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Coccolithophore assemblage response to Black Sea water inflow into the North Aegean Sea (NE Mediterranean)

Karatsolis, B.-Th.^a, Triantaphyllou, M.V.^a, Dimiza, M.D.^a, Malinverno, E.^b, Lagaria, A.^c, Mara, P.^c, Archontikis, O.^c, Psarra, S.^c

^aFaculty of Geology & Geoenvironment, National and Kapodistrian University of Athens, Athens, Greece

^bDepartment of Earth and Environmental Sciences, University of Milano-Bicocca, Milan, Italy

^cInstitute of Oceanography, Hellenic Centre for Marine Research, Crete, Greece

Abstract

This study aims to presents the species composition of living coccolithophore communities in the NE Aegean Sea, investigating their spatial and temporal variations along a north-south transect in the area receiving the inflowing surface Black Sea Water (BSW) over the deeper Levantine Water (LW) layer. Coccolithophores in the area were relatively diverse and a total of 95 species over 3 sampling periods studied were recognized using Scanning Electron Microscope (SEM) techniques. R-mode hierarchical cluster analysis distinguished two coccolithophore Groups (I, IIa, IIb, IIc) with different ecological preferences. *Emiliania huxleyi* was the most abundant species of Group I, whereas *Syracosphaera* spp., *Rhabdosphaera* spp. and holococcolithophores were prevailing in the highly diversified Group II assemblages. Biometric analysis conducted on *E. huxleyi* coccoliths from Aegean water column and Black Sea sediment trap samples, indicated that during autumn, NE Aegean specimens in samples under BSW influence were featured by unimodal distribution concerning the coccolith relative tube width, with values similar to those provided by the Black Sea specimens. In early spring, coccoliths in the stations with increased BSW influx displayed a bimodal pattern of relative tube width with smaller values found mostly in the surface layers, while the distribution became again unimodal and dominated by larger values within the deeper LW layers. In the summer period, the typical LW holococcolithophore species (Group II) presented low cell numbers in the surface layer (<20 m), which is their usual ecological niche in the Aegean Sea, compared to greater depths, therefore marking LW mass flowing beneath the less saline

BSW surface lid. In contrast to Black Sea early summer bloom conditions, *E. huxleyi* was almost absent in the NE Aegean during the summer sampling period.

Key words: Coccolithophores; abundance; diversity; Black Sea; Aegean Sea; water masses

1. Introduction

Coccolithophores constitute a significant component of the marine phytoplankton and are very sensitive to the changing environmental conditions of the upper water column. In particular, through photosynthesis and calcification, they play a vital role in the biogeochemical cycles and the global climate system and make an important contribution to translocation of the inorganic carbon produced in pelagic areas to the ocean floor and thus to the sedimentary archive (e.g., [Westbroek et al., 1993](#); [Haidar and Thierstein, 2001](#); [Rost and Riebesell, 2004](#)). They are considered to be the most productive calcifying organisms on earth, as they produce minute calcium carbonate plates called coccoliths at some stage in their life cycle (e.g., [Young, 1994](#)). Many coccolithophore species have a complex life cycle that involves the sequential production of holococcoliths and heterococcoliths. Heterococcoliths, produced during the diploid life-cycle stage, are formed from complex shaped crystal units and their biomineralization occurs intracellularly (e.g., [Manton & Leedale, 1969](#); [Young et al., 1999](#)). Holococcoliths document the motile haploid life-cycle phase and are formed of numerous extracellularly calcified minute euhedral crystallites ([Manton & Leedale, 1963](#); [Rowson et al., 1986](#)). Although traditionally considered that most coccolithophore species prefer warm, low productivity regions ([McIntyre and Bé, 1967](#); [Honjo and Okada, 1974](#)), it is evident that except of certain taxa being important bloom-producers at high latitudes (e.g., [Westbroek et al., 1993](#)), nearly all species are also thriving in the high productivity areas such as upwelling regions (e.g., [Sprengel et al., 2000](#); [Saavedra-Pellitero et al., 2013](#)). Coccolithophores are among the dominant primary producers, presenting a high number of species (> 100) in the oligotrophic eastern Mediterranean Sea (e.g., [Knapperstbusch, 1990](#); [Kleijne, 1993](#); [Triantaphyllou et al., 2004](#); 2010; [Malinverno et al., 2003](#); 2009; 2014; [Dimiza et al., 2008](#), 2015; [Ignatiades et al., 2009](#)) with a strong seasonal variability and regional patchiness. Coccolithophores of the photic zone in the Aegean Sea indicate a close relationship between coccosphere densities and surface water circulation, with sea temperature gradient and nutrient availability affecting species composition. They are relatively diverse and dominated mostly by

heterococcolithophore species *Emiliania huxleyi*, *Syracosphaera* spp., Rhabdosphaeraceae and numerous holococcolithophores ([Dimiza et al., 2015](#)).

In this study, we investigate the seasonal and spatial distribution of coccolithophores in the NE Aegean water column from samples obtained along a transect evidencing hyposaline Black Sea Water (BSW) influx, and discuss the potential effects of *Emiliania huxleyi*-dominated BSW mass on the highly diversified Aegean coccolithophore assemblages. In order to achieve this goal, we investigated relations among abundance, species variation and distribution of coccolithophore species. Diversity indices and multivariate analysis were used in order to interpret the distribution patterns and to infer the ecology of living coccolithophore communities. Comparative biometric analyses on *E. huxleyi* coccoliths from Aegean samples and Black Sea sediment trap data were applied to detect the presence and origin of different morphotypes that potentially mark the BSW inflow.

1.1 Oceanographic setting

The Aegean Sea (NE Mediterranean) is featured by complex bathymetry and fluvial freshwater inputs essentially constrained only in the northern part, due to numerous major rivers from the surrounding areas of the Balkans and Turkey (e.g., [Lykousis et al., 2002](#); [Roussakis et al., 2004](#)). The entire Aegean Sea, in general, and the north Aegean basin, in particular, is featured by an overall cyclonic upper thermohaline circulation ([Lykousis et al., 2002](#)) and the dual flow between NE Mediterranean and Black Seas through the Dardanelles and the Bosphorus Straits (Fig. 1a, b). It represents an oligotrophic region that may display mesotrophic characteristics during the productive spring period ([Siokou-Frangou et al., 2002](#); [Ignatiades et al., 2002](#); [Zervoudaki et al., 2011](#); [Lagaria et al., this issue](#)). During winter, the low-salinity surface BSW (<31) tends to flow northwest of Limnos Island with minimum inflow rates; maximum inflow takes place during summer but the blowing northerly winds, the Etesians, deflect its presence to some extent south of Limnos ([Lascaratos, 1992](#); [Zodiatis, 1994](#); [Zervakis et al., 2000](#)). The warmer and more saline (>39) Levantine Water (LW) masses occupy surface layers in the absence of BSW, flowing northward along the eastern Aegean Sea, following the general cyclonic circulation (e.g., [Zervakis et al., 2004](#)). The upper layer in the south Aegean is occupied mostly by the surface saline waters of Levantine origin, entering through the eastern Cretan Arc Straits and the low-

salinity Modified Atlantic Waters (MAW) that intrude through the Antikithira Straits and occasionally via the Kassos Straits (Fig. 1b, e.g., [Lykousis et al., 2002](#)).

