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# Coccolithophore haploid and diploid distribution patterns in the Mediterranean Sea: can a haplo-diploid life cycle be advantageous under climate change?

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Coccolithophores are unicellular pelagic algae, capable of calcification. In the Mediterranean Sea, several species have a well-known haplo-diploid life cycle, alternating the production of different types of calcite plates, the holo- and hetero-coccoliths. We analyzed the distribution of both phases along a W-E Mediterranean transect during April 2011 and May 2013 (spring season), following strong environmental gradients in salinity, oxygen and nutrient concentration, temperature, carbonate chemistry and fluorescence. The proportion of holococcolithophores:heterococcolithophores of selected species varies not only vertically through the water column, but also longitudinally, following the main environmental gradients. Based on the environmental affinities of the coccolithophore life phases, we conclude that a dimorphic life cycle might provide the ability to adapt to the south-eastern (SE) Mediterranean environment, in conditions characterized by surface water with relatively high calcite saturation state, high temperature, stratification and nutrient limitation, and support the survival of species whose diploid phases are in contrast adapted to Atlantic or south-western (SW) Mediterranean conditions. Thus, a haplo-diploid life cycle could provide a way to adapt to environmental changes.

**KEYWORDS:** coccolithophore; holococcolithophore; Mediterranean; warming; acidification

## INTRODUCTION

Coccolithophores are unicellular pelagic calcifying phytoplankton belonging to the phylum Haptophyta and the class Prymnesiophyceae. The products of their calcification consist of minute and elaborated calcite plates, known as coccoliths, which constitute the cell exoskeleton (coccosphere). Coccolithophores are usually outcompeted by other groups of phytoplankton; however, they can occasionally dominate when a bloom of *Emiliania huxleyi* occurs, in either eutrophic or oligotrophic oceanic regions (Tyrrell and Merico, 2004; Lessard *et al.*, 2005). They have a poorly documented life cycle, characterized by the secretion of two different types of coccoliths (heterococcoliths and holococcoliths): the correspondent life phases were originally considered different species. The discovery of their alternation was first detected in *Coccolithus pelagicus*, having a motile and a non-motile stage (Parke and Adams, 1960), with heterococcoliths produced during the diploid phase, and holococcoliths during the haploid phase (Billard, 1994). Thought to be a transition between the two life phases (Geisen *et al.*, 2004), the presence of both stages on a single coccosphere cell has been repeatedly found in natural populations, especially in the Mediterranean Sea (Kleijne, 1991; Thomsen *et al.*, 1991; Samtleben and Schröder, 1992; Alcober and Jordan, 1997; Cortés, 2000; Cros *et al.*, 2000; Cortés and Bollmann, 2002; Cros and Fortuño, 2002; Geisen *et al.*, 2002; Triantaphyllou and Dimiza, 2003; Triantaphyllou *et al.*, 2004, 2009, 2014; Malinverno *et al.*, 2008; Frada *et al.*, 2009; Triantaphyllou, 2010; Karatsolis *et al.*, 2014; Šupraha *et al.*, 2014). The haplo-diploid nature of hetero-holococcolith alternation was later confirmed, through DNA content analysis by flow-cytometry, for four coccolithophore species (Houdan *et al.*, 2004). The triggers behind coccolithophore phase change are still under discussion. It may be stimulated by exposure to extreme environmental conditions and guarantee the survival of coccolithophore species in a changing oligotrophic habitat (Valero *et al.*, 1992; Cros *et al.*, 2000; Houdan *et al.*, 2005, 2006; Cros and Estrada, 2013). Holococcolithophores (HOLs) are especially abundant in the upper photic zone of subtropical oceans (Winter *et al.*, 1979; Kleijne, 1991). Based on their distribution, it has been hypothesized that this phase exhibits a preference for warm, oligotrophic, stratified waters (Cros and Estrada, 2013) and in fact they are common in the Mediterranean Sea during summer (Dimiza *et al.*, 2008; Malinverno *et al.*, 2009; Šupraha *et al.*, 2016). Only a few previous studies have focused on the distribution of two life phases of the same coccolithophore species (Cros and Estrada, 2013; Dimiza *et al.*, 2015; Šupraha *et al.*, 2016). In the last decades, sea surface temperature has been increasing in the Mediterranean Sea (Belkin, 2009; Calvo *et al.*, 2011), in

association with water column stratification and a deepening of the thermocline (Lejeune *et al.*, 2010). Moreover, since the beginning of the industrial era, anthropogenic CO<sub>2</sub> released into the atmosphere has been sequestered by surface waters, contaminating the whole basin and lowering the pH by 0.055–0.156 units (Hassoun *et al.*, 2015a). Such changes are expected to impact the marine community, including coccolithophores, but the response involves complex mechanisms on multiple trophic layers (Portner *et al.*, 2014). The environmental control on haploid and diploid phases in coccolithophores is still largely unknown and field studies can shed light on their ecology. The present work is the first attempt to characterize the distribution of HOLs along a large biochemical and physical gradient in the Mediterranean Sea, in order to underpin the potential triggers of coccolithophore life phase change in the natural environment. General observations, based on seven selected species (*Calcidiscus leptoporus*, *Coccolithus pelagicus*, *Coronosphaera mediterranea*, *Syracosphaera bannockii*, *S. histrica*, *S. molischii*, *S. pulchra*), were reinforced analyzing the distribution of both life phases of *Coccolithus pelagicus* subsp. *braanudii*, *Calcidiscus leptoporus*.

## Oceanographic setting

This study focuses on data collected during two oceanographic cruises, in April 2011 and May 2013 in the southern basins of the Mediterranean Sea, including the Gibraltar Strait, the Alboran Sea, the southern Alguero-Balearaic Basin, the Sicily Strait, the southern Ionian Sea and the Levantine Basin. The Mediterranean is a semi-enclosed basin, connected to the Atlantic Ocean and the Black Sea through respectively the Gibraltar and the Dardanelles Straits; and artificially to the Red Sea through the Suez Canal. The main water exchange occurs in the west, through the Gibraltar Strait, where the low-salinity and nutrient-rich Atlantic waters enter the Mediterranean. The Atlantic waters flow superficially, due to their low density, and propagate towards the Levantine Basin, gradually losing nutrients and increasing their salinity and temperature (Wüst, 1961; Malanotte-Rizzoli and Hecht, 1988). The Mediterranean is characterized by strong longitudinal environmental gradients (Crombet *et al.*, 2011; Ribera d'Alcalà, 2003; Skliris, 2014; Tanhua *et al.*, 2013a, 2013b). In April 2011 and May 2013, waters in the south-eastern basins were much warmer, saltier and oligotrophic than those in the south-western basins (Oviedo *et al.*, 2015), see also supplementary figures online, Fig. S1, S2. Carbonate ion (CO<sub>3</sub><sup>2-</sup>) concentration and pH (Fig. S3), as well as total alkalinity, were all higher in the Eastern Mediterranean during both cruises (Tanhua *et al.*, 2013; Álvarez *et al.*, 2014; Gemayel *et al.*, 2015; Oviedo *et al.*, 2015; Hassoun *et al.*, 2015b).

