

# Holo-heterococcolithophore life cycles: ecological implications

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**ABSTRACT:** Many coccolithophores have complex life cycles with haploid and diploid stages bearing structurally different coccolith types (holococcoliths and heterococcoliths, respectively). Laboratory studies suggest that holo- and heterococcolithophores may occupy distinct ecological niches, but the potential ecological implications of the existence of haploid and diploid stages are poorly known. We present here a study of holo- and heterococcolithophore distributions in the Catalano-Balearic Sea, during 2 cruises (MESO-96, from 18 June to 3 July, and FRONTS-96, from 16 to 21 September) that covered 2 intervals of the stratification period of 1996. We define a holo-coccolithophore prevalence index (HOLP index), calculated for each coccolithophore-containing sample, as the percent ratio between the number of holococcolithophores and the total number of holo- and heterococcolithophores belonging to families with alternation of holo- and heterococcolithophore life stages (coccolithophores having HOL-HET life cycles; Total\_HHLC). In both cruises, the distribution of holo- and heterococcolithophores and the HOLP index indicated a preference of the holococcolithophores for shallower waters and of the heterococcolithophores for deeper layers. This segregation may be linked to a differentiation of ecological niches, with the haploid holococcolithophores occupying the more oligotrophic upper layers and the diploid heterococcolithophores inhabiting relatively rich deeper waters.

**KEY WORDS:** Holococcolithophore prevalence index · Haploid-diploid life cycles · Coccolithophores · NW Mediterranean

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## INTRODUCTION

Coccolithophores are haptophytes that at some stage of their life cycle possess a cell cover consisting of calcified scales (coccoliths). They are a major component of the oceanic phytoplankton and play an important role in the marine biogeochemistry of carbonate and, therefore, in the carbon cycle (Westbroek et al. 1994, Rost & Riebesell 2004).

Two structurally different types of coccoliths, heterococcoliths and holococcoliths, are recognizable among the coccolithophores; the heterococcoliths are formed of crystal units of variable shape and size, while the holococcoliths consist of numerous minute (<0.1 µm) crystallites (Young et al. 1999). A clear demonstration that holo- and heterococcolithophores could be life-

cycle phases of the same species occurred when culture studies by Parke & Adams (1960) showed that monoclonal strains of the heterococcolithophore *Coccolithus pelagicus* gave rise to a holococcolithophore that could be assigned to the genus *Crystallolithus*. Further evidence concerning coccolithophore life cycles was gathered from work on coastal coccolithophores of the Pleurochrysidaceae and Hymenomonadaceae. These families present an alternation of a motile heterococcolithophore phase and a non-motile non-calcifying benthic phase (Gayral & Fresnel 1983, Fresnel 1989, Fresnel & Billard 1991). Nuclear staining and chromosome counting showed that the heterococcolithophore phase is diploid, while the benthic phase is haploid (Rayns 1962, Fresnel 1994). Other studies indicated that the organic

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body scales of the haploid and diploid phases were different and that the same scale types occurred, respectively in the holo- and heterococcolithophore phases of *C. pelagicus* (Manton & Leedale 1969, Gayral & Fresnel 1983, Fresnel & Billard 1991), providing support to the hypothesis that coccolith and body scale type could be associated with different ploidy phases (Billard 1994). Later studies using flow-cytometric DNA analysis confirmed this hypothesis and showed that a similar phenomenon occurred with the coccolithophores *Calcidiscus leptoporus* and *Coronosphaera mediterranea* (Houdan et al. 2004). The study of coccolithophore life cycles is difficult because only a small number of species are maintained in culture and direct observations of phase changes are rare (Rowson et al. 1986, Noël et al. 2004). Nevertheless, combination coccospheres, which bear both hetero- and holococcoliths and have been interpreted as cells in a life-cycle transition (Geisen et al. 2004), are sporadically recorded in natural samples (e.g. Thomsen et al. 1991, Kleijne 1993, Cros et al. 2000, Cortés & Bollman 2002, Geisen et al. 2002, Triantaphyllou et al. 2004, Frada et al. 2009), providing an additional source of information. It is now recognized that many families of coccolithophores, including Helicosphaeraceae, Syracosphaeraceae, Rhabdosphaeraceae, Coccolithaceae, Calcidiscaceae, Papposphaeraceae and Pontosphaeraceae (Billard & Inouye 2004, Frada et al. 2009) have a life cycle with holococcoliths in the haploid phase and heterococcoliths in the diploid phase. The finding that some heterococcolithophores appear to be associated with 2, or even 3, holococcolithophore 'species', which has been attributed to ecophenotypic variations in the holococcoliths and cryptic or pseudo-cryptic speciation in the diploid phase (Geisen et al. 2002, 2004, Saez et al. 2003), complicates even more an understanding of the significance of these life histories.