The north Aegean Sea is considered as one of the most important areas for dense water formation in the eastern Mediterranean region (e.g., [Zervakis et al., 2000](#); [Theocharis et al., 2014](#); [Velaoras et al., 2014](#)). Currently, the influence of the low-salinity BSW and the high-salinity LW is very important on the stratification frequency over the major North Aegean deep basins ([Androulidakis et al., 2012](#)). Increased BSW spreading during winter may affect the mixed layer depth, promoting the stratification of the water column and dense water formation activity; the existence of the low density BSW surface layer acts as an insulating lid that impedes air–sea interactions thus hindering the formation of dense water over the area it covers ([Zervakis et al., 2000](#)). The available salinity profiles of the area used in the present study, provided substantial information about the seasonal presence of BSW in the NE Aegean. The permanent halocline that separates BSW and LW was observed at all seasons, with the exception of stations southern of Limnos Island (stations AMT6, AMT7; Fig. 1c), that were totally mixed, in March 2014 (Lagaria et al., this issue). Obviously, the increased stratification and steeper halocline during autumn and summer (October 2013 and July 2014; Lagaria et al., this issue) are due to a combination of increased buoyancy input of 'younger', less saline (32 to <36) BSW through the Dardanelles Straits that mostly occupies the upper 20 m of the water column (Fig. 1c), and local warming of the surface layers ([Zervakis and Georgopoulos, 2002](#); [Androulidakis et al., 2012](#); [Kokkini et al., this issue](#)).

2. Material and methods

2.1 Sample collection and laboratory analysis

The present study focuses on the analysis of 72 seawater samples from 4 stations (Fig. 1b) 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 AegeanMarTech sampling station, 6 standard depths were sampled within the photic layer (3-75 m), adjusted to bottom topography and bathymetry (Table 1). Several physico-chemical parameters including temperature, salinity, oxygen, chlorophyll-a concentrations, and mineral nutrients were measured at all stations and depths (Appendix A; for details see Lagaria et al., this issue).

For coccolithophore analysis, 2 liters of seawater per sample were filtered on Whatman cellulose nitrate filters (47 mm diameter, 0.45 μm pore size), using a Whatman membrane filter holder and vacuum filtration system; particular caution was taken for even distribution of the filtered material. Salt was removed by washing the filters with 2 ml of mineral water. The filters were oven dried and stored in plastic Petri dishes. A piece of each filter, approximately $8 \times 8 \text{ mm}^2$, 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 (National and Kapodistrian University of Athens, Faculty of Geology and Geoenvironment) and all the individual coccospheres occurring on the studied filter area were identified and counted at 1200x. At least 200 coccospheres have been determined per sample, except of samples with extremely low densities. Coccolithophore absolute abundance (number of coccospheres l^{-1}) was calculated following the methodology of [Jordan and Winter \(2000\)](#), by scaling up the raw counts from a known scanned area, using the equation: $A = N \times S / V$, 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^{-1} . Identification and taxonomy of species was mostly based on the taxonomic references of [Young et al. \(2003\)](#), [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](#)).

At least twenty *E. huxleyi* coccoliths attached on coccospheres were measured per sample in the NE Aegean, except of cases with very low densities; in total 60 coccospheres have been considered for the layers 3-20 m and 20-75 m respectively. Biometric analyses of *E. huxleyi* relative tube width were performed in the NE Aegean samples (this study) and compared to available measurements from Black Sea sediment trap samples ($42^{\circ} 58'00''\text{N}$ $29^{\circ} 29'00''\text{E}$ 16/10/2007-01/09/2008; [Bouloubassi et al., 2010](#); [Triantaphyllou et al., 2014](#)) (Fig. 1a). In addition, several existing *E. huxleyi* biometric data from the Aegean Sea ([Triantaphyllou et al., 2010](#)) and measurements from station Hermione-HAS (February 2012; [Dimiza et al., 2015](#)) have been incorporated to our data set (Fig. 1b, Table 1). Coccoliths attached on coccospheres from the Aegean Sea (in total 925) and Black Sea sediment trap (in total 121), 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).

2.2 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 in all studied samples. 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.

3. Results

Physico-chemical data for each sampled station, e.g., temperature, salinity, oxygen, concentrations of chlorophyll-a and nutrients are shown in Appendix A. Cell densities of heterococcolithophores and holococcolithophores recorded during all sampling periods in the studied areas are presented in Appendix B. A total of 95 coccolithophore species have been recognized in the samples analyzed. All of them, separated in heterococcolithophores and holococcolithophores, are listed alphabetically with full citations in Appendix C.

3.1 Total abundance and diversity of coccolithophore assemblages

In samples of October 2013, coccolithophore abundance was highest in the surface waters (3 m layer) at sampling stations AMT6 and AMT7 (13×10^3 and 6×10^3 coccospheres l^{-1} , respectively). The rest of the studied stations displayed abundances $< 5 \times 10^3$ coccospheres l^{-1} and total absence of coccospheres has been recorded in samples AMT2-20 m and 50 m depth (Fig. 2, Appendix B). 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. 2).

The highest coccolithophore absolute abundance was found in March 2014 (up to 99×10^3 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. 2).

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

3.2 Species composition

In October 2013, *E. huxleyi* type A (the only *E. huxleyi* morphotype observed in all samples, Plate 1) displayed high abundances at sampling stations AMT6 and AMT7 (max= 13×10^3 coccospheres l^{-1} at station AMT6-3 m depth, representing $\sim 50\%$ of the assemblage; the species was totally absent in sample AMT2-10 m). Concerning species composition (Plate 2), *Syracosphaera* spp. were generally represented by low abundances (max= 2×10^3 coccospheres l^{-1} at station AMT6-3 m depth; Fig. 3), with *Syracosphaera pulchra* (max=22%) being the most common species (Fig. 4). Another important component, *Umbellosphaera tenuis* comprised about 19% of the assemblage (Fig. 4).

Emiliania huxleyi was the most abundant species in March 2014 varying between 6×10^3 coccospheres l^{-1} (station AMT6-75 m; Fig. 3) and 85×10^3 coccospheres l^{-1} (station AMT3-20 m; Fig. 3), therefore constituting on average $>80\%$ of the coccolithophore assemblage (Fig. 4). *Syracosphaera* spp. were relatively common (min= 0.2×10^3 coccospheres l^{-1} at station AMT3-75 m and max= 12×10^3 coccospheres l^{-1} 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 represented (min= 0.1×10^3 coccospheres l^{-1} at station AMT6-75 m and max= 6×10^3 coccospheres l^{-1} at station AMT6-20 m depth), mostly by *Algirosphaera robusta* (max=15%). Between 10 and 30 m water layer, holococcolithophores were comprising up to 10% of the total assemblage (station AMT3-10 m). Among them *Syracosphaera histrica* HOL (max=35%) was the most

frequent species, and *Syracosphaera amoena* HOL (max=10%) and *Syracolithus dalmaticus* (max=7%) were also represented in common abundances.

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; being 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*) occurred in all stations with absolute abundance values $< 2 \times 10^3$ coccospheres l^{-1} . Holococcolithophores were relatively well represented in the summer coccolithophore assemblages with maxima of 6×10^3 coccospheres l^{-1} (station AMT3-20 m) (Fig. 3), 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 coccolithophore taxa involved in the same life-cycle were synonymized ([Triantaphyllou et al., 2015](#)).

3.3 Statistical and biometric analyses

R-mode hierarchical cluster analysis on NE Aegean coccolithophore assemblages (Fig. 5) enabled us to recognize two groups, one of which can be subdivided into three subgroups, comparable to those described by [Dimiza et al. \(2015\)](#) for the Aegean Sea region. Group I is characterized by *E. huxleyi*, *A. robusta*, *S. nodosa*, and *S. dalmaticus*. Group II consisted of three subgroups (Fig. 5).

The biometric analyses of *E. huxleyi* coccoliths from Aegean waters and Black Sea sediment trap samples (Fig. 6), showed consistent difference in relative tube width among different sample sets. Specimens from the Black Sea (bearing stable morphometric features year round), the NE Aegean stations AMT6,7 (October 2013) and other Aegean sites sampled during the warm season, presented unimodal distribution with low values (ranging between ~ 0.10 - 0.20 ; Fig. 6a). Specimens from all the investigated winter-early spring Aegean sites including stations AMT2, 3 (20-75 m depth, March 2014; Figs. 6b, 7a) and stations AMT6, 7 (3-75 m; Figs. 6b, 7a) had

an unimodal distribution shifted to higher values (ranging between ~0.20-0.50). In contrast, samples from stations AMT2, 3 (<20 m depth) during March 2014 were characterized by a bimodal distribution (peaks at ~0.10 and ~0.40; Figs. 6b, 7a).