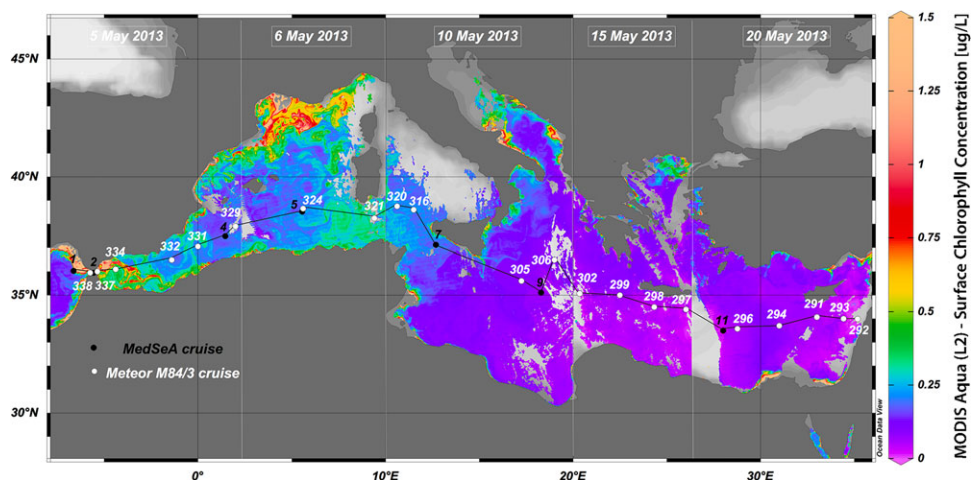
Finally, an eastward deepening of the Deep Chlorophyll Maximum (DCM) and of the 0.1% limit of PAR was observed during the 2013 MedSeA cruise (Fig. S4).

## METHOD

Twenty-seven oceanographic stations, distributed along a west-east (W-E) transect extending between 36.03°N–6.64°W and 33.99°N–35.17°E, were considered in this study (Fig. 1, Table S1). Such stations were surveyed during the Meteor M84/3 (6–28 April 2011) and the 2013 MedSeA (1–31 May 2013) cruises. A profiling CTD (Sea-Bird Electronics 991) recorded temperature, salinity, fluorescence and dissolved oxygen concentration for each station during both cruises. Data relative to the 2013 MedSeA cruise CTD data can be downloaded from PANGAEA (Ziveri and Grelaud, 2015). Nutrient concentrations during this last cruise were measured with a Bran + Luebbe3 AutoAnalyzer, according to Grasshoff *et al.* (1999), after onboard filtration through glass fiber filters (Whatman GF/F; 0.7  $\mu\text{m}$ ) and storage at  $-20^{\circ}\text{C}$ . Detection limits were 0.02 and 0.01  $\mu\text{M}$  for nitrate plus nitrite ( $\text{NO}_3^- + \text{NO}_2^-$ ) and phosphate ( $\text{PO}_4^{3-}$ ) concentrations, respectively. The quality of the nutrient measurements was confirmed by the results of the inter-comparison exercises (QUASIMEME). Total alkalinity and dissolved inorganic carbon data for this same cruise are available in PANGAEA (Goyet *et al.*, 2015). CO2Sys (Lewis and Wallace, 1998) was used to calculate the remaining variables of the carbonate system: equilibrium constants (Mehrbach *et al.*, 1973) were refitted (Dickson and Millero, 1987; Álvarez *et al.*, 2014). The methods used during the M84/3 cruise to obtain nutrient and carbonate system

variables have been previously described (Tanhua *et al.*, 2013; Rahav *et al.*, 2013; Oviedo *et al.*, 2015); all CTD and bottle data regarding this cruise are also available online (<http://cchdo.ucsd.edu/cruise/06MT20110405>; [http://cdiac.ornl.gov/oceans/Coastal/Meteor\\_Med\\_Sea.html](http://cdiac.ornl.gov/oceans/Coastal/Meteor_Med_Sea.html)).

A total of 107 seawater samples were analyzed for coccolithophores. Sample depth ranged between 0 and 175 m (as described in Table S1). During both cruises, seawater was collected with Niskin bottles; 2.5–5 l were filtered per sample on cellulose acetate-nitrate filters (Millipore,  $\varnothing$  47 mm, 0.45  $\mu\text{m}$ ) using a hydraulic vacuum pump (Eyela, A-1000S), and oven-dried at  $40^{\circ}\text{C}$  for 8–12 h. From each phytoplankton sample, a piece of filter was radially cut, mounted on a stub and gold-coated, before analysis at 3000 $\times$  magnification with a Zeiss EVO MA 10 scanning electron microscope. A minimum of 80–100 coccospheres (maximum 420) were counted per sample, along a longitudinal transect of at least 5 mm, for an average of 4.1 ml of water. Heterococcospheres and holococcospheres were identified down to species level (Cros and Fortuño, 2002; Young *et al.*, 2003), based also on the Nannotax3 website (<http://ina.tmsoc.org/Nannotax3/>). Upper and lower confidence limits were calculated (Bollmann *et al.*, 2002); corresponding errors oscillated between 149–1258 cells  $\text{l}^{-1}$  for samples containing, respectively,  $5.6 \times 10^3$  and  $4.1 \times 10^5$  cells  $\text{l}^{-1}$ . Only values that exceeded the maximum counting error per sample were subsequently considered for statistical analysis and interpretation. Coccolithophore absolute abundance data concerning the M84/3 cruise were previously published (Oviedo *et al.*, 2015). In order to express the prevalence of the HOL phase, we used the HOLP index (Cros and Estrada, 2013):  $\text{HOLP} = 100 \times (\text{Total HOLs}/\text{Total}$



**Fig. 1.** Surface Chlorophyll Concentration during May 2013, at the time of the MedSeA cruise. Superimposed are the Meteor M84/3 and MedSeA stations considered for this work.

HHLC), where Total\_HHLC is the number of coccolithophores (of both life phases) that belong to species with an established life cycle, excluding those species with a potentially non-calcified haploid phase (e.g. *E. huxleyi*). In the calculation of the HOLF index were included the most abundant holo-heterococcolithophore species, listed in Table S2. The HOLF index can vary between values of 0 (absence of HOLF) and 100 (absence of heterococcolithophores).

PAST 3.14 (Hammer *et al.*, 2001) was used to perform all statistical analysis. First, in order to characterize the coccolithophore life cycle phases along the transect, we performed a hierarchical cluster analysis on the 2013 MedSeA and M84/3 cruise stations. The analysis was constrained by longitude, and based on the average HOLF index calculated between 5 and 100 m, excluding a few stations for which only one sample depth was available. Based on the dendrogram of the cluster analysis (Fig. 2), we assigned the 2013 MedSeA and M84/3 cruise stations to the SW or to the SE Mediterranean (Figs 3, 5, 6). The mean coccolithophore abundances and HOLF indices in the SW and SE Mediterranean were compared by *t*-test; means were considered statistically different at *P*-values  $\leq 0.05$ .

