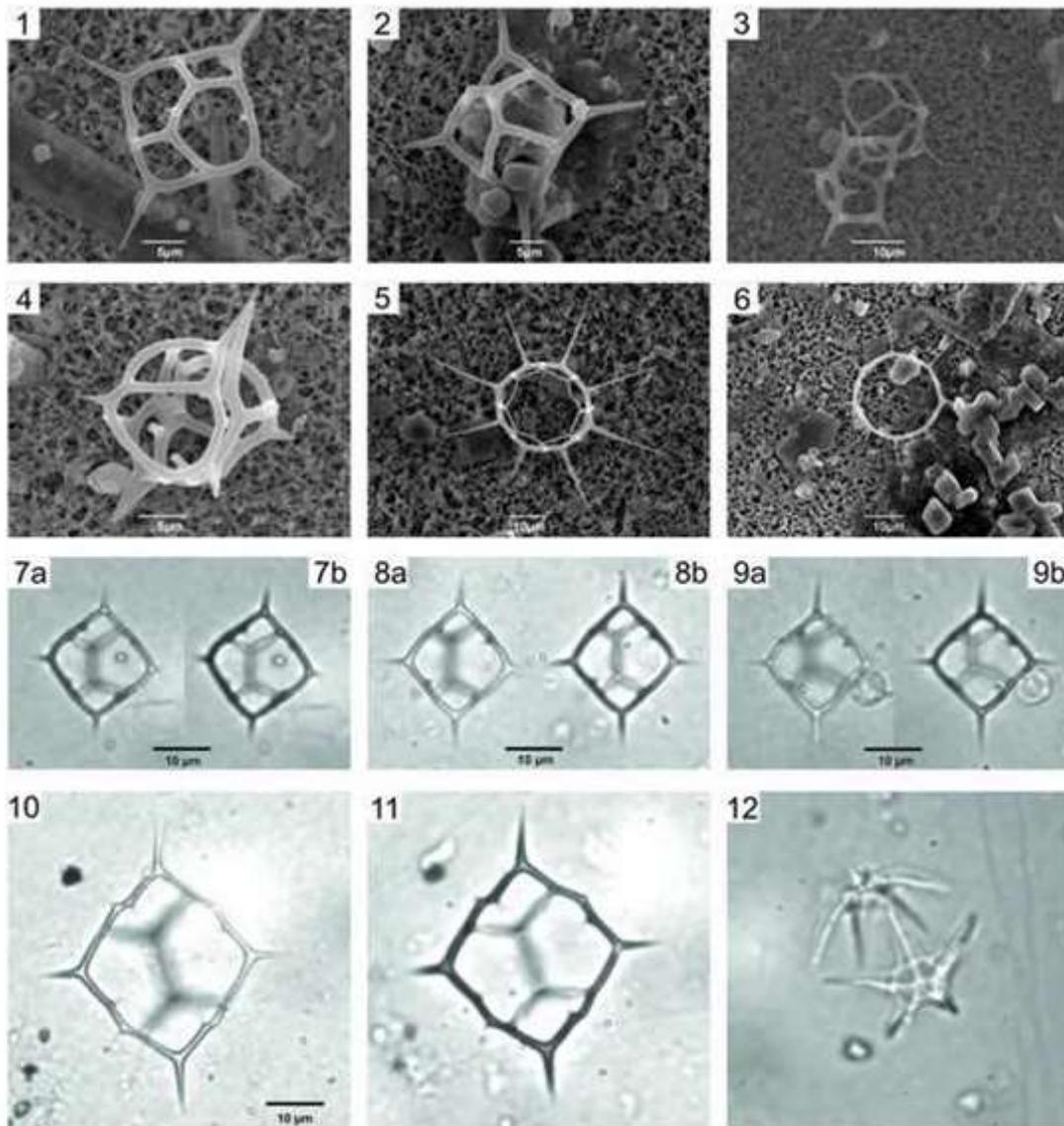
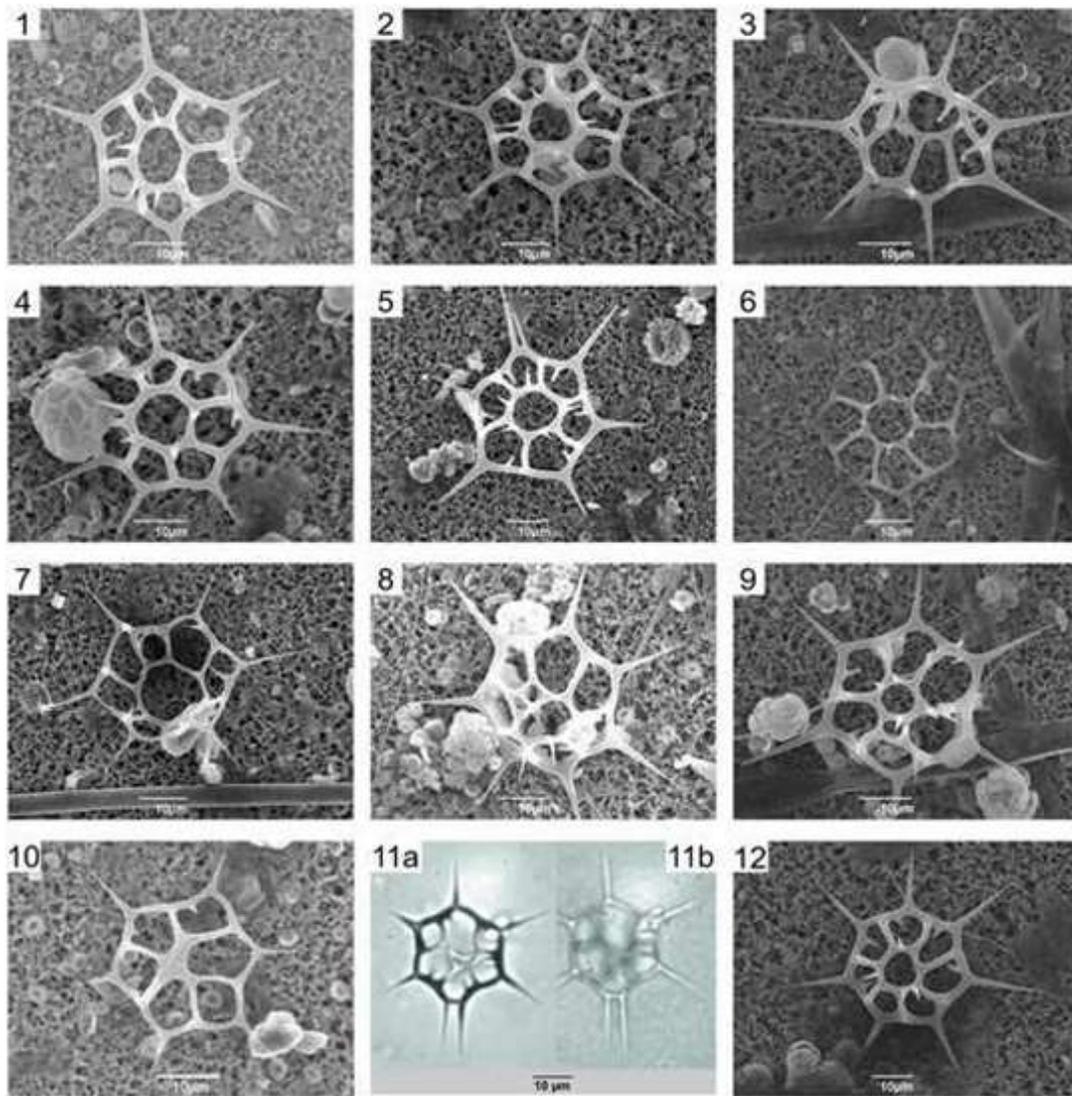