The salinity profiles of the area revealed the presence of a permanent halocline that separates BSW and LW at ~20 m depth, with the exception of stations AMT6, 7, that were totally mixed, in March 2014 (Fig. 1c). In accordance, the percentage of the total standing stock for each of the clustered species Groups was estimated for the surface low salinity BSW layer (3-20 m) and the deeper LW layer (20-75 m) (Fig. 7b).

Coccolithophore assemblages were compared to physico-chemical parameters using the Spearman's correlation coefficient (Table 2). *Emiliania 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. Out of Group II, only *S. molischii*, *S. protrudens* and *S. histrica* HOL are negatively correlated with temperature; in contrast, the remaining species of Group II are featured by an opposite pattern. 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* were negatively correlated with depth, while *A. robusta* HOL, *H. vercellii* and *S. amoena* HOL displayed significant negative values with salinity.

4. Discussion

4.1. *Emiliania 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). More recently, different views have been expressed suggesting factors other than carbonate chemistry to affect coccolith calcification degree (Smith et al., 2012; [Berger et al., 2014](#); Young et al., 2014).

In the temperate 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; the size increase of both coccospheres and 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 with 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 that both in October 2013 and July 2014 the NE Aegean assemblage was dominated by lightly calcified morphotypes (Fig. 6a), although very rare in the latter, as already documented for the warm season Aegean Sea *E. huxleyi* populations within the LW masses ([Triantaphyllou et al., 2010](#)). Similar morphotypes are the dominant element of Black Sea coccolithophore assemblage year round (e.g., Triantaphyllou et al., 2014). On the contrary, in specimens from Aegean winter waters under maximum BSW influence, relative tube width values were characterized by bimodal distribution in surface BSW-dominated waters and unimodal distribution, dominated by higher values in the underlying LW layers (Figs. 6b, 7a). The high values of relative tube width are hence corresponding to overcalcified *E. huxleyi* coccoliths that are recorded all over the Aegean Sea in LW mass during the winter period ([Triantaphyllou et al., 2010](#)).

Obviously, 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 the Aegean specimens. Culture experiments and genetic analysis are needed to test the reliability of both hypotheses and test any association of the different morphotypes with the carbonate chemistry of the Aegean Sea water masses.

4.2 Coccolithophore assemblages in the different NE Aegean water masses

In October and July, *E. huxleyi* abundances decreased with increasing depth (Fig. 3), 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 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. 1c), associated with the dominance of Group I (Fig. 7b). Notably, a ‘fresher’ BSW mass of even lower salinity (32) was recorded in the first few meters of the water column around station AMT6 (Fig. 1c) with the highest coccolithophore total standing stock (13×10^3 coccospheres l^{-1} ; sample AMT6-3 m; Fig. 3), dominated by *E. huxleyi* (Fig. 3) lightly calcified morphotypes similar to those of Black Sea waters (Figs. 6a, 7a). 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) constructed a low abundance-high diversity assemblage (Figs. 2-4) that inhabited the BSW-influenced upper 20 m of the water column (Figs. 1c, 7b). However, both Groups IIa and IIb showed significantly higher absolute abundances below 20 m depth (Fig. 3). 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. 5), 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 2) 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.

Emiliania huxleyi was practically absent in the summer NE Aegean samples (Fig. 3) 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](#)) and other summer water samples ([Dimiza et al., 2008, 2015](#)). 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](#), this issue), 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., this issue](#)). 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. 7b), all studied stations in NE Aegean displayed elevated concentrations of *E. huxleyi* (Fig. 3), along with positive correlations with nutrients (Fig. 4; Table 2). 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. 1c; [Lagaria et al., 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. 7b). In contrast, stations AMT2 and AMT3 north of Limnos Island, exhibited a surficial thin (~10 m) BSW influenced layer (34-36 psu, Fig. 1c). The smaller lower 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. 7b), 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-Frangou et al., 2002](#)), accounting for significant export from the surface BSW layer, thus 'fertilizing' the underlying water masses with organic matter ([Frangoulis et al., 2010](#); [Lagaria et al., this issue](#)). Extant silicoflagellate populations studied in the same samples in March 2014 ([Malinverno et al., 2016](#)), provided extra evidence of the influence of cold and low salinity BSW on organisms as they displayed high abundance and peculiar morphologies of *Stephanocha speculum*, with skeletal characters reported so far only from the cold high-latitudes in both hemispheres but also recovered from Black Sea waters ([Triantaphyllou et al., 2014](#)). Such morphologies were instead rare at station AMT6 that was not at all influenced by the BSW flux in March 2014.

Coccolithophore data from the Ionian Sea as also from stations KM3NET-NSR and Hermione-HAS in the south Aegean (Figs. 1a, b, Table 1) 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. 1b). Coccolithophore assemblages affected by the Atlantic waters have been previously reported for the western Mediterranean consisting of *Gephyrocapsa* species (*G. oceanica*, *G. muelleriae*, *G. ericsonii*; e.g., [Knappertsbusch, 1993](#); [Cros, 2001](#)). Recently, [Oviedo et al. \(2015\)](#) proposed the tracers for Atlantic water influx, being mostly present until ~10° E after the Sardinia Channel and in the Tyrrhenian Sea in early spring. Notably, data from the 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 the less saline MAW 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 (e.g., Triantaphyllou et al., 2014).

5. Conclusions

BSW inflow affects different parts of the NE Aegean in respect to season variations; inflow rates show strong seasonal and interannual variability, reflected on coccolithophore assemblages, which are comprised by two groups with different ecological affinities. The main conclusions of the present study in a transect of stations sampled along a pattern of varying BSW influx, can be summarized as follows:

- During autumn (October 2013), BSW was mainly present as a surface less saline and cooler lid at station AMT6 (south of Limnos Island). The coccolithophore assemblage was dominated by *E. huxleyi*. The biometric analysis of Aegean *E. huxleyi* coccoliths revealed an unimodal distribution concerning the coccolith relative tube width, with values similar to those provided by specimens from Black Sea sediment traps (peak relative tube width ranging between ~0.10-0.20). Group IIc (*U. tenuis* and *S. pulchra*) marked the assemblage reflecting the influence of LW masses.
- In early spring (March 2014), BSW was mostly influencing northern stations AMT2 and AMT3 <20 m depth, north of Limnos Island. All stations and both BSW and LW masses were featured by increased concentrations of Group I and especially *E. huxleyi* that showed positive correlation with nutrients associated with significant export from the surface BSW layer. The biometric analysis of *E. huxleyi* coccoliths proved that the stations with increased BSW influx (AMT2, AMT3 <20 m) displayed a bimodal pattern of relative tube width; the distribution becoming unimodal and dominated by larger values in the LW water layers (AMT2, AMT3 20-75 m; AMT6, AMT7 3-75 m). The lower relative tube width values found mostly in the surface layers are well comparable to the Black Sea *E. huxleyi* measurements (peak at ~0.10).
- 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 (Groups IIa, b) 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.

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FIGURE CAPTIONS

Fig. 1 Geographical location of the stations discussed in the present study: **a.** (circle) Black Sea sediment trap site ($42^{\circ} 58'00''\text{N}$ $29^{\circ} 29'00''\text{E}$, 1000 m depth; Triantaphyllou et al., 2014) and (stars) sampled stations from the Ionian Sea (Malinverno et al., 2003). **b.** Stations in the Aegean Sea (circles: present study; stars: previous expeditions. For stations code numbers and sampling details see Table 1), and the main patterns of sea water surface circulation (BSW: Black Sea Water, LW: Levantine Water, MAW: Modified Atlantic Water). **c.** Salinity profiles conducted during the performed AegeanMarTech oceanographic cruises and the location of the studied stations (from Lagaria et al., this issue).

Fig. 2 Total density and Shannon-Wiener diversity index (H') for the investigated stations AMT2, AMT3, AMT6, AMT7 during the different seasonal samplings.