Then, the biological and environmental data were compared through Spearman correlation analysis. A first analysis was run between the total HOLF population, the HOLF index and the environmental parameters (Table I). A second

analysis was run between the absolute abundance of seven selected species, for both the HOLF and heterococcolithophore (HET) forms (*Calcidiscus leptoporus*, *Coccolithus pelagicus*, *Coronosphaera mediterranea*, *Syracosphaera bannockii*, *S. histrica*, *S. molischii* and *S. pulchra*) and the environmental parameters (Table II). Those species were selected for having an established taxonomy (both hetero- and holococcoliths forms are known) and a high average relative abundance in our samples (Table S2). Correlation coefficients (*r*) with *P*-values  $\leq 0.05$  were considered statistically significant. Abundance data for all species are available on PANGAEA (doi: 10.1594/PANGAEA.875202; 10.1594/PANGAEA.875924).

## RESULTS

### Absolute abundances and HOLF index

During the two W-E trans-Mediterranean samplings in 2011 and 2013, the total coccolithophore concentrations in the upper 100 m (including both HOLF and heterococcolithophore specimens, Fig. 3) ranged between  $5.7 \times 10^3 \text{ cells l}^{-1}$  and  $4.1 \times 10^5 \text{ cells l}^{-1}$ . The lowest values were recorded at station 5 (southern Alguero-Balearic Basin, May 2013), while the highest values were recorded at station 337 (Gibraltar Strait, April 2011). The mean coccolithophore concentration in the first 100 m of the water column was significantly (*t*-test *P* = 0.04) higher in the SW than in the SE Mediterranean stations. Heterococcolithophores were the largely dominating coccolithophore life phase; their distribution drove the total coccolithophore population (Fig. 3) and they were recorded in all samples from 0 to 175 m depth. Minimum and maximum abundances in the upper 100 m were similar to those of the total population ( $5.7 \times 10^3 \text{ cells l}^{-1}$ ;  $3.2 \times 10^5 \text{ cells l}^{-1}$ ). Their mean concentration in the first 100 m of the water column was significantly (*t*-test *P* = 0.02) higher in the SW than in the SE Mediterranean stations. HOLF had a patchy distribution, but were particularly abundant between 0 and 50 m depth (Fig. 3). Their highest concentration was recorded at station 337 ( $8.6 \times 10^4 \text{ cells l}^{-1}$ , April 2011). Their mean concentration was higher in the SW than in the SE Mediterranean, but the difference was not statistically significant (*t*-test *P* = 0.52). Overall,

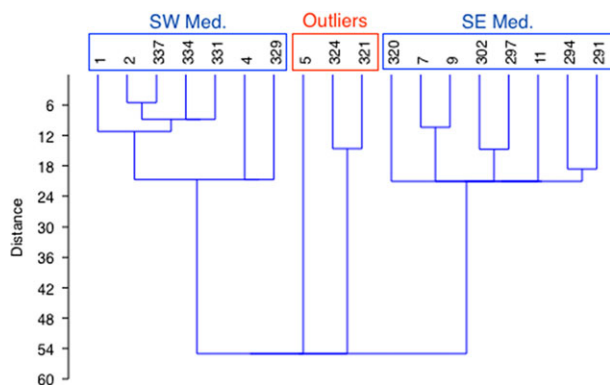


Fig. 2. Dendrogram from the cluster analysis of MedSeA and M84/3 stations, based on the average HOLF index between 5 and 100 m.

Table I: Environmental correlations of absolute total HOLF abundance (Tot. HOLF; N = 97) and of the HOLF index (N = 94)

	T	Sal	NO <sub>x</sub>	PO <sub>4</sub> <sup>3-</sup>	pH	pCO <sub>2</sub>	CO <sub>3</sub> <sup>2-</sup>	PAR%
Tot. HOLF	<b>**0.35</b>	0.00	<b>**−0.45</b>	<b>**−0.40</b>	<b>**−0.26</b>	<b>**−0.25</b>	<b>**0.26</b>	<b>**0.66</b>
HOLF index	<b>**0.41</b>	0.03	<b>**−0.52</b>	<b>**−0.44</b>	−0.14	−0.11	<b>**0.30</b>	<b>**0.68</b>

PAR% values were available only for the MedSeA cruise data set (N = 41 in relation to Tot. HOLF; N = 38 in relation to HOLF). Significant Spearman correlation coefficients are in bold (\**P*  $\leq 0.05$ , \*\**P*  $\leq 0.01$ ). NO<sub>x</sub> stays for the sum of [NO<sub>2</sub><sup>-</sup>] and [NO<sub>3</sub><sup>-</sup>].



Table II: Environmental correlations of selected species, calculated for the absolute abundance of each phase

	T	Sal	NO <sub>x</sub>	PO <sub>4</sub> <sup>3-</sup>	pH	pCO <sub>2</sub>	CO <sub>3</sub> <sup>2-</sup>	PAR%
<i>C. leptopus</i> HOL	<b>*0.25</b>	<b>**0.31</b>	<b>**−0.30</b>	−0.17	0.01	−0.11	<b>**0.34</b>	0.28
<i>C. leptopus</i> HET	−0.17	<b>**−0.28</b>	<b>**0.29</b>	<b>**0.35</b>	−0.19	0.15	<b>**−0.38</b>	−0.25
<i>C. pel.subsp.braarudii</i> HOL	0.01	<b>**−0.39</b>	−0.10	0.03	−0.07	<b>**−0.27</b>	−0.15	0.31
<i>C. pel.subsp.braarudii</i> HET	<b>*−0.21</b>	−0.05	<b>**0.25</b>	<b>**0.26</b>	0.13	<b>**0.26</b>	<b>*−0.25</b>	−0.24
<i>C. mediterranea</i> HOL	0.02	−0.11	<b>**−0.28</b>	−0.09	−0.23	<b>**−0.23</b>	0.04	<b>**0.45</b>
<i>C. mediterranea</i> HET	0.12	−0.13	−0.15	0.04	−0.15	−0.01	−0.01	0.28
<i>S. bannockii</i> HOL	0.19	<b>*−0.25</b>	0.09	0.00	0.12	0.01	−0.05	<b>**0.51</b>
<i>S. bannockii</i> HET	−0.09	−0.05	−0.08	−0.07	0.04	<b>*−0.20</b>	0.02	−0.05
<i>S. histrica</i> HOL	0.03	−0.06	−0.05	−0.10	0.12	−0.14	0.05	0.16
<i>S. histrica</i> HET	0.11	0.20	<b>*−0.22</b>	−0.14	0.10	−0.18	<b>*0.23</b>	0.09
<i>S. molischii</i> HOL	0.12	<b>**0.29</b>	<b>**−0.32</b>	<b>**−0.41</b>	<b>**−0.32</b>	−0.17	<b>**0.27</b>	0.20
<i>S. molischii</i> HET	<b>**−0.31</b>	<b>**−0.32</b>	<b>*0.20</b>	<b>**0.26</b>	−0.15	−0.03	<b>**−0.36</b>	−0.07
<i>S. pulchra</i> HOL	<b>**0.26</b>	0.19	<b>**−0.57</b>	<b>**−0.59</b>	−0.09	<b>**−0.25</b>	<b>**0.35</b>	<b>**0.47</b>
<i>S. pulchra</i> HET	<b>**0.26</b>	<b>**0.31</b>	<b>**−0.36</b>	<b>**−0.32</b>	<b>*0.23</b>	<b>**−0.35</b>	<b>**0.45</b>	0.09