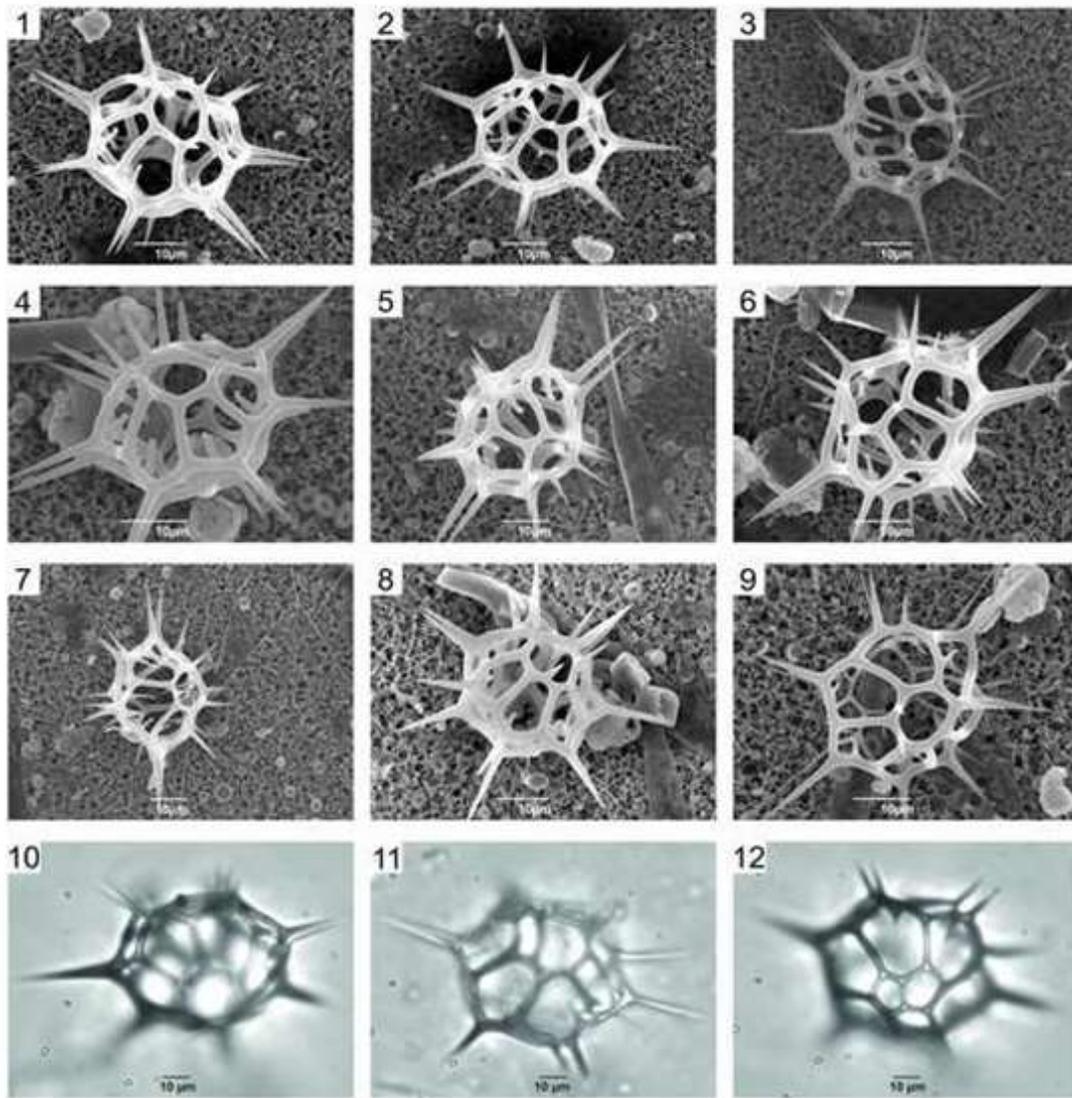