Coccolithophores tend to occupy ecological environments with an intermediate degree of fertility, midway between the extremes represented by turbulent, nutrient-rich waters, which favour diatom growth, and stratified, nutrient-poor waters, where dinoflagellates and other flagellates tend to dominate (Margalef 1978, Estrada et al. 1999). Within this general trend, coccolithophores present a wide spectrum from r-selection to k-selection (Brand 1994) and their coccolith shape has been related to their nutrient affinity (Young 1994). Field observations indicate that heterococcolithophores such as *Emiliania huxleyi*, which can form extensive blooms, and *Coccolithus pelagicus* may be found in environments with a certain degree of nutrient availability (Balch et al.

1992, Cachao & Moita 2000) while holococcolithophores tended to occur in more oligotrophic waters and appeared to prefer the upper part of the euphotic zone (Kleijne 1993, Cros 2002, Triantaphyllou et al. 2002, Dimiza et al. 2008a). Detailed taxonomic studies of the distribution of holococcolithophores in the field are not common, in part because holococcolithophores degrade easily in water samples and cannot be reliably identified with standard optical microscopy. In a study of samples from weather stations in the western Atlantic, Okada & McIntyre (1979) found that the holo- and heterococcolithophore stages of *C. pelagicus* were respectively more abundant at the surface and 100 m of depth, and that the relative proportion between both forms changed markedly along a seasonal cycle. Based on samples of the northern north Atlantic, Balestra et al. (2004) reported also that the holococcolithophore phase of *C. pelagicus* tended to occur at shallower levels of the water column.

Houdan et al. (2006) used laboratory experiments with cultures to show that the haploid and diploid stages of *Coccolithus braarudii* and *Calcidiscus leptoporus* corresponded to different ecological strategies, with the haploid phase, flagellated and capable of mixotrophy, exploiting a more oligotrophic niche than the diploid phase, which grew faster in nutrient-rich media. However, the potential ecological implications of the existence of haploid and diploid stages are poorly known and there is a need of comparative studies of holo- and heterococcolithophore distributions in natural ecosystems.

In this work, we present a study of holo- and heterococcolithophore distributions in the Catalano-Balearic Sea during 2 different periods of the year, emphasizing the comparison between holo- and heterococcolithophore stages of the same species, and discuss the ecological interpretation of our findings. Because of the spotty distribution or lack of the holo- or heterococcolithophore phase cells of many of the species, we propose a numerical index (HOLP index) as a tool to evaluate the general prevalence of the holococcolithophore phase in the water column.

## MATERIALS AND METHODS

### Sea water samples

The studied samples were collected during the cruises MESO-96 and FRONTS-96, carried out in the Catalano-Balearic Sea (NW Mediterranean) on board the RV 'García del Cid', from 18 June to 3 July 1996

and from 16 to 21 September 1996, respectively. Fig. 1 shows the positions of the sampled stations and Table 1 details the geographic positions of the stations and the date and time when they were visited.

At each station, vertical profiles of temperature, salinity and fluorescence were obtained with a Neil Brown Mark III CTD probe. Water samples were obtained at selected depths between 5 and, usually, 100 m of depth (one sample was taken from 160 m) using a rosette with Niskin bottles, attached to the CTD system. Chlorophyll *a* (chl *a*) concentration was determined by measuring the fluorescence of acetonic extracts (Yentsch & Menzel 1963) with a Turner Designs fluorometer. Analyzed chl *a* concentrations were used to calibrate the fluorescence readings of the CTD system. For coccolithophore enumeration, about 200 cm<sup>3</sup> of sea water were filtered, using a vacuum pump, onto a polycarbonate membrane filter of 0.8 µm pore size and 25 mm diameter, placed on top of a cellulose ester filter of 3 µm pore size. Salt was removed by washing the filters with about 1 to 2 cm<sup>3</sup> of bottled drinking