Fig. 3 Absolute abundances of *Emiliana huxleyi*, *Syracosphaeraceae*, *Rhabdosphaeraceae*, holococcolithophores and other species for the investigated stations AMT2, AMT3, AMT6, AMT7 during the different seasonal samplings.

Fig. 4 Relative abundance of the major coccolithophore species.

Fig. 5 Hierarchical cluster analysis (centroid linkage method, distance metric is 1-Pearson correlation coefficient) based on the abundances of the most important coccolithophore species.

Fig. 6 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 (3-75 m, October 2013) in respect to specimens from Black Sea (year-round) and other Aegean sites during the warm season (summer-autumn). **b.** Relative tube width bimodal distribution during the cold season (winter-spring); ranging between ~ 0.10 - 0.20 , in samples from stations AMT2, 3 (< 20 m, March 2014) and Black Sea (year-round), ranging between ~ 0.20 - 0.50 , in samples AMT2, 3 (20-75 m, March 2014), AMT6, 7 (3-75 m, March, 2014) and other Aegean sites. The larger values are corresponding to *E. huxleyi* overcalcified coccoliths that are

recorded all over the Aegean Sea within LW masses during the winter-spring period (Triantaphyllou et al., 2010). The different *E. huxleyi* morphotypes documented in the Aegean and Black Seas are shown in Plate I.

Fig. 7 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.

TABLE CAPTIONS

Table 1. 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.

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. 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. 5).

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

PLATE CAPTIONS

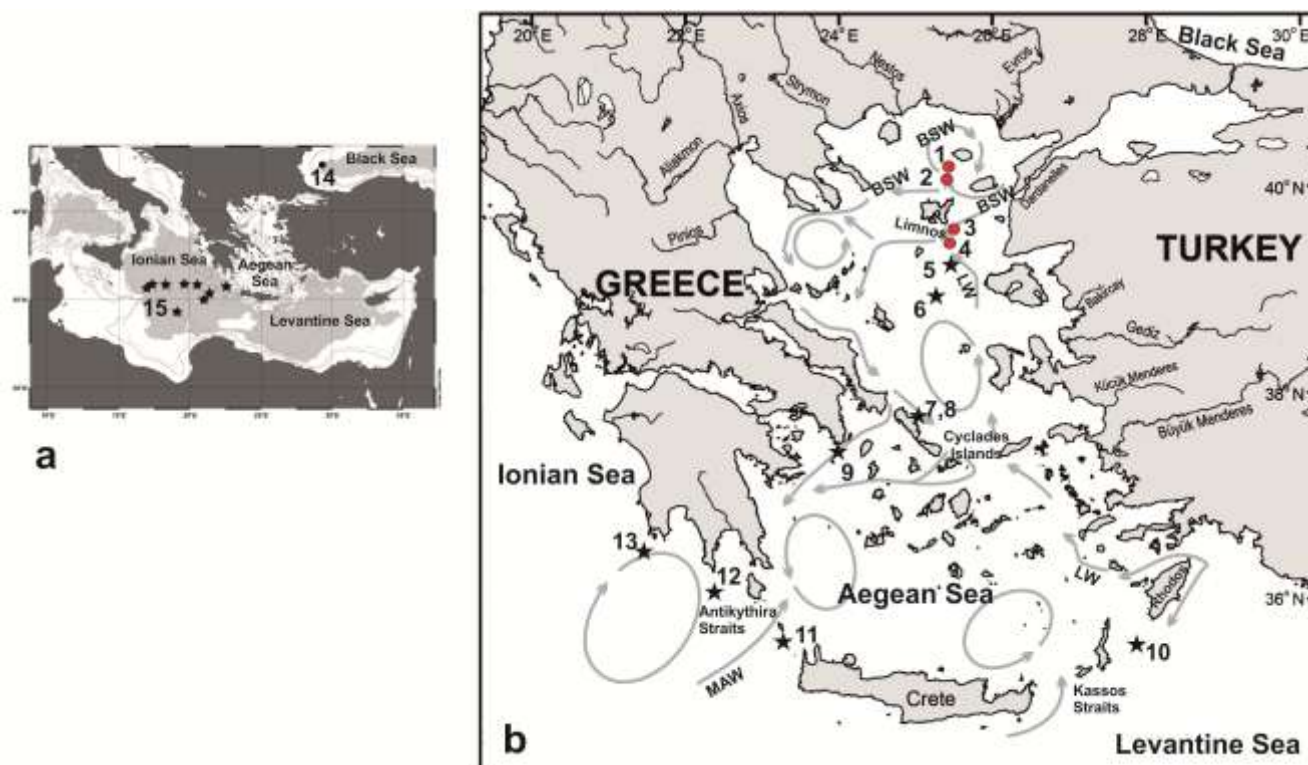
Plate 1. Figs 1, 4, 5, 8 *Emiliana huxleyi* lightly calcified morphotypes from Black Sea and Aegean Sea assemblages. **Figs 2, 3, 6, 7** *Emiliana huxleyi* overcalcified morphotypes from Aegean Sea assemblages.

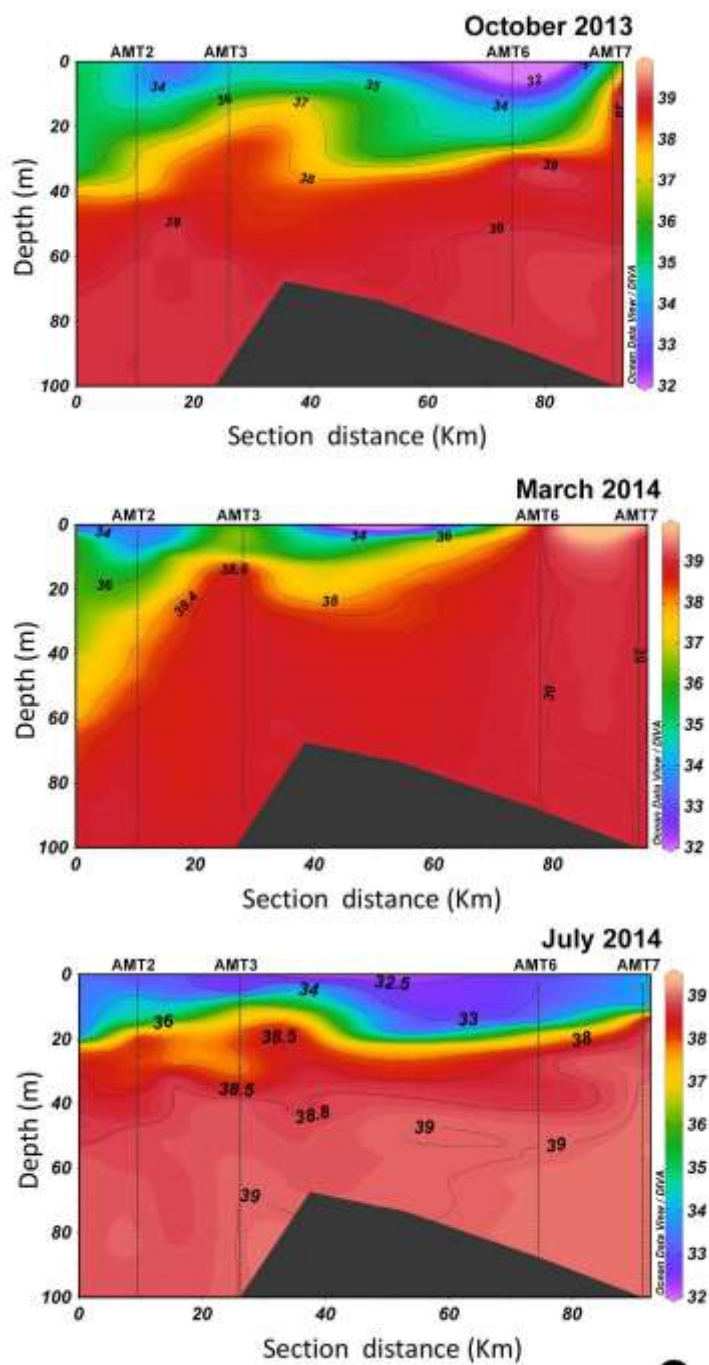
Plate 2. Group I. 1. *Emiliana huxleyi* (Lohmann) Hay and Mohler var. *huxleyi*, AMT3, 3 m, March 2014. **2.** *Algirosphaera robusta* (Lohmann) Norris, AMT3, 50 m, March 2014. **3.** *Syracosphaera nodosa* Kamptner, AMT6, 3 m, March 2014. **4.** *Syracolithus dalmaticus* (Kamptner) Loeblich and Tappan, AMT6, 10 m, March 2014. **Group IIa. 5.** *Syracosphaera pulchra* Lohmann, AMT6, 3 m, October 2013. **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.