Significant Spearman correlation coefficients are in bold ( $N = 91$ ;  $*P \leq 0.05$ ,  $**P \leq 0.01$ ). PAR% values were available only for the MedSeA cruise data set ( $N = 37$ ).

heterococcolithophores largely dominated the coccolithophore assemblage. Two coccolithophore species (*Calcidiscus leptopus* and *Coccolithus pelagicus* subsp. *braarudii*) were represented mainly by their HOL stage along the transect (Fig. 4). *Calcidiscus leptopus* HET and *Coccolithus pelagicus* subsp. *braarudii* HET were found only in the SW Mediterranean, where they reached respectively a maximum of  $\approx 2000$  cells  $l^{-1}$  and  $\approx 250$  cells  $l^{-1}$ . *Coccolithus pelagicus* subsp. *braarudii* HOL was found prevalently in the SW Mediterranean, reaching a maximum of 1300 cells  $l^{-1}$  at M84/3 cruise station 334. On the other hand, *Calcidiscus leptopus* HOL was usually found in the SE Mediterranean, reaching a maximum of 4000 cells  $l^{-1}$  at M84/3 cruise station 302. The HOLF index, indicating the incidence of the HOLF phase, varied greatly in our samples: between 0 (only heterococcolithophores) and 100 (only HOLs) at both the SW and SE Mediterranean stations (Fig. 5). The mean HOLF index calculated for the first 100 m of the water column was significantly higher ( $t$ -test  $P = 0.04$ ) at the SE than at the SW Mediterranean. Stations 5, 324 and 321 were not included in either of the two main groups identified by the cluster analysis, due to their distinctive HOLF indexes. The high values of station 5 (average = 89.13) contrasted with the lower values registered in the SW Mediterranean cluster (average = 34.11) and in stations 324 and 321 (average = 27.26). The average HOLF index at stations 324 and 321 was also considerably lower than that registered in the SE Mediterranean cluster (average = 53.86). In particular, very high HOLF index values (=100) were registered within the first 10 m of station 5 (Fig. 5), due to an almost mono-specific bloom of *Syracosphaera bannockii* HOL (=69–73% of the total coccospheres); this form was scarce in all other samples, oscillating between

0 and 16%. On the other hand, *S. bannockii* HET was not found at station 5 and it was rare along the remaining stations, never representing more than 2% of the total coccolithophore community.

Stations 5, 324 and 321 were adjacent to each other, defining an interval of high HOLF index variability between 5.55 and 9.40°E; having being isolated as outliers, they were excluded from the calculations presented in Figs 3, 5, 6, and from the correspondent  $t$ -tests.

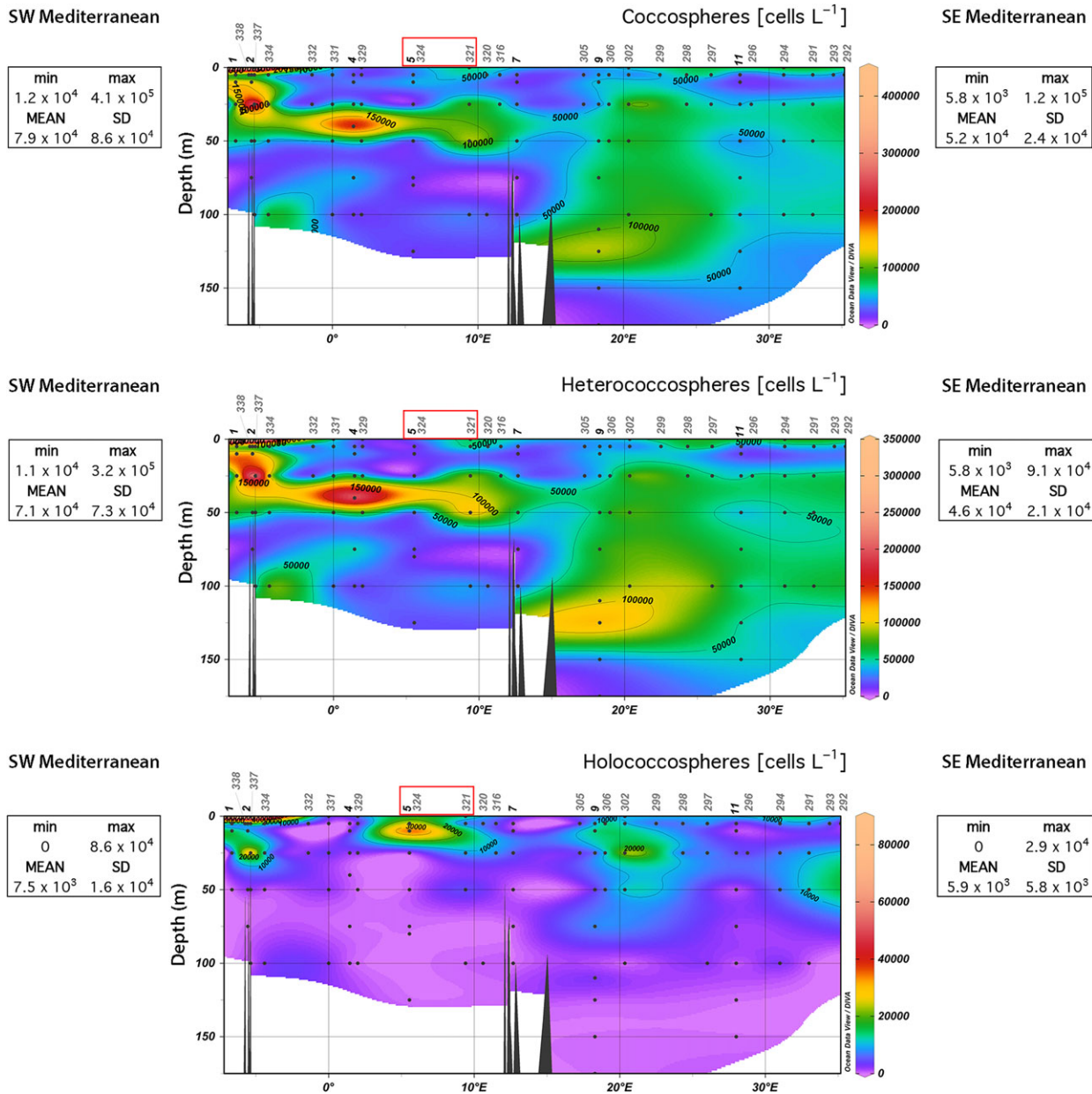
### Environmental correlations

HOL absolute abundance (Tot. HOL) and the HOLF index were both negatively correlated with  $[NO_3^- + NO_2^-]$ ,  $[PO_4^{3-}]$ , and positively with  $[CO_3^{2-}]$ . Other significant correlations included: temperature, pH, pCO<sub>2</sub> and PAR% for Tot. HOL; temperature and PAR% for the HOLF index (Table I). Figure 6 illustrates the variability of the main environmental and coccolithophore parameters within the 2013 MedSeA cruise stations analyzed (excluding station 5).