Plate 2 – 1-4, 7-9: *Dictyocha stapedia*, 1, 2) typical morphologies of *D. stapedia*, SEM, apical view (2AMT-6, 20 m); 3) thick skeleton, abapical view (left) and thin skeleton, apical view (right), SEM (2AMT-2, 30 m); 4) double skeleton, SEM, lateral view (2AMT-2, 30 m); 7-9) LM photos at high and low focus (7, 2AMT-2, 20 m; 8, 2AMT-3, 10 m; 9, 2AMT-2, 30 m); 5, 6) *Octactis pulchra*, SEM: 5, double skeleton, apical axis view; 6, single skeleton, abapical view 10,11) *Dictyocha aculeata*, LM, focus on basal ring (10) and apical bar (11), apical view (2AMT-3, 50 m); 12) *Actiniscus*, LM (2AMT-2, 20 m).



*Plate 3 – Morphologies of *Stephanocha speculum* from the Northeast Aegean (1-11) and Western Black Sea (12). 1) 6-sided, elongated apical ring, apical view (2AMT-3, 10 m); 2) 7-sided, apical view (2AMT-6, 10 m); 3) 7-sided, lateral view (2AMT-3, 10 m); 4) 7-sided, open basal ring, apical view (2AMT-6, 10 m); 5) 6-sided with an additional vestigial basal ring spine, apical view (2AMT-2, 20 m); 6) 7-sided, large, with twisted struts, apical view (2AMT-2, 20 m); 7) 6-sided with one additional strut, abapical view (2AMT-2, 20 m); 8) 7-sided with tripartite apical ring, apical view (2AMT-3, 20 m) 9) 6-sided, with very small apical ring and spines on the struts, apical view (2AMT3-10); 10) 6-sided with aberrant apical ring transformed in a plate (2AMT-3, 2 m); 11) 6-sided with an additional basal ring spine and deformed apical ring (LM, 2AMT-2, 2 m); 12) 7-sided, with rotated struts, Black Sea specimen (BS3-22).*