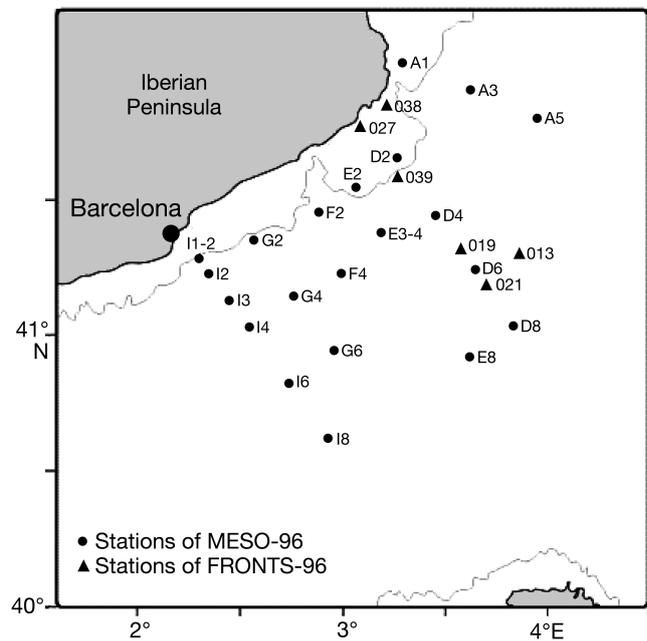


Fig. 1. Position of the stations sampled in the cruises MESO-96 and FRONTS-96

Table 1. Geographic position, date and time of visit of the sampled stations

Station	Date	Time (GMT)	Latitude (N)	Longitude (E)	Sampling depth (m)
<b>Cruise MESO-96</b>					
A1	18-6-1996	22:39 h	42° 00.0'	3° 17.3'	5,40,70,100
A3	19-6-1996	01:38 h	41° 54.0'	3° 37.1'	5,40,70,100
A5	19-6-1996	11:57 h	41° 48.0'	3° 56.9'	5,40,70,100
D2	21-6-1996	06:36 h	41° 38.9'	3° 15.0'	5,40,70,100
D4	21-6-1996	03:31 h	41° 26.6'	3° 26.5'	5,40,70,100
D6	20-6-1996	23:55 h	41° 14.3'	3° 38.0'	5,40,70,100
D8	20-6-1996	20:34 h	41° 02.0'	3° 49.5'	5,40,70,100
E2	30-6-1996	12:45 h	41° 33.0'	3° 03.0'	5,40,70,100
E3-4	01-7-1996	08:12 h	41° 23.0'	3° 10.2'	5,40,70,100
E8	02-7-1996	08:30 h	40° 55.1'	3° 36.6'	5,70,100
F2	23-6-1996	23:58 h	41° 27.2'	2° 52.0'	5,40,70,100
F4	24-6-1996	04:12 h	41° 13.7'	2° 59.7'	5,40,70,100
G2	24-6-1996	09:15 h	41° 20.9'	2° 33.7'	5,20,40,50,70,100
G4	24-6-1996	17:27 h	41° 08.6'	2° 45.2'	5,40,70,100
G6	25-6-1996	09:43 h	40° 56.3'	2° 56.7'	5,40,70,100
I1-2	29-6-1996	12:05 h	41° 17.0'	2° 17.8'	5,40,70,100
I2	24-6-1996	23:00 h	41° 13.9'	2° 20.7'	5,40,70,100
I3	28-6-1996	08:06 h	41° 07.7'	2° 26.5'	5,40,70,100
I4	25-6-1996	02:09 h	41° 01.6'	2° 32.2'	5,40,70,100
I6	25-6-1996	05:27 h	40° 49.3'	2° 43.7'	5,40,70,100
I8	27-6-1996	10:13 h	40° 37.0'	2° 55.2'	5,40,70,100
<b>Cruise FRONTS-96</b>					
O13	16-9-1996	03:12 h	41° 17.8'	3° 51.2'	10,30,60,66,75,90
O19	17-9-1996	10:55 h	41° 19.3'	3° 33.5'	5,30,57,100
O21	17-9-1996	11:55 h	41° 11.7'	3° 41.6'	20,30,50,68,90
O27	18-9-1996	10:39 h	41° 46.7'	3° 03.9'	5,10,20,30,45
O38	20-9-1996	15:23 h	41° 51.0'	3° 12.0'	15,35,45,60
O39	21-9-1996	08:51 h	41° 35.3'	3° 15.8'	10,30,40,50,70,160

water. The filters were air dried and stored under partial vacuum in hermetically sealed boxes until preparation for the scanning electron microscope (SEM).