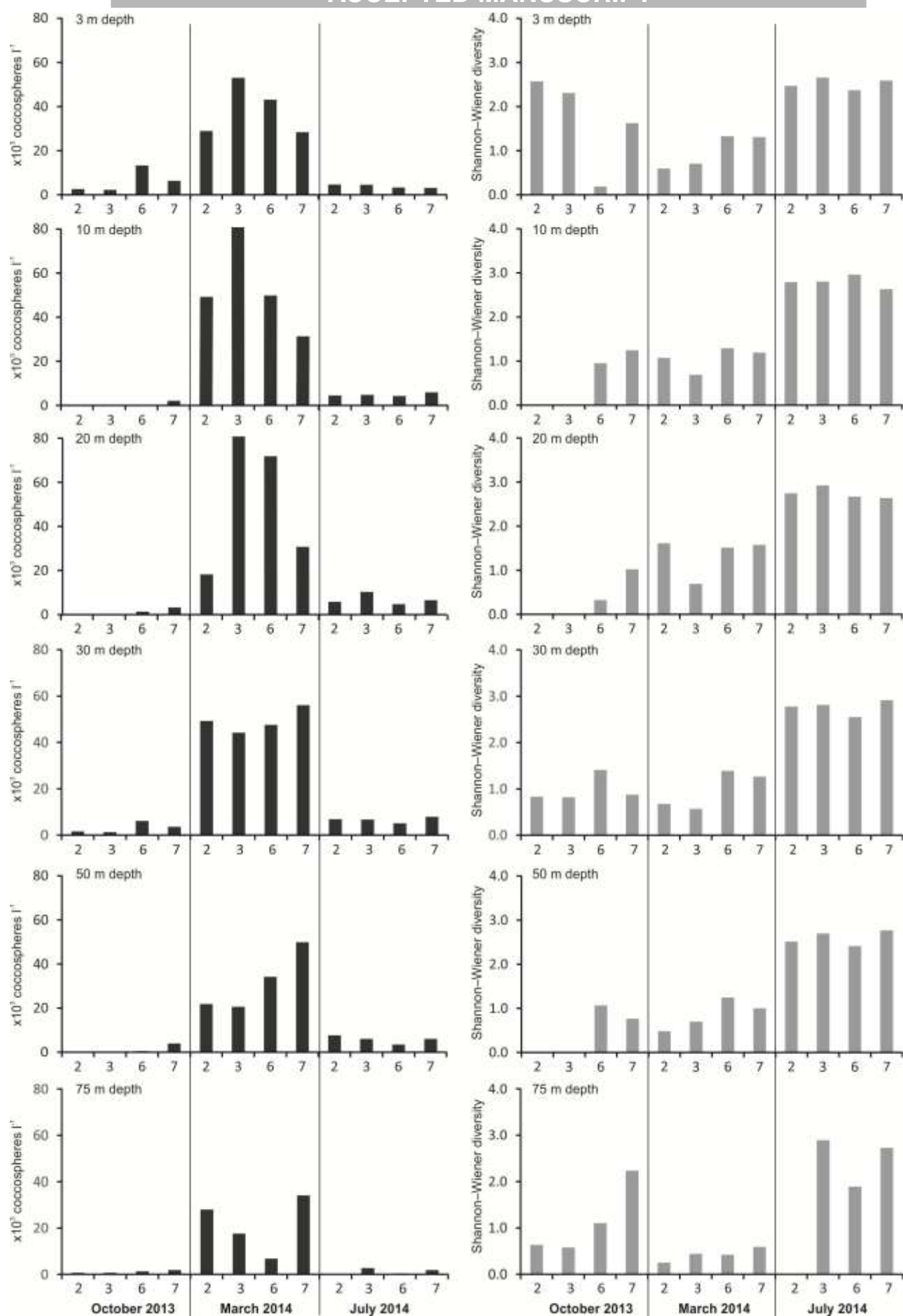
Highlights

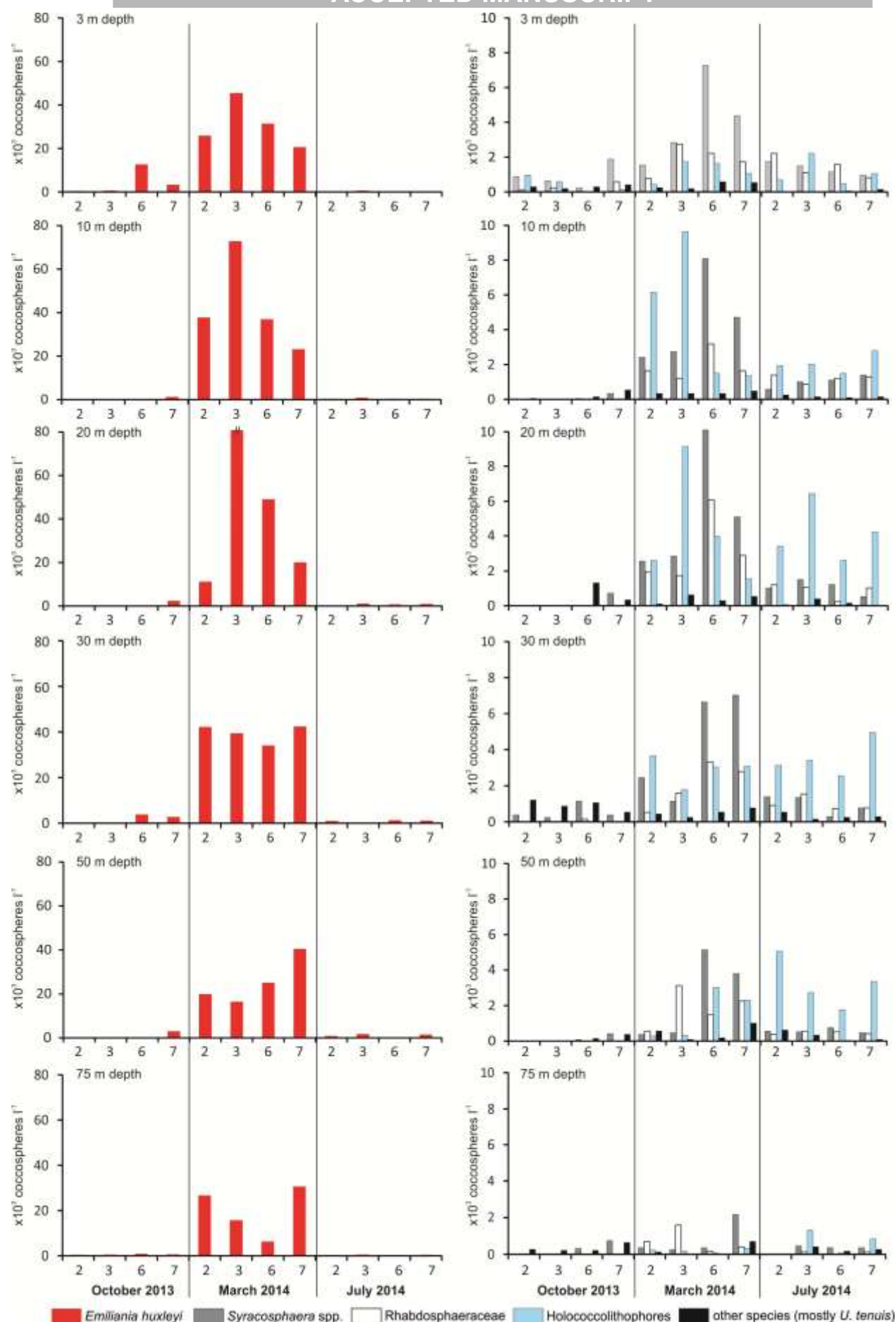
- species composition of living coccolithophore communities in the NE Aegean Sea
- spatial and temporal coccolithophore variations in the NE Aegean area, receiving the inflowing surface less saline Black Sea Water over the deeper Levantine Water layer
- biometric analyses on *Emiliania huxleyi* morphology in the NE Aegean Sea further complicate the so far documented seasonal variation in the Aegean sea coccoliths, now involving Black Sea morphotypes in addition to the Aegean specimens