*Plate 4 - Double skeletons of Stephanocha speculum from the Northeast Aegean. 1) 7-sided doublet, oblique apical axis view (2AMT-2, 20 m); 2) 7-sided doublet, oblique apical axis view (2AMT-2, 20 m); 3) 7-sided doublet, with slightly thinner skeleton on top, oblique apical axis view (2AMT-2, 30 m); 4, 5, 6) 7-sided doublets, lateral view (2AMT-3, 10 m); 7) doublet formed by a 7-sided skeleton (left) and 6-sided skeleton (right) with open basal ring, lateral view (2AMT-3, 2 m); 8) 7-sided doublet; the skeleton on top has an open basal ring, oblique apical axis view (2AMT-6, 20 m); 9) 7-sided doublet: the skeleton on top is thick and has an additional spine, the skeleton on the bottom is very thin. Note that corners are not always coincident on the two skeletons of the doublet; apical axis view (2AMT-2, 20 m); 10) 7-sided doublet, showing the two skeletons connected at the corners but not along the sides, LM, lateral view (2AMT-2, 2 m); 11, 12) 7-sided doublet, same specimen: 11, focus on the lower skeleton, 7-sided and with bipartite apical ring; 12, focus on the upper skeleton, 6-sided and with tripartite apical ring; LM, oblique apical axis view (2AMT-2, 10 m).*