In both the SW and SE Mediterranean basins, a “high HOLF layer” was identified. The high HOLF layer corresponds to a depth interval characterized by HOLF index values  $\geq 45$ , and containing 75% of the HOL cells.

The high HOLF layer extended within the upper 10 m of the superficial water column in the SW basin, and within the upper 25 m in the SE basin. These depth intervals were characterized by the co-occurrence of relatively low nutrient concentrations, high PAR%, high temperature and high  $[CO_3^{2-}]$ , as shown in Table III.

In the SE Mediterranean, the HOLF layer occurred above the DCM (positive peak of fluorescence around 50 m; Fig. 6) and within the upper part of the thermocline (interval characterized by a particularly strong



**Fig. 3.** MedSeA and M84/3 combined data on absolute abundance of total coccopheres, heterococcosphere and holococcospheres. The minimum (min), maximum (max), mean (MEAN) and standard deviation (SD) values refer to the first 100 m of the water column in the SW and SE Mediterranean. Stations 5, 321 and 324 are treated as outliers and excluded from the calculations.

decline in temperature with depth). In the SW Mediterranean, the HOLP layer still occurred above the DCM, but coinciding with a superficial positive peak in fluorescence; within this depth interval strong variations in temperature were not observed.

In conclusion, there seems to be a consistent relationship between the position of the HOLP layer, the depth of the DCM and the thermocline, but only in the oligotrophic SE Mediterranean.

Cell concentrations, for both the diploid and haploid phases, of the seven selected species (*Calcidiscus leptoporus*, *Coccolithus pelagicus*, *Coronosphaera mediterranea*, *Syracosphaera bannockii*, *S. histrica*, *S. molischii* and *S. pulchra*) showed distinct correlations with the environmental parameters (Table II). It is important to note that for a given species, the correlation with the environmental parameters exhibited opposite sign whether the haploid or the diploid phase is considered, with the exception of *S. pulchra*:

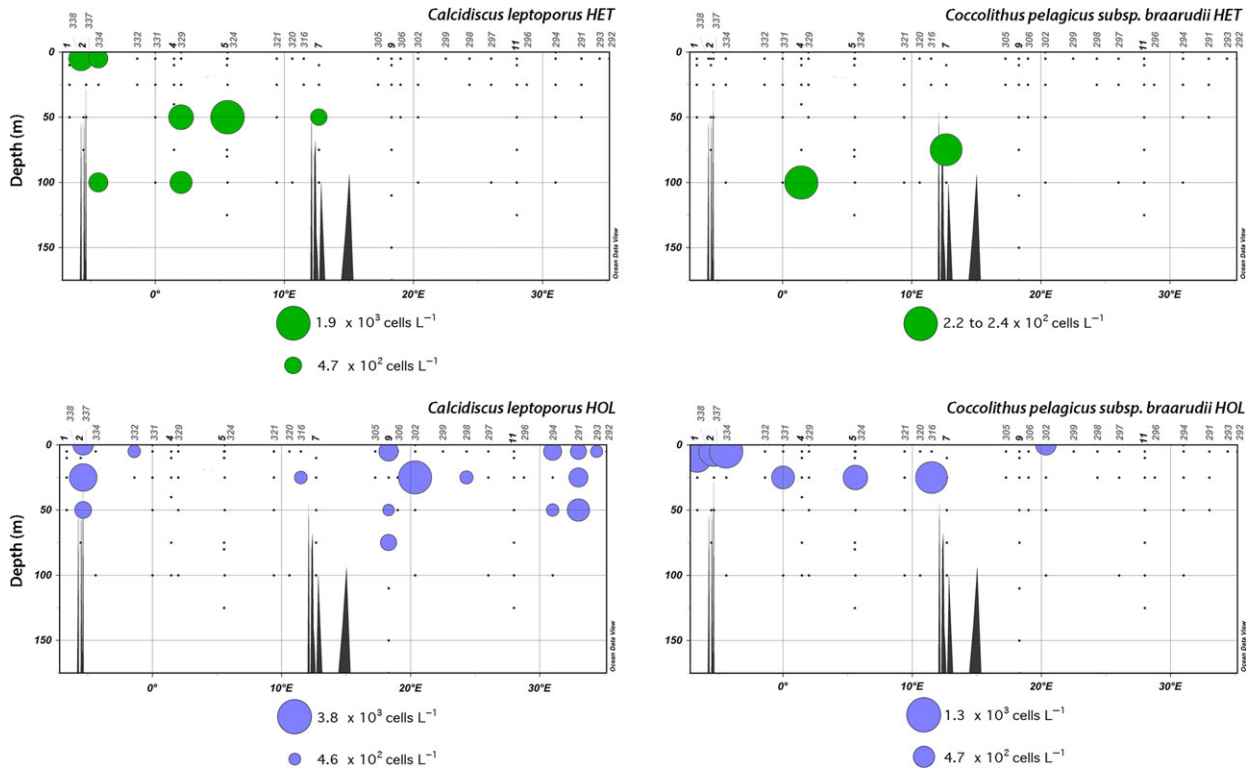


Fig. 4. Absolute abundance of the HET and HOL forms of *Cacidiscus leptoporos* (left) and *Coccolithus pelagicus* subsp. *braarudii* (right).