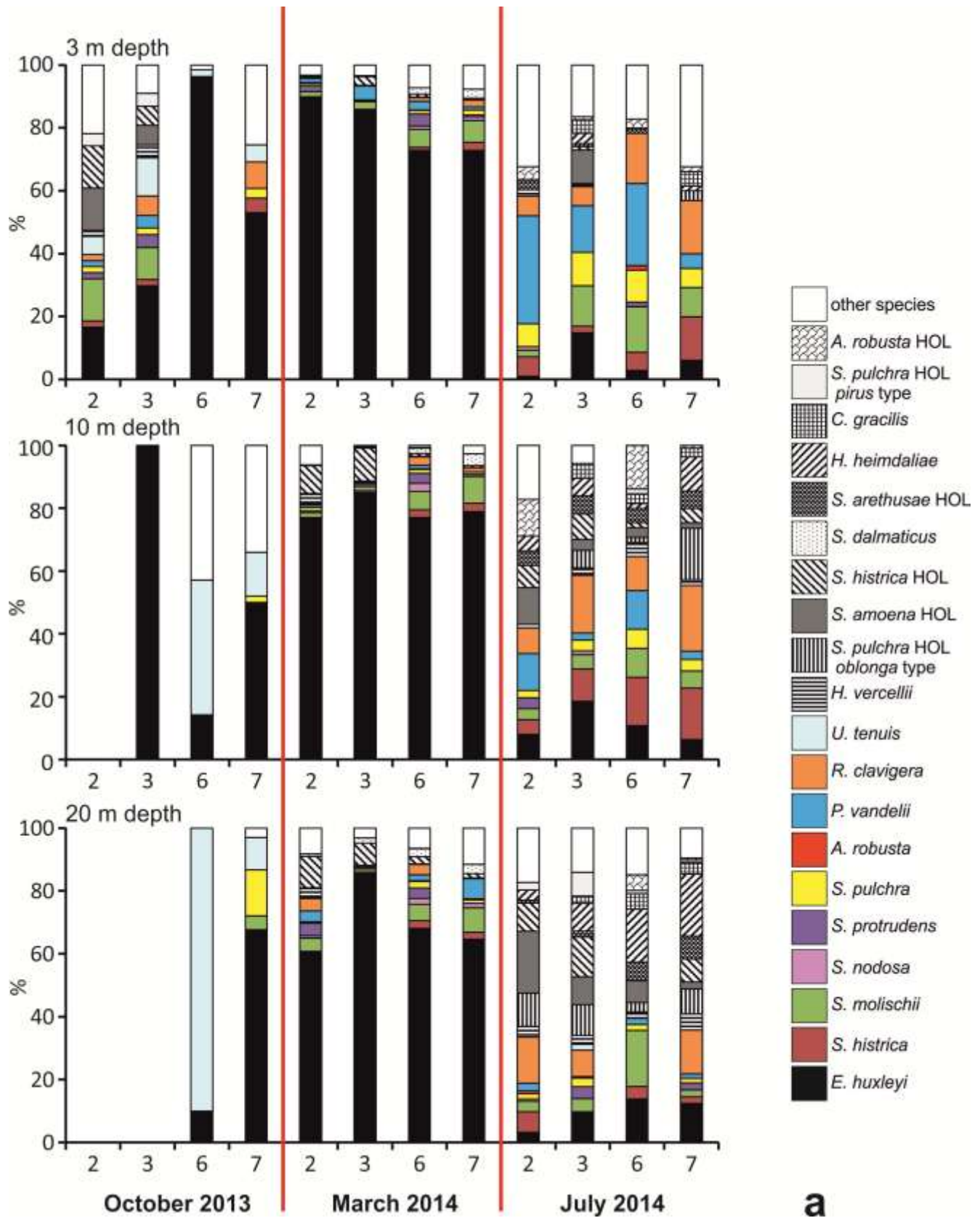


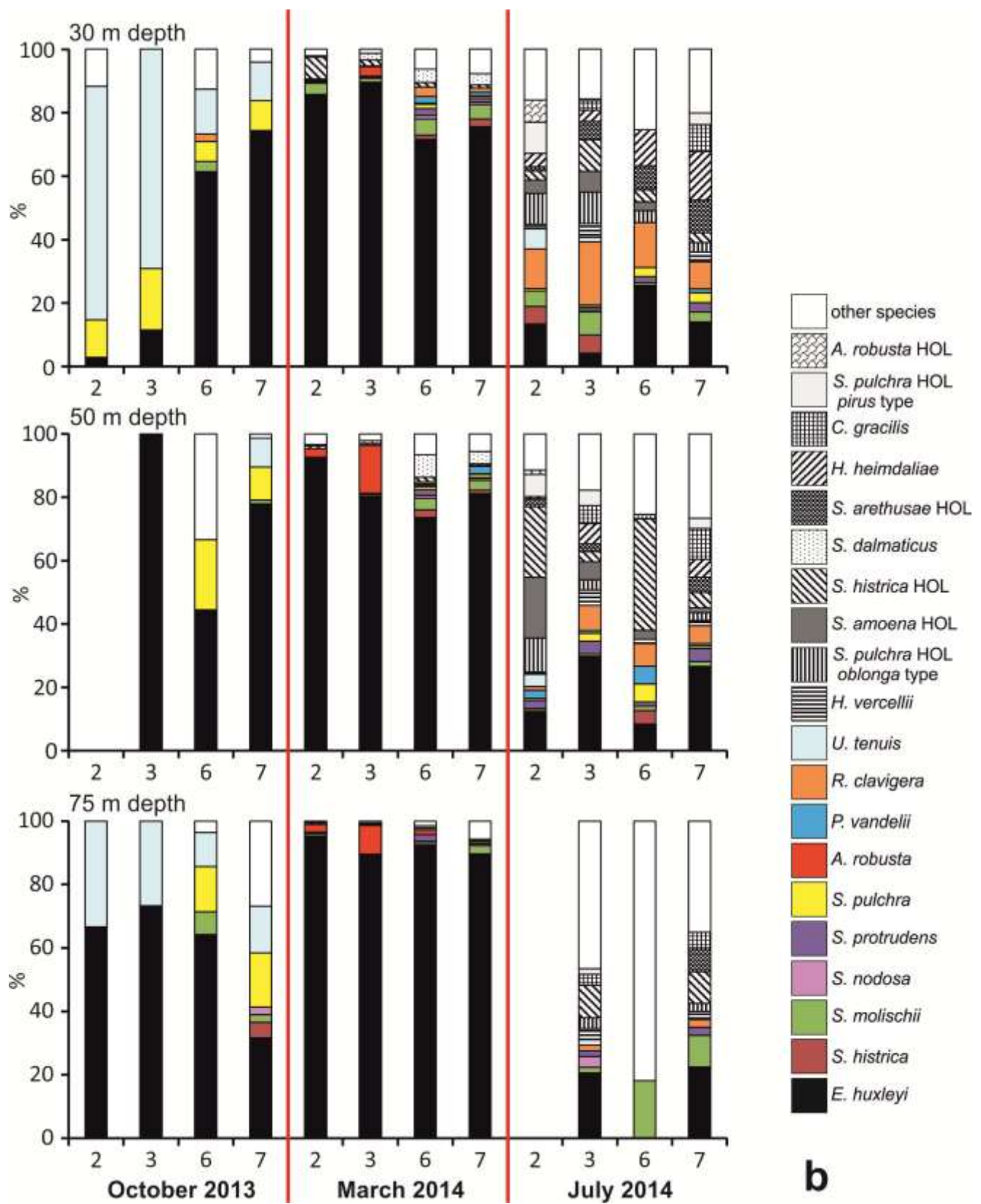


C





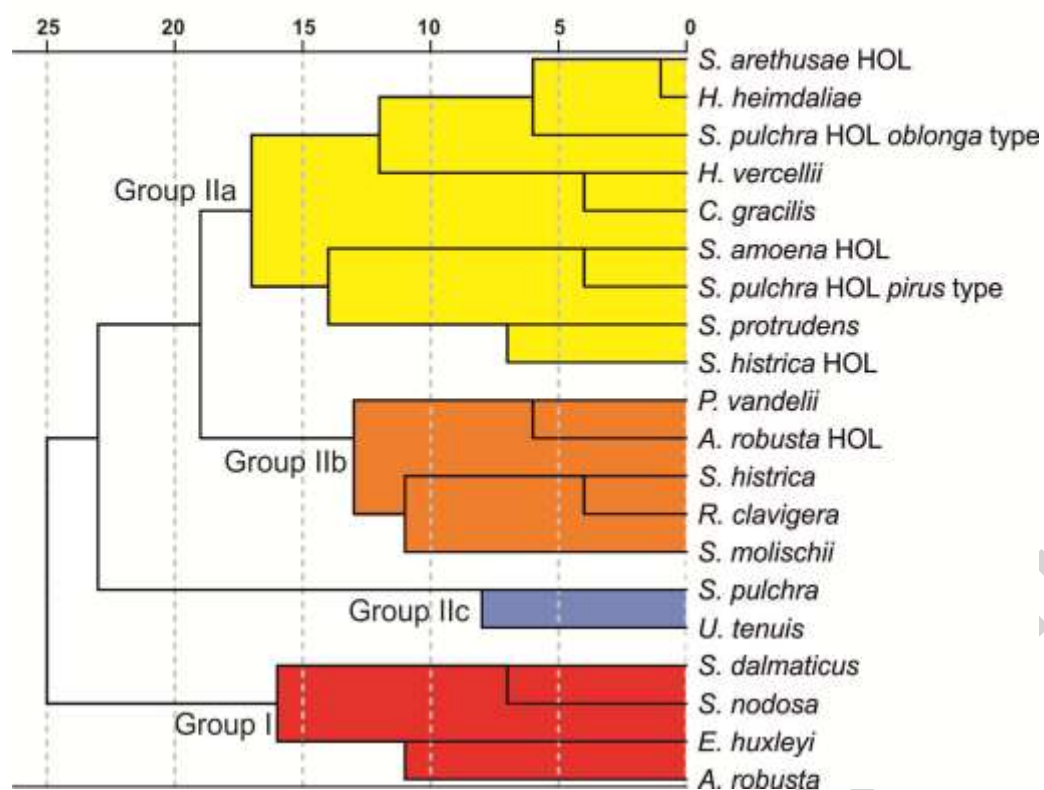


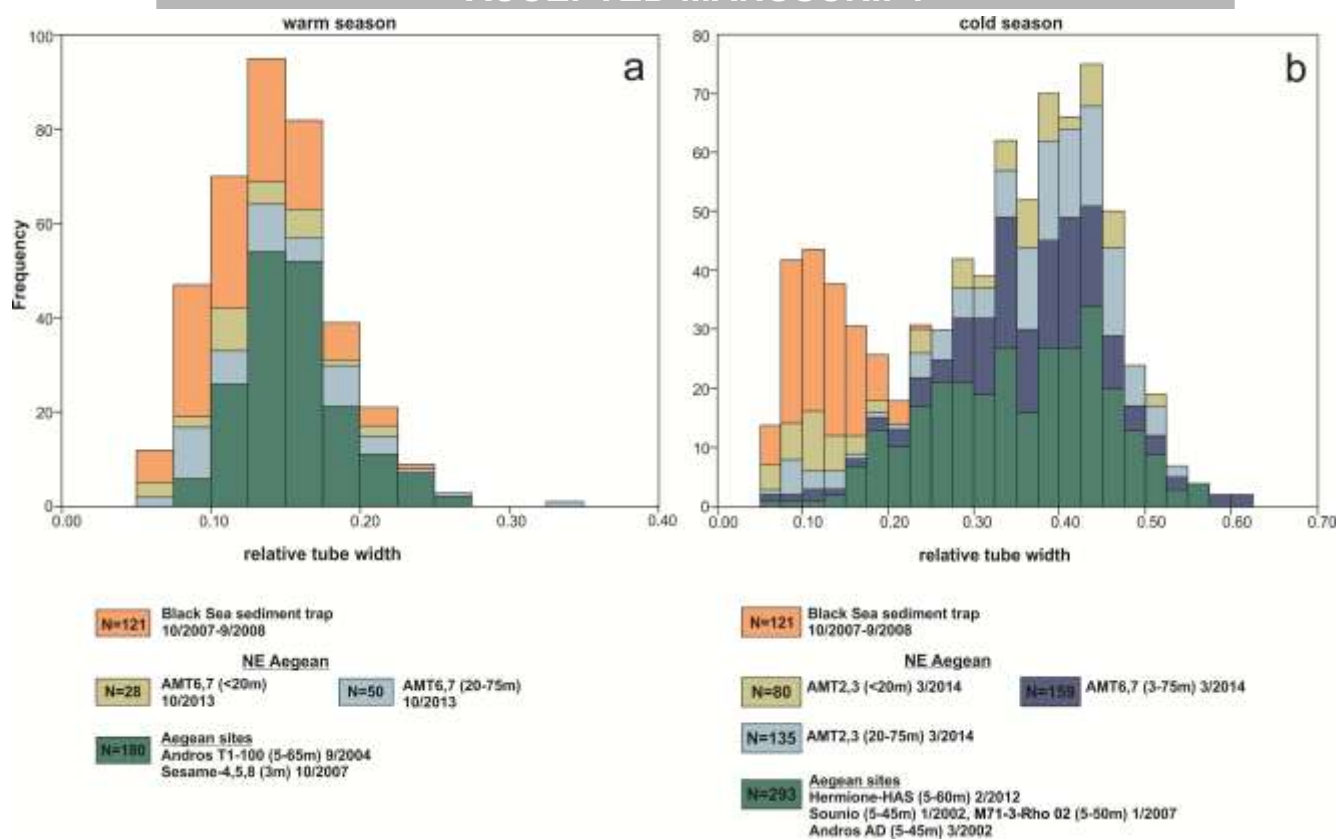


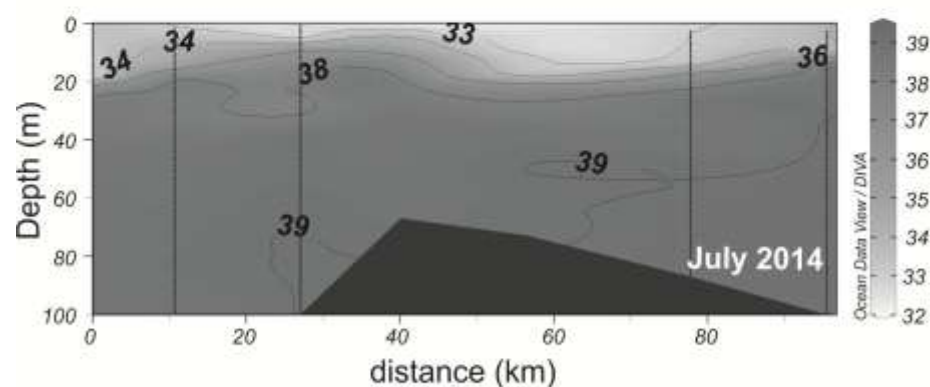
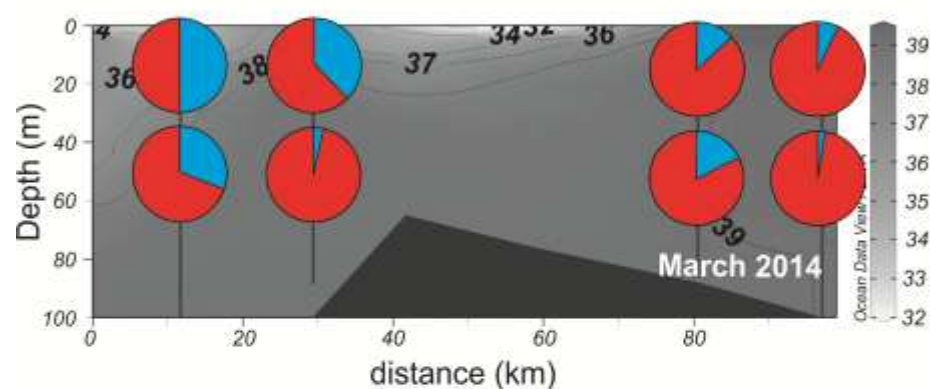