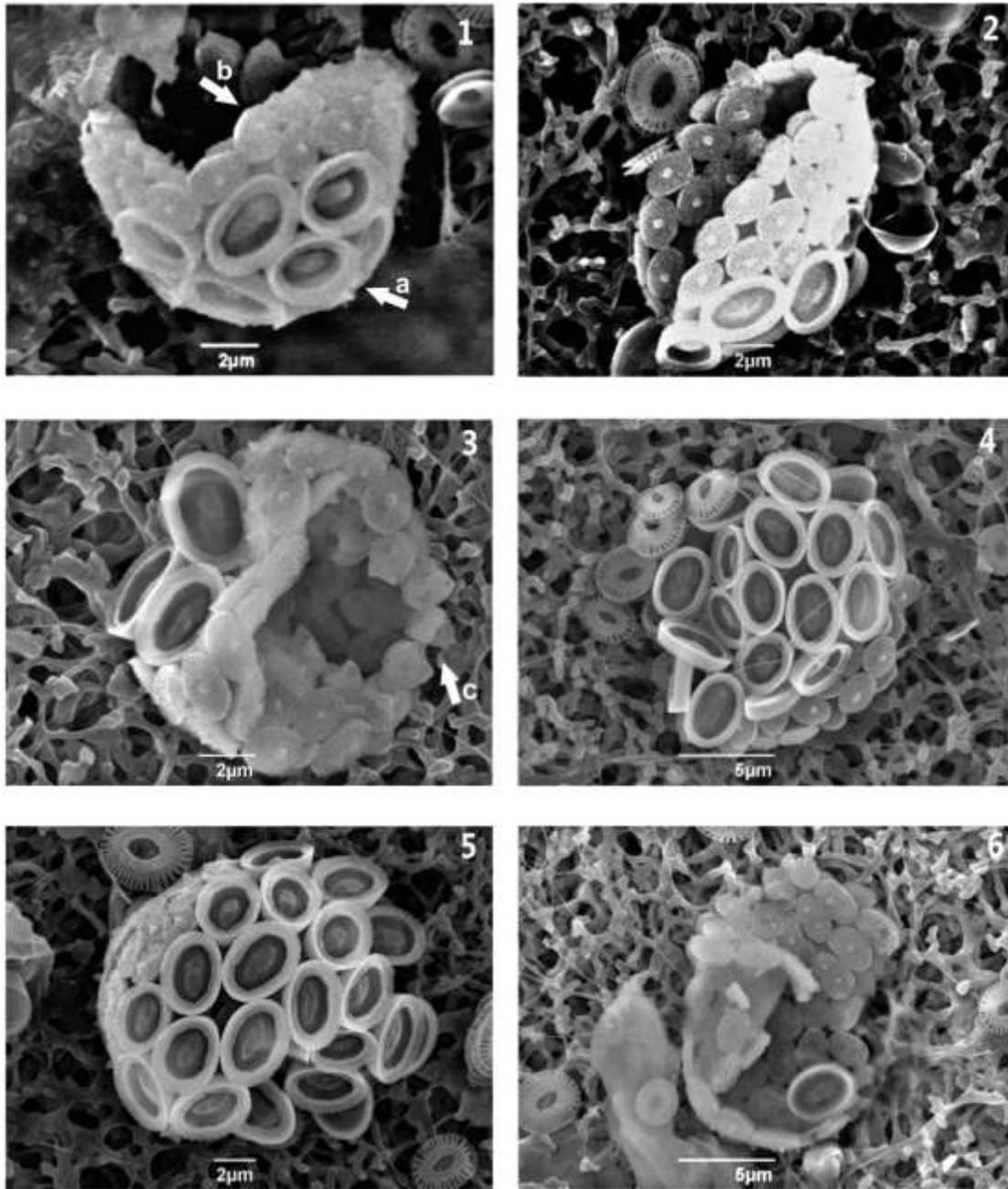


Plate 5

*Syracosphaera mediterranea* [combination coccospheres involving: (a) *Syracosphaera mediterranea* HET (“*Coronosphaera mediterranea*”) with (b, c) *Syracosphaera mediterranea* HOL (“*Zygosphaera hellenica*”)]

- |   |                         |   |                         |
|---|-------------------------|---|-------------------------|
| 1 | <i>sample 2AMT6-20m</i> | 4 | <i>sample 2AMT7-30m</i> |
| 2 | <i>sample 2AMT3-13m</i> | 5 | <i>sample 2AMT7-50m</i> |
| 3 | <i>sample 2AMT7-14m</i> | 6 | <i>sample 2AMT7-50m</i> |

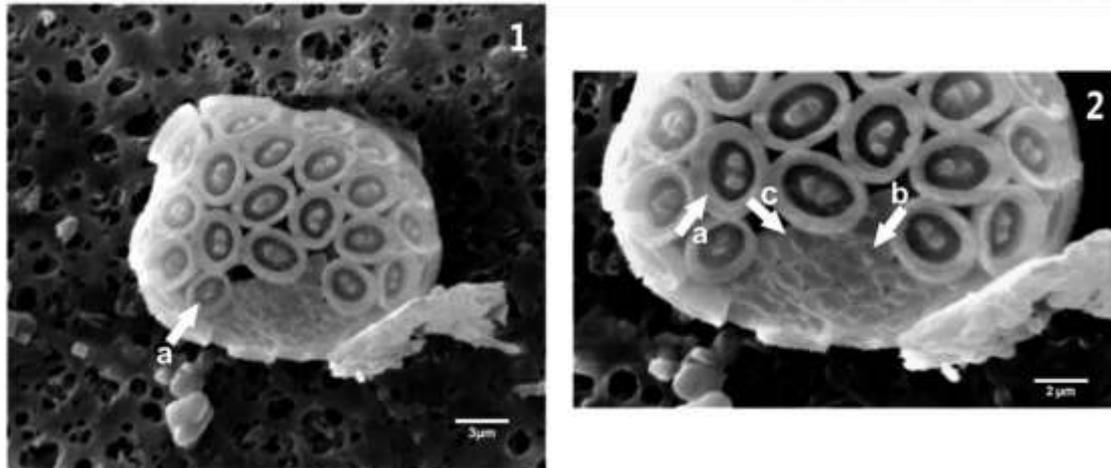


Plate 6

*Syracosphaera mediterranea* [combination coccospheres involving: (a) *Syracosphaera*

1 sample LB7-11m

2 sample LB7-11m, detailed view of  
*Syracosphaera mediterranea* HOL  
 (“*Calyptrolithophora gracillima*”)  
 holococcoliths - “*Coronosphaera*  
*mediterranea* HET”