### Analytical SEM methodology

A part of the membrane filter was placed on a SEM stub and coated with a film (of about 150 Å) of gold or gold-palladium, to avoid electric charges; the sputter coater used was a Polaron SC-500. The examination and counting of the specimens as well as the microphotographs were made with a Hitachi S-570 scanning electron microscope. Detailed explanations of the SEM methodologies can be found in Cros & Fortuño (2002).

### Holococcolithophore prevalence index (HOLP index)

Let Total\_HHLC be the total number of coccolithophores (including both holo- and heterococcolithophores) belonging to families with recognized Holococcolithophore–Heterococcolithophore (Hol-Het) Life Cycles (Calcidiscaceae, Coccolithaceae, Helicosphaeraceae, Papposphaeraceae, Pontosphaeraceae, Rhabdosphaeraceae and Syracosphaeraceae).

The index of holococcolithophore prevalence, HOLP, is defined for each coccolithophore-containing sample, when total HHLC > 0, as:

$$\text{HOLP} = 100 * \frac{\text{Total Holococcolithophores}}{\text{Total\_HHLC}}$$

If HHLC = 0, then HOLP = 0

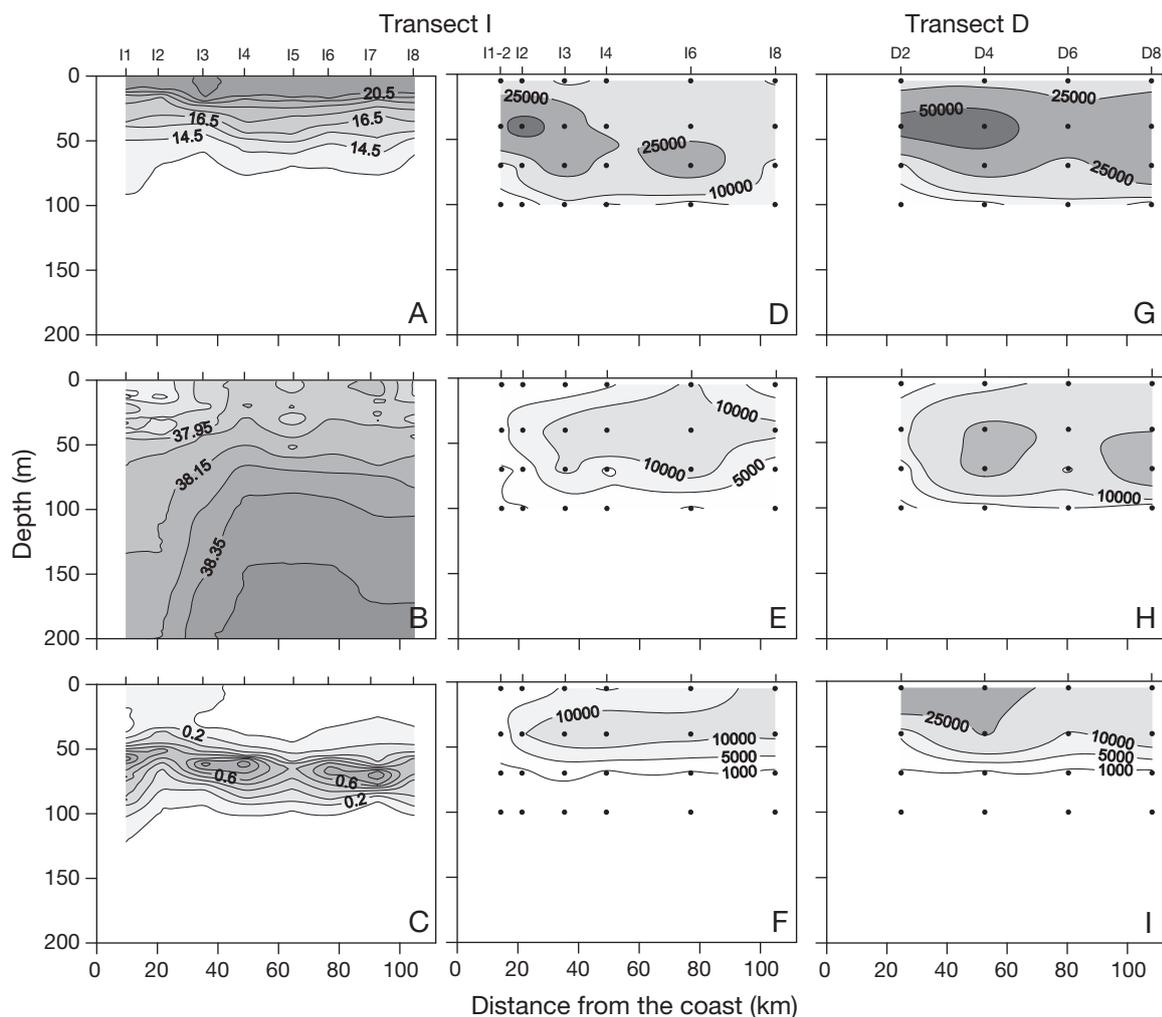


Fig. 2. Cruise MESO-96: Distribution of (A) temperature, (B) salinity, (C) chl a concentration in transect I. In (D) and (G), respectively: Distribution of total heterococcolithophores (cells l<sup>-1</sup>) in transects I and D. In (E) and (H), respectively: Distribution of heterococcolithophores (cells l<sup>-1</sup>) from families with holococcolithophore–heterococcolithophore life cycles (Het\_HHLC) in transects I and D. In (F) and (I), respectively: Distribution of total holococcolithophores (cells l<sup>-1</sup>) in transects I and D