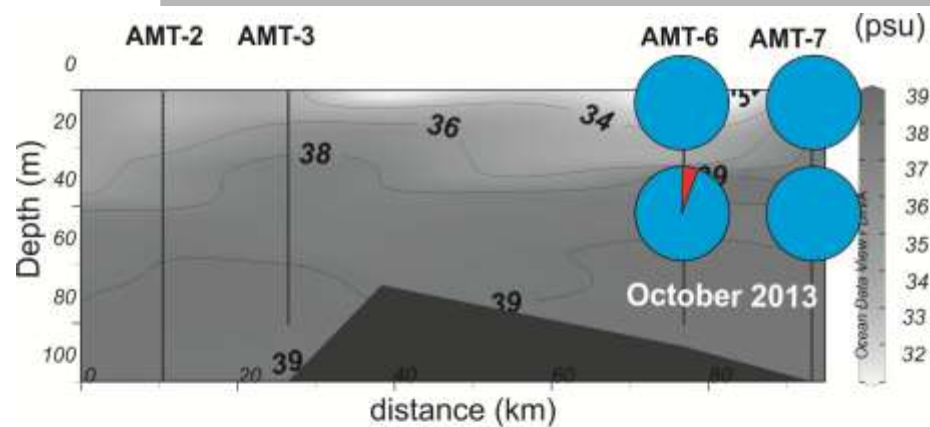
HIERARCHICAL CLUSTER ANALYSIS

Dendrogram using Centroid Linkage

Rescaled Distance Cluster Combine

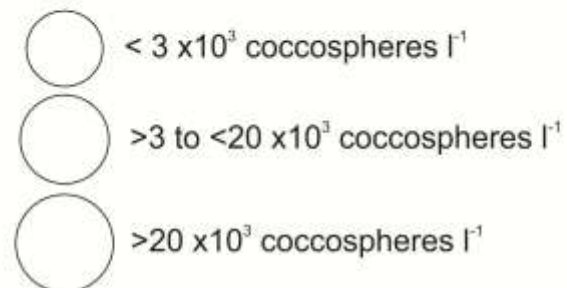
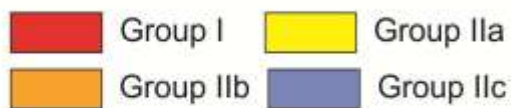
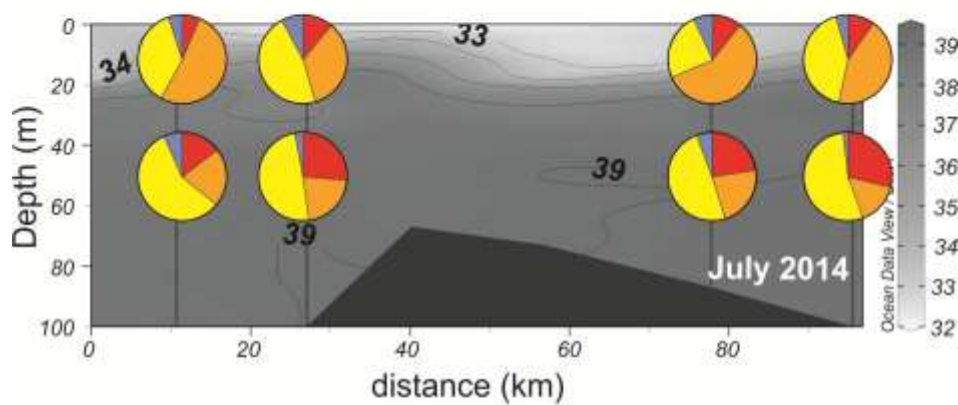