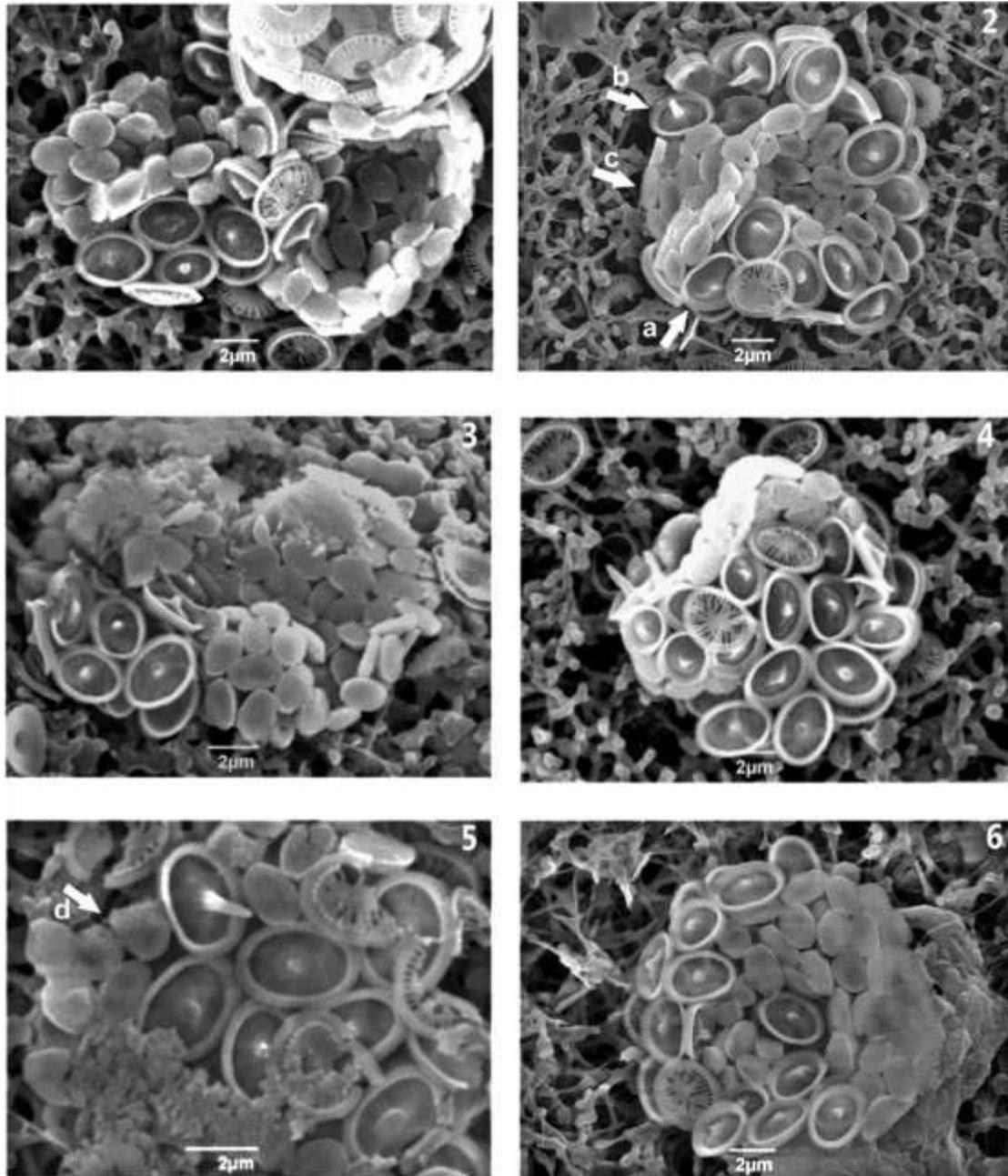


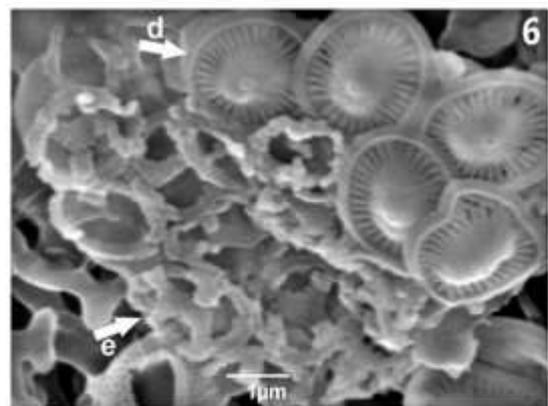
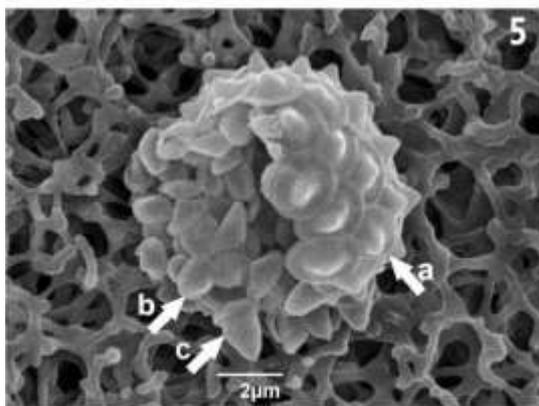
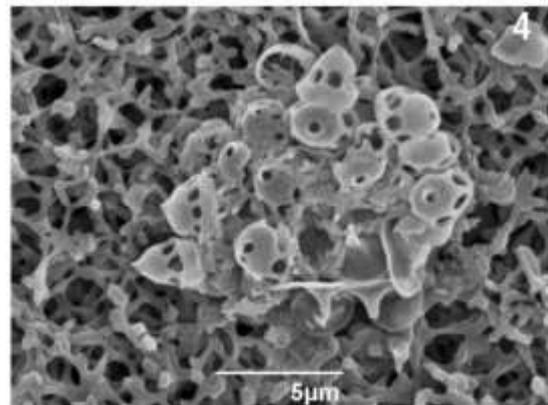
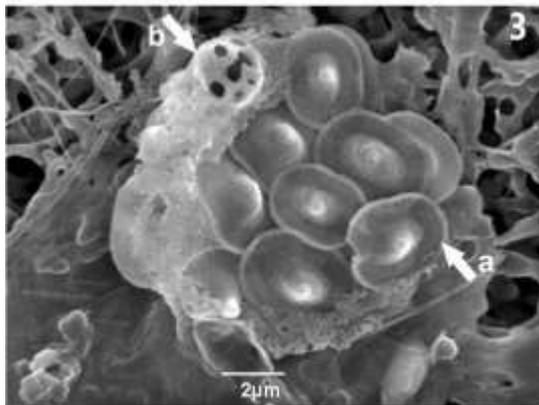
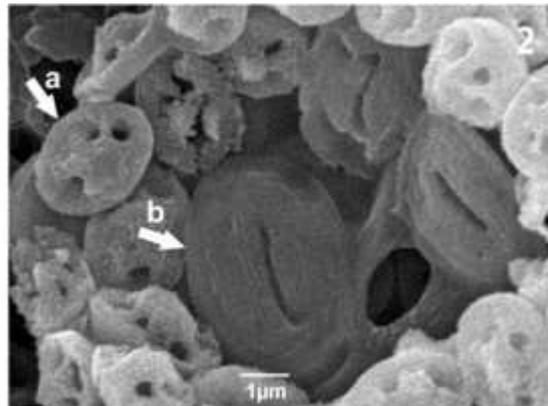
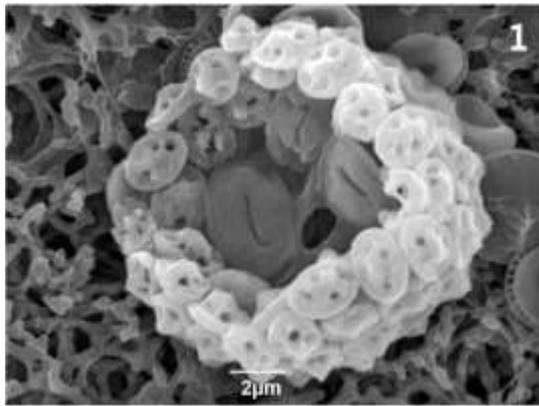
Plate 7

*Syracosphaera histrica* [combination coccospheres involving: (a, b) *Syracosphaera histrica* HET with (c, d) *Syracosphaera histrica*

HOL (“*Calyptrolithophora papillifera*”)]

- |   |                         |   |                         |
|---|-------------------------|---|-------------------------|
| 1 | <i>sample 2AMT3-10m</i> | 4 | <i>sample 2AMT3-20m</i> |
| 2 | <i>sample 2AMT7-30m</i> | 5 | <i>sample 2AMT6-20m</i> |
| 3 | <i>sample 2AMT3-20m</i> | 6 | <i>sample 2AMT7-20m</i> |





*Plate 9*

*Helicosphaera dalmatica* [combination coccosphere involving: (a) *Syracolithus dalmaticus* with (b) *Helicosphaera pavimentum*]