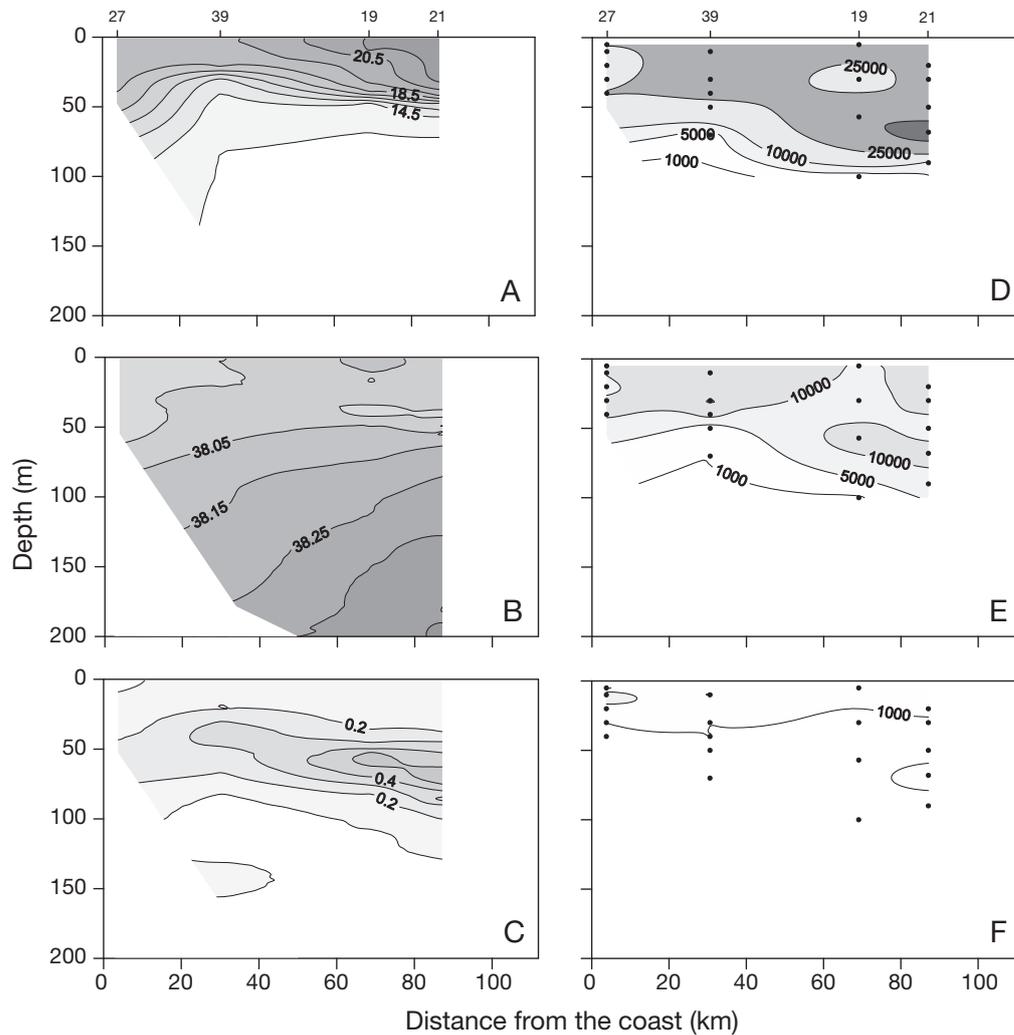


Fig. 3. Cruise FRONTS-96: Distribution of (A) temperature, (B) salinity, (C) chl *a* concentration, (D) total heterococcolithophores (cells  $l^{-1}$ ), (E) heterococcolithophores (cells  $l^{-1}$ ) from families with holococcolithophore–heterococcolithophore life cycles, (F) holococcolithophores

The values are given as the means and the standard errors of the subsets of HOLP values belonging to the depth intervals (in m): 5–25, 26–50, 51–80, 81–160 (see Fig. 7). The corresponding points are located at the mean depth of the observations included in each subset.

## RESULTS

### Hydrographic features during the MESO-96 and FRONTS-96 cruises

As can be seen in Figs. 2 & 3, during both cruises there was marked stratification of the water column, with a sharper thermocline in June and July (MESO-96). The signature of the Catalan Front was apparent in the salinity distributions, approximately 40 to

50 km offshore of the continental coast. At the surface, chl *a* concentrations ranged between 0.1 and 0.2  $mg\ m^{-3}$  at most stations of FRONTS-96, but did not exceed 0.1  $mg\ m^{-3}$  offshore of the Catalan Front in MESO-96. The deep chl *a* maximum (DCM), which occurred in both cruises at depths of 50 to 70 m (Figs. 2C & 3C), was more marked in MESO-96, with chl *a* concentrations reaching up to 1.37  $mg\ m^{-3}$ , in comparison with only 0.60  $mg\ m^{-3}$  in FRONTS-96.

### Hetero- and holococcolithophore diversity and distribution

The total number of heterococcolithophore and holococcolithophore taxa was, respectively, 56 and 33 in MESO-96, and 47 and 18 in FRONTS-96. Holococ-

Table 2. Relative abundance of heterococcolithophores and holococcolithophores represented in MESO-96 cruise: heterococcolithophore (HET)-holococcolithophore (HOL) life cycle pairs, most abundant hetero- and holococcolithophores either without a recognized pair or with a pair not found in our samples, and most abundant heterococcolithophores without a HET-HOL life cycle