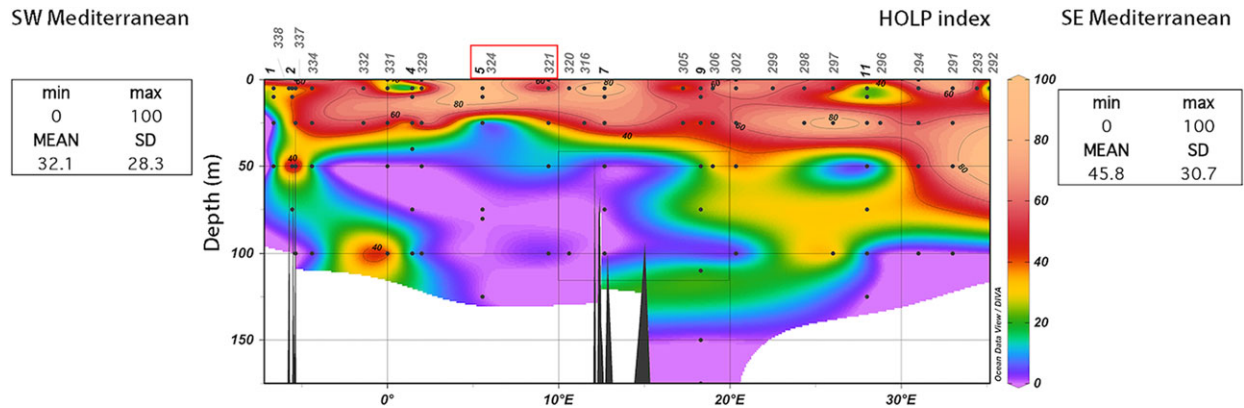
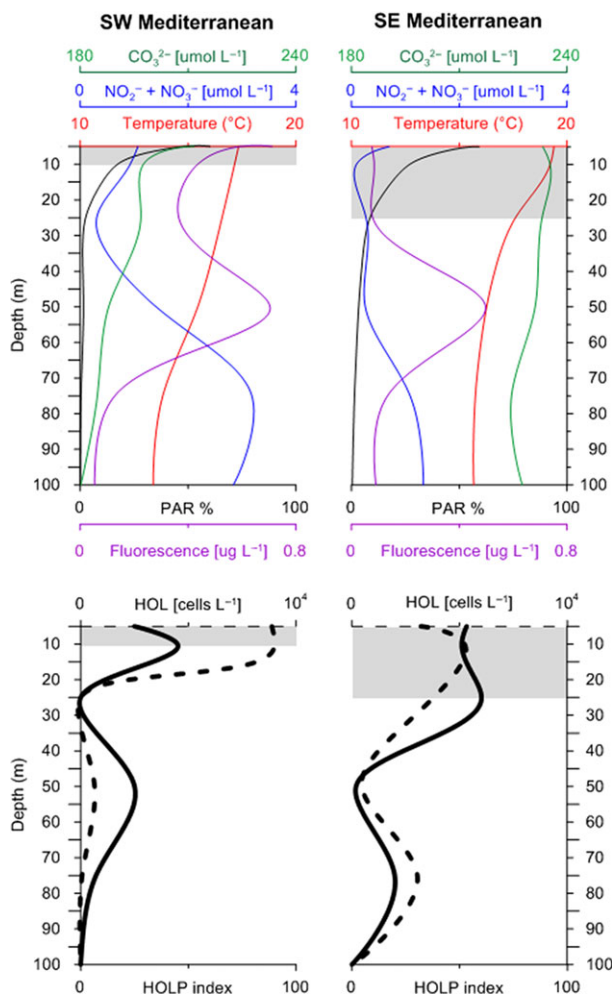


Fig. 5. HOLP index along the transect. The minimum (min), maximum (max), mean (MEAN) and standard deviation (SD) values refer to the first 100m of the water column in the SW and SE Mediterranean. Stations 5, 324 and 321 are treated as outliers and excluded from the calculations.

its heterococcolithophore and HOL stages were similarly correlated with temperature,  $[\text{NO}_3^- + \text{NO}_2^-]$ ,  $[\text{PO}_4^{3-}]$ ,  $\text{pCO}_2$  and  $[\text{CO}_3^{2-}]$ .

Among the outliers (Fig. 2), station 5 had the highest average HOLP index (89.13), while stations 324 and 321 had lower values (respectively 34.57 and 19.95). The gradual decrease in HOLP index of the stations of the outlier group, corresponded to gradual

increases in the range of  $[\text{NO}_3^- + \text{NO}_2^-]$ ,  $[\text{PO}_4^{3-}]$  and slight decreases in  $[\text{O}_2]$  and pH. In station 5,  $[\text{NO}_3^- + \text{NO}_2^-] = 0.02\text{--}0.70 \mu\text{mol l}^{-1}$ ,  $[\text{PO}_4^{3-}] = 0\text{--}0.02 \mu\text{mol l}^{-1}$ ,  $[\text{O}_2] = 236.60\text{--}249.07 \mu\text{mol kg}^{-1}$ ,  $\text{pH} = 8.09\text{--}8.14$ ; in station 324,  $[\text{NO}_3^- + \text{NO}_2^-] = 0\text{--}2.00 \mu\text{mol l}^{-1}$ ,  $[\text{PO}_4^{3-}] = 0\text{--}0.10 \mu\text{mol l}^{-1}$ ,  $[\text{O}_2] = 216.00\text{--}234.50 \mu\text{mol kg}^{-1}$ ,  $\text{pH} = 7.90\text{--}8.00$ ; in station 321,  $[\text{NO}_3^- + \text{NO}_2^-] = 0.10\text{--}5.20 \mu\text{mol l}^{-1}$ ,  $[\text{PO}_4^{3-}] =$



**Fig. 6.** Average  $[\text{CO}_3^{2-}]$ ,  $[\text{NO}_2^- + \text{NO}_3^-]$ , temperature, PAR%, fluorescence, HOL absolute abundance (HOL, dotted line) and HOLP index (solid line) in the first 100 m of the SW (left) and SE Mediterranean (right) during the MedSeA cruise. The interval in gray delimits the “high HOLP layer”.

$0\text{--}0.20 \mu\text{mol l}^{-1}$ ,  $[\text{O}_2] = 180.00\text{--}238.50 \mu\text{mol kg}^{-1}$ ,  $\text{pH} = 7.90\text{--}8.00$ .

## DISCUSSION

HOLs are particularly abundant in the Mediterranean Sea, more than in adjacent regions like the Atlantic or the Red Sea (Kleijne, 1991), especially in summer (Dimiza *et al.*, 2008; Malinverno *et al.*, 2009; Cros and Estrada, 2013; Šupraha *et al.*, 2016). Our results from a Mediterranean longitudinal transect, characterized by strong biogeochemical differences (in nutrients, temperature, carbonate system), showed that they reached maximum density always at shallower depths than the