1 sample 2AMT3-20m 2 sample 2AMT3-20m (detailed view)

*Cyrtosphaera aculeata* [possible combination coccospheres involving:

(a) *Cyrtosphaera aculeata* HET with (b) *Holococcolithophora heimdaliae*]

3 sample 2AMT6-2m 4 sample 3AMT6-20m

*Acanthoica* combination coccosphere [heterococcoliths (a, d) of *Acanthoica* sp. with (b, c) cap-shaped to cone-shaped holococcoliths of a dimorphic *Sphaerocalyptra* species or (e) incomplete holococcoliths]

5 sample 3AMT6-30m 6 sample 2AMT6-10m

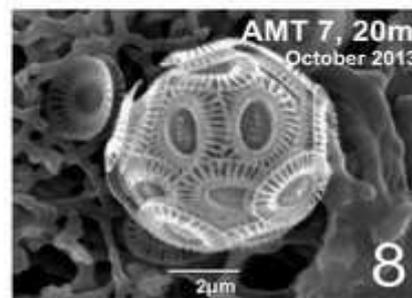
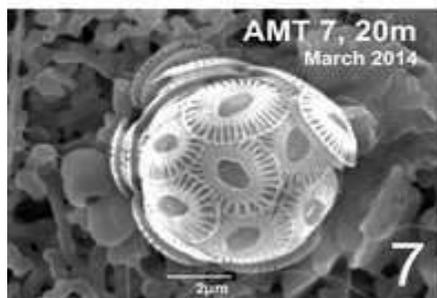
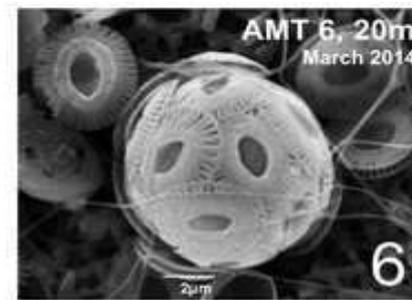
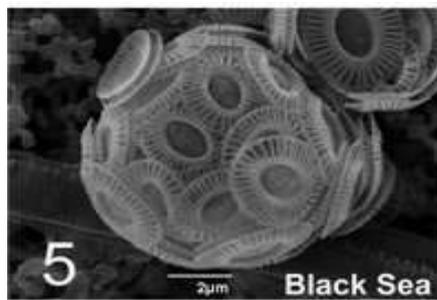
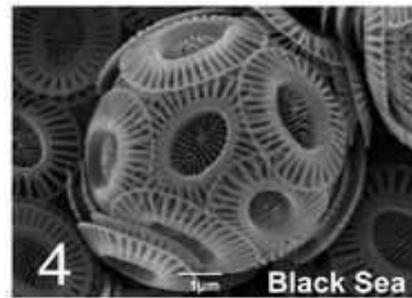
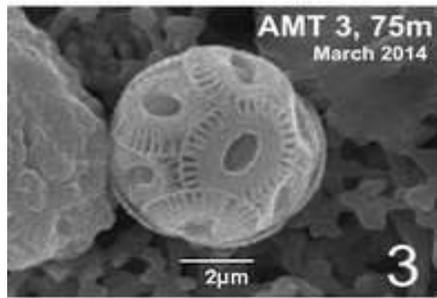
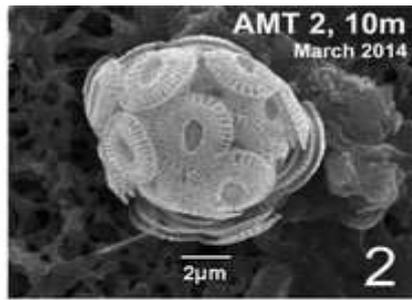
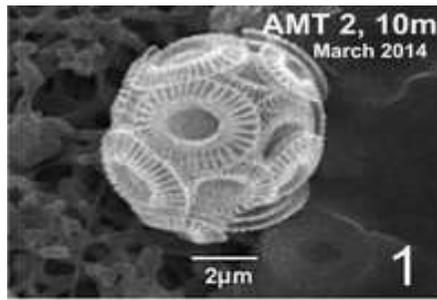


Plate 10 Figs. 1, 4, 5, 8. *Emiliana huxleyi* lightly calcified morphotypes from Black Sea and Aegean Sea assemblages. Figs. 2, 3, 6, 7. *Emiliana huxleyi* overcalcified morphotypes from Aegean Sea assemblages.

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