HET-HOL life cycle pairs			
Heterococcolithophores	%	Holococcolithophores	%
<i>Acanthoica quattrosospina</i> <sup>a</sup>	0.52	<i>A. quattrosospina</i> HOL, formerly <i>Sphaerocalyptra</i> sp. <sup>a</sup>	0.21
<i>Algirosphaera robusta</i>	0.52	<i>Sphaerocalyptra quadridentata</i> <sup>b</sup>	0.52
<i>Rhabdosphaera clavigera</i>	2.97	<i>Sphaerocalyptra quadridentata</i> <sup>b</sup>	
<i>Coronosphaera mediterranea</i> <sup>c</sup>	0.06	<i>C. mediterranea</i> HOL, formerly <i>Calyptrolithina wettsteinii</i>	1.31
<i>Coronosphaera mediterranea</i> <sup>c</sup>		<i>C. mediterranea</i> HOL, formerly <i>Zygosphaera hellenica</i>	0.15
<i>Helicosphaera carteri</i> <sup>c</sup>	3.18	<i>H. carteri</i> HOL, formerly <i>Syracolithus catilliferus</i>	2.29
<i>Helicosphaera carteri</i> <sup>c</sup>		<i>H. carteri</i> HOL, formerly <i>Syracolithus confusus</i>	0.42
<i>Syracosphaera anthos</i> <sup>a</sup>	0.21	<i>S. anthos</i> HOL, formerly <i>Periphyllophora mirabilis</i> <sup>a</sup>	0.33
<i>Syracosphaera amoena</i> <sup>c</sup>	0.17	<i>S. amoena</i> HOL, formerly <i>Corisphaera</i> sp. type A of Kleijne	0.19
<i>Syracosphaera amoena</i> <sup>c</sup>		<i>S. amoena</i> HOL formerly <i>Zygosphaera bannockii</i>	0.02
<i>Syracosphaera delicata</i> <sup>a</sup>	0.02	<i>S. delicata</i> HOL, formerly <i>Corisphaera</i> sp. type B of Kleijne	0.10
<i>Syracosphaera didyma</i> <sup>d</sup>	0.42	<i>Homozygosphaera arethusae</i> <sup>d</sup>	2.56
<i>Syracosphaera halldalii</i> <sup>e</sup>	1.35	<i>S. halldalii</i> HOL, formerly <i>Calyptrolithina divergens</i> var. <i>tuberosa</i>	0.17
<i>Syracosphaera histricea</i> <sup>d</sup>	0.23	<i>Calyptrolithophora papillifera</i> <sup>d</sup>	1.68
<i>Syracosphaera molischii</i> <sup>d</sup>	4.18	<i>Anthosphaera fragaria</i> <sup>d</sup>	0.69
<i>Syracosphaera nana</i> <sup>a</sup>	0.29	<i>S. nana</i> HOL <sup>a</sup>	0.19
<i>Syracosphaera nodosa</i> <sup>d</sup>	0.29	<i>Helladosphaera cornifera</i> <sup>d</sup>	5.65
<i>Syracosphaera pulchra</i> <sup>a</sup>	0.98	<i>S. pulchra</i> HOL, formerly <i>Calyptosphaera oblonga</i> <sup>a</sup>	0.10
<b>SUBTOTAL</b>	<b>15.39</b>	<b>SUBTOTAL</b>	<b>16.58</b>
<b>Most abundant HETs without HOL pair</b>		<b>Most abundant HOLs without HET pair</b>	
<i>Rhabdosphaera xifos</i>	5.15	<i>Holococcolithophora heimdaleae</i>	2.64
<i>Umbellosphaera tenuis</i>	2.74	<i>Sphaerocalyptra</i> cf. <i>adenensis</i>	2.10
<b>SUBTOTAL</b>	<b>7.89</b>	<b>SUBTOTAL</b>	<b>4.74</b>
<b>Most abundant HETs without HET-HOL life cycle</b>			
<i>Emiliana huxleyi</i>	31.81		
<i>Gephyrocapsa ericsonii</i>	6.54		
<b>SUBTOTAL</b>	<b>38.35</b>	<b>SUM OF SUBTOTALS</b>	<b>82.95</b>
<sup>a</sup> HET-HOL pair established in Cros et al. (2000) <sup>b</sup> Holococcolithophore that has been related to 2 heterococcolithophores (Cros & Fortuño 2002, Triantaphyllou & Dimiza 2003) <sup>c</sup> Heterococcolithophore that has been related to 2 or more holococcolithophores (Cros et al. 2000, Geisen et al. 2002, Dimiza et al. 2008b) <sup>d</sup> Pair subject to confirmation (Cros et al. 2000) <sup>e</sup> HET-HOL pair established in Triantaphyllou et al. (2004)			

colithophores represented 29.8% of the coccospheres in MESO-96 and 4.8% in FRONTS-96. In some cases, one heterococcolithophore (or holococcolithophore) morphotype was recorded in association with, respectively, 2 or more holococcolithophore (or heterococcolithophore) morphotypes; however, for simplicity, we will refer to these associations as 'pairs'. Tables 2 & 3 show the relative abundance of the hetero- and holococcolithophores represented in the samples. The most abundant species in both cruises was *Emiliana huxleyi*, which does not have a hetero-holococcolithophore life cycle (Tables 2 & 3). Detailed abundance data for the taxa considered here can be found in Tables S1 & S2 in the Supplement at [www.int-res.com/articles/suppl/m492p057\\_supp.xls](http://www.int-res.com/articles/suppl/m492p057_supp.xls).