heterococcolithophores (Figs 3, 6) and had a wider vertical distribution in the SE Mediterranean. HOLs preferential distribution in shallow waters has been previously noticed in the Mediterranean (Cros *et al.*, 2000; Triantaphyllou *et al.*, 2002; Dimiza *et al.*, 2004, 2015; Malinverno *et al.*, 2009; Cros and Estrada, 2013; Oviedo *et al.*, 2015), and might be justified by an affinity for nutrient-poor, high-light conditions. In fact, haploid cell replication involves lower energetic costs than that of diploid cells (Lewis, 1985); the former also tend to be smaller, meaning higher surface-volume ratios and enhanced nutrient absorption (Karl *et al.*, 2001); in the case of *E. huxleyi*, it has been demonstrated that the haploid phase is more resistant to phosphorus limitation (Rokitta *et al.*, 2016), a condition which is accentuated in the upper photic zone of the Mediterranean Sea, particularly in the SE basin (Fig. S2). Due to their ultra-oligotrophic character, the waters of the photic zone in the SE basin tend to be more transparent than those in the SW Mediterranean, allowing deeper light penetration and the formation of a DCM (see fluorescence and 0.1% PAR limit in Fig. S4). HOLs might have an advantage over heterococcolithophores there, being better adapted to high light intensities: the crystalline structure of some holococcoliths has been shown to be extremely effective in backscattering harmful UV radiation (Quintero-Torres *et al.*, 2006). HOL preference for the upper photic zone is supported also by the distributions of *C. pelagicus* subsp. *braarudii* and *C. leptoporus*. The holococcospheres and heterococcospheres of these species had different vertical distributions, reflecting the general trends discussed above (Fig. 4). However, while *C. pelagicus* subsp. *braarudii* was found almost exclusively in the SW basin, together with the diploid form of *C. leptoporus*, the haploid form of *C. leptoporus* was prevalently found in the SE Mediterranean. Under controlled culture conditions, both *C. pelagicus* subsp. *braarudii* HOL and *C. leptoporus* HOL grow faster than their diploid counterparts under nutrient depletion (Houdan *et al.*, 2006), while *C. pelagicus* subsp. *braarudii* HET and *C. leptoporus* HET are considered high-productivity indicators, typical of temperate zones (Cachão and Moita, 2000; Renaud and Klaas, 2001; Renaud *et al.*, 2002; Saez *et al.*, 2003). Thus, the longitudinal extension of these species and their life phases might reflect the negative W-E gradient in  $[\text{NO}_3^- + \text{NO}_2^-]$  and  $[\text{PO}_4^{3-}]$  (Fig. S2): *C. pelagicus* subsp. *braarudii* and *C. leptoporus* seem to cope better with the Mediterranean environment when in their haploid forms; also, our data suggest that, among them, only the haploid phase of *C. leptoporus* might be able to reach significant concentrations in the SE basin. The idea that the two coccolithophore life phases might exploit separate ecological niches (Cros



Table III: Average values of the main environmental parameters in the MedSeA cruise stations (\*at 5 m; values at 10 m not available)

Stations	Depth interval (m)	T (°C)	NO <sub>x</sub> [ $\mu\text{mol L}^{-1}$ ]	PO <sub>4</sub> [ $\mu\text{mol L}^{-1}$ ]	CO <sub>3</sub> <sup>2-</sup> [ $\mu\text{mol kg}^{-1}$ ]	PAR%
1, 2, 4	5–10	17.25	0.50	0.04	204.63	38.76
1, 2, 4	25–100	14.81	1.94	0.12	187.42	1.09
5	5–10	17.41	*0.16	*0.02	211.33	31.85
5	25–80	14.54	0.47	0.02	204.01	2.12
7, 9, 11	5–25	18.74	0.35	0.02	233.85	31.58
7, 9, 11	50–100	15.90	0.89	0.04	227.73	1.69

and Estrada, 2013; Oviedo *et al.*, 2015; Šupraha *et al.*, 2016) is supported by the distributions we observed along the transect (Figs 3, 4), as well as by their distinct environmental correlations (Table II). The environmental correlations relative to the abundance of the haploid stage agreed in sign among species, but varied in intensity, perhaps due to slightly different ecological requirements (Cros, 2001). In this work, the HOLP index was used (Cros and Estrada, 2013; Šupraha *et al.*, 2016) to understand the triggers of coccolithophore life phase change. We found that the HOLP index increased eastward (Fig. 5); moreover, the absolute HOL abundance and the HOLP index were tested against the same set of environmental variables and showed similar correlations (Table I), although the only significant carbonate system parameter to which the HOLP index was correlated was carbonate ion concentration. The environmental correlations of the HOLP index reflect natural longitudinal gradients that co-vary and therefore it is difficult to disentangle the real triggers of phase change solely from our data. A haplo-diploid life cycle is considered to be advantageous in a temporally and spatially variable environment (Stebbins and Hill, 1980; Valero *et al.*, 1992; Hughes and Otto, 1999; Noël *et al.*, 2004). Also, genomic evidence suggests that some strains of *E. huxleyi* adapted to low environmental variability and low biotic pressure may have totally abandoned this life strategy (von Dassow *et al.*, 2015). Although such observations have been made solely on *E. huxleyi* (Noelaerhabdaceae), other coccolithophore species may adapt to similar environmental conditions and might have lost their ability to change phase.

Life phase transformations are likely triggered by exogenous factors (environmental stimuli), but endogenous mechanisms might avoid this energetically costly process for short-termed environmental changes (Houdan *et al.*, 2004). The Mediterranean exhibits seasonal changes, strong biogeochemical gradients and mesoscale structures like gyres and eddies (Millot, 1987, 1991; Bergamasco and Malanotte-Rizzoli, 2010; Shaltout and Omstedt, 2014), and can therefore be considered a variable environment.

Stations 5, 321 and 324 of the 2013 MedSeA and M84/3 cruises could not be assigned to either the SW nor

to the SE Mediterranean based on their average HOLP index (Fig. 2), corresponding perhaps to areas of marked environmental instability. Extremely high HOLP index values (100) were observed at 5 and 10 m depth at the MedSeA 2013 cruise station 5. This station stood out for its low superficial nutrient concentrations (phosphate and nitrate plus nitrite), lower salinity, higher dissolved oxygen, higher [CO<sub>3</sub><sup>2-</sup>] and higher pH than other stations of the MedSeA (Fig. S1, S2, S3). When compared with the other two outlier stations (324 and 324), the most striking environmental differences consisted again in lower nutrient concentrations and in slightly higher dissolved oxygen and pH. The maximum fluorescence was found deeper at station 5 than at adjacent stations on the same cruise. Such environmental conditions suggest that the water column at station 5 during the sampling was likely stratified, perhaps due to the persistence of an anticyclonic eddy in this area, slightly detectable in the satellite derived Chl *a* concentration data (Fig. 1), although no similar structures were recognizable from altimetric data. In the core of an anticyclonic eddy, water stratification can take place creating negative anomalies in superficial nutrient concentrations and a DCM (Brenner *et al.*, 1991; Krom *et al.*, 1992). This water mass stays relatively isolated from its surroundings, and can serve as a distinct ecological niche (Margalef, 1978; Bracco *et al.*, 2000), regulating the local coccolithophore distribution (Garcia-Soto *et al.*, 1995; Vaillancourt *et al.*, 2003; Cokacar *et al.*, 2004; Read *et al.*, 2007). The positive HOLP index anomaly at station 5 was due to a monospecific bloom of *S. bannockii* HOL (= 69–73% of the total assemblage). Both life phases have been identified in the North and South Atlantic (Balestra *et al.*, 2004; Boeckel and Baumann, 2008; Poulton *et al.*, 2010; Charalampopoulou *et al.*, 2011; Daniels *et al.*, 2014) and Mediterranean (Cros *et al.*, 2000; Geisen *et al.*, 2002; Malinverno, 2003; Oviedo *et al.*, 2015; Šupraha *et al.*, 2016) living assemblages. A relative abundance of *S. bannockii* similar to that at station 5, was observed previously only in the Bay of Biscay, during April 2010 (Daniels *et al.*, 2014), but in that case it was represented by a mix of the two phases, with a dominance of heterococcolithophores. During the MedSeA cruise (station 5), the diploid phase was completely absent, and it was very rare in the closest Meteor M84/3 cruise samples

(station 324), occurring only at 25 m with a concentration of 678 cells  $l^{-1}$ . The presence of both life phases during the bloom of *S. bannockii* in the Bay of Biscay, was interpreted either as an indication of similar ecologies for the two phases, or as an ongoing adaptation of cells to lower nutrient conditions (Houdan *et al.*, 2006). Based on our observations for the MedSeA and Meteor M84/3 cruises, we suggest that the two life phases of this species should have different ecologies, and that probably the contemporary observation of *S. bannockii* HET and HOL in the Bay of Biscay was conducted during the adaptation of this species to a seasonal environmental change.