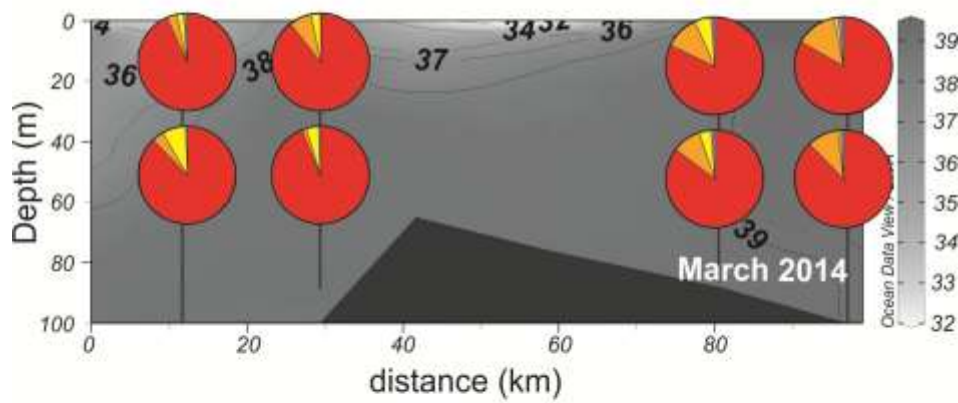
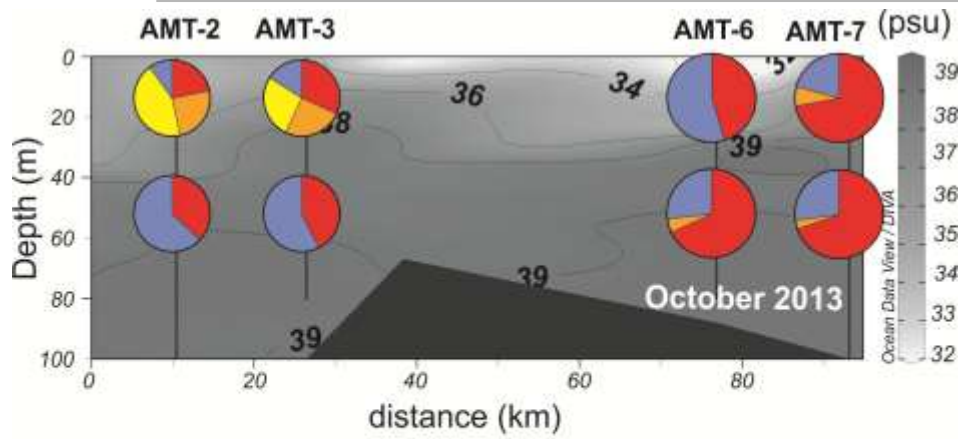






- *E. huxleyi* lightly calcified coccoliths (relative tube width <0.20)
- *E. huxleyi* overcalcified coccoliths (relative tube width between ~0.20-0.50)

a



b