The distribution of total heterococcolithophores, heterococcolithophores belonging to Hol-Het life cycle families (abbreviated as Het\_HHLC) and holococcolithophores is represented in Figs. 2 & 3. The distributions of some hetero-holococcolithophore pairs of each cruise are shown in Figs. 4 to 6. The choice of these examples took into account, first, that the association was a well-established one, with only one heterococcolithophore morphotype involved, and second, that the heterococcolithophore or both phases presented relatively high abundances. The concentration of total heterococcolithophores and Het-HHLC was broadly similar in the 2 cruises, while that of holococcolithophores was markedly lower in FRONTS-96. Population

Table 3. Relative abundance of heterococcolithophores and holococcolithophores represented in FRONTS-96 cruise: heterococcolithophore (HET)-holococcolithophore (HOL) life cycle pairs, most abundant hetero- and holococcolithophores either without a recognized pair or with a pair not found in our samples, and most abundant heterococcolithophores without a HET-HOL life cycle

<b>HET-HOL life cycle pairs</b>			
Heterococcolithophores	%	Holococcolithophores	%
<i>Coronosphaera mediterranea</i> <sup>c</sup>	0.31	<i>C. mediterranea</i> HOL, formerly <i>Calyptrolithina wettsteinii</i> <sup>c</sup>	0.19
<i>Helicosphaera carteri</i> <sup>a</sup>	0.56	<i>H. carteri</i> HOL, formerly <i>Syracolithus catilliferus</i> <sup>a</sup>	0.06
<i>Scyphosphaera apsteinii</i> <sup>b</sup>	0.06	<i>S. apsteinii</i> HOL, formerly <i>Syracolithus schilleri</i> <sup>b</sup>	0.13
<i>Syracosphaera anthos</i> <sup>a</sup>	0.31	<i>S. anthos</i> HOL, formerly <i>Periphylliphora mirabilis</i> <sup>a</sup>	0.19
<i>Syracosphaera amoena</i> <sup>c</sup>	0.06	<i>S. amoena</i> HOL, formerly <i>Corisphaera</i> sp. type A of Kleijne <sup>c</sup>	0.06
<i>Syracosphaera didyma</i> <sup>d</sup>	0.44	<i>Homozygosphaera arethusae</i> <sup>d</sup>	0.88
<i>Syracosphaera histrica</i> <sup>d</sup>	1.00	<i>Calyptrolithophora papillifera</i> <sup>d</sup>	0.19
<i>Syracosphaera molischii</i> <sup>d</sup>	1.69	<i>Anthosphaera fragaria</i> <sup>d</sup>	0.31
<i>Syracosphaera nodosa</i> <sup>d</sup>	0.25	<i>Helladosphaera cornifera</i> <sup>d</sup>	0.38
<b>SUBTOTAL</b>	<b>4.68</b>	<b>SUBTOTAL</b>	<b>2.39</b>
<b>Most abundant HETs without HOL pair</b>		<b>Most abundant HOLs without HET pair</b>	
<i>Rhabdosphaera clavigera</i>	7.27	<i>Holococcolithophora sphaeroidea</i>	0.38
<i>Syracosphaera ossa</i>	3.01	<i>Corisphaera</i> spp.	1.13
<i>Syracosphaera pulchra</i>	1.76	<b>SUBTOTAL</b>	<b>1.51</b>
<i>Umbellosphaera tenuis</i>	7.59		
<b>SUBTOTAL</b>	<b>19.63</b>		
<b>Most abundant HETs without HET-HOL life cycle</b>			
<i>Emiliana huxleyi</i>	45.45		
<i>Gephyrocapsa ericsonii</i>	13.29		
<b>SUBTOTAL</b>	<b>58.74</b>	<b>SUM OF SUBTOTALS</b>	<b>86.95</b>
<sup>a</sup> HET-HOL pair established in Cros et al. (2000) <sup>b</sup> HET-HOL pair established in Frada et al. (2009) <sup>c</sup> Heterococcolithophore that has been related to 2 or more holococcolithophores (Cros et al. 2000, Geisen et al. 2002, Dimiza et al. 2008b) <sup>d</sup> Pair subjected to confirmation (Cros et al. 2000)			

densities of total heterococcolithophores and Het\_HHLC tended to present subsurface maxima in June (MESO-96) and to be highest in the near surface layers in September (FRONTS-96). *Emiliana huxleyi*, which dominated the heterococcolithophore community, was generally more abundant around 40 to 60 m of depth (data not shown). As can be seen in Figs. 2F,I & 3F, the holococcolithophores were found in the upper meters of the water column, while the heterococcolithophores, although sometimes present