A high absolute abundance of *S. bannockii* HOL has been previously reported in summer in the Mediterranean, positively correlated with temperature and negatively with nutrient concentration (Šupraha *et al.*, 2016). During our sampling in 2011 and 2013, *S. bannockii* did not show any strong correlations with these same variables (Table II), likely due to its low occurrence along the remaining transect; however, there was a strong negative anomaly in nutrient concentration at station 5. Adding to the absence of any evident temperature anomaly between this location and the adjacent stations (Fig. S1), we think that the bloom of *S. bannockii* HOL was mainly triggered by nutrient limitation. Although there is no apparent influence of temperature on the *S. bannockii* HOL bloom we observed, this parameter might still influence the main vertical and longitudinal trends in abundance (Dimiza *et al.*, 2008; Cros and Estrada, 2013; Oviedo *et al.* 2015, 2016; Šupraha *et al.*, 2016) and affect the HOLP index. Another possible factor explaining the dominance of *S. bannockii* HOL at station 5 might be the stable conditions (low turbulence) of the water column in this area. Experimental evidence indicates in fact that the haploid phases of coccolithophores tend to be inhibited by turbulence (Houdan, 2003; Houdan *et al.*, 2005, 2006). The carbonate system also helps to explain both the general HOLP index trends and the anomaly at station 5. Carbonate ion ( $CO_3^{2-}$ ) concentration was the only carbonate system parameter to show significant correlations with the HOLP index (Table I). It tended to increase eastward and upward in the water column; it was also visibly higher in station 5 than in adjacent stations of the SW Mediterranean, but lower than in the SE basin (Fig. S3), indicating that, likely, it has a secondary effect. The interaction between the coccolithophore life cycle and the carbonate system has been scarcely studied until present. Coccolithophores produce coccoliths made of calcium carbonate in an intracellular vesicle through mechanisms which are still not fully understood (Taylor *et al.*, 2017). HOLs could have been negatively affected by low carbonate saturation levels during the Paleocene Eocene Thermal Maximum (PETM), but only when coupled with

exceedingly high, non-optimal temperatures, which could have increased the energetic cost of extra-cellular calcification (Gibbs *et al.*, 2015). Probably, the cells need to absorb both  $CO_3^{2-}$  and  $HCO_3^-$  to calcify in seawater, at  $pH > 7$  (Ziveri *et al.*, 2012). A connection between HOL distribution and the carbonate system was suspected earlier (Oviedo *et al.*, 2015), but it was difficult to differentiate the role of the different components; on the other hand, based on the preference of some heterococcolithophore species for the Eastern Mediterranean, an enhanced ability to utilize  $CO_3^{2-}$  for calcification in respect to Western Mediterranean species was hypothesized (Oviedo *et al.*, 2015). Culture experiments have additionally demonstrated species-specific and strain-specific effects of the carbonate system on coccolithophore calcification (Langer *et al.*, 2006, 2009; Fiorini *et al.*, 2011a, 2011b), dependent on the collection site of the strains (Rickaby *et al.*, 2016). Calcification itself is a highly energy consuming process (Monteiro *et al.*, 2016), and its function can change between HOLs and heterococcolithophores, which seem to inhabit very different nutrient regimes. It is thus plausible that the haploid phase of coccolithophores might be more efficient in calcifying in high  $[CO_3^{2-}]$  conditions, such as those of the upper photic zone of the SE Mediterranean, rather than the diploid phase. Light sensibility seems to explain the vertical distribution of HOLs, but it does not seem to be the best explanation for the longitudinal differences in HOLP index along the 2013 MedSeA cruise transect (the only cruise for which PAR% values were available, Fig. 6): the average PAR% in the first 100 m was very similar for the two main basins. The same was probably valid for the Meteor M/84 cruise. Finally, it is worth noticing that some environmental and biological dynamics which were not measured during the cruises might also have an important role, such as the rate and quality of viral infection (Frada *et al.*, 2008, 2012): haploid cells of *E. huxleyi* can appear in post-bloom conditions, after heterococcolithophores have been decimated by virus infections. Overall, this study contributes to the understanding of the coccolithophore haploid and diploid distribution patterns in a changing ocean. Ocean warming, associated to surface water column stratification and nutrient limitation, might further stimulate the haploid phase over the diploid phase, elevating the relative abundance of HOLs in the Mediterranean Sea. The frequency of diploid-haploid transformations could increase in a warming scenario, constituting a survival strategy for species whose diploid phases are adapted to cooler and nutrient-richer conditions; however, ocean acidification and the associated decrease in seawater carbonate ion concentration might negatively affect HOLs. More studies are needed to confirm the potential influence of the carbonate system on the coccolithophore life cycle.

## CONCLUSIONS

The coccolithophore haploid and diploid life cycle is a successful ecological strategy in the Mediterranean Sea, probably crucial for the eastward propagation of species whose diploid forms are adapted to North Atlantic or SW Mediterranean conditions (e.g. *Calcidiscus leptopus*, *Coccolithus pelagicus* subsp. *braarudii*). HOLs tend to concentrate in the upper photic zone (first 10–25 m) and usually constitute a minority of the total population, but their number can locally increase and surpass that of heterococcolithophores, even within the SW basin. The distribution of total HOLs and their prevalence index (HOLP) suggest an adaptation of this life phase to oligotrophic, warm waters, rich in carbonate ions; light intensity is also a potential factor explaining their superficial distribution. The unusually high HOLP indices ( $\approx 100$ ) observed in the upper 10 m of one SW Mediterranean station, caused by a mono-specific bloom of *S. bannockii* HOL, indicates that nutrient-limitation might be sufficient to trigger a diploid-haploid transformation, at least in this species.

Based on the IPCC projections (IPCC, 2013), by the year 2100 the Mediterranean Sea may experience a pH reduction of 0.245–0.462 in the Western Basin and of 0.242–0.457 in the Eastern Basin (Goyet *et al.*, 2016), while regionally averaged temperatures could increase by 6°C (Sakalli, 2017). These changes are expected to cause shifts in the carbonate system, including a drop in carbonate ion concentration, and a decrease in surface nutrient concentrations (Gruber, 2011). Overall, a haplo-diploid life cycle might support the survival of coccolithophore species under warming and increasingly water-stratified conditions; however, the influence of the carbonate system on their life cycle should be clarified to make realistic projections.

## SUPPLEMENTARY DATA

Supplementary data is available at *Journal of Plankton Research* online.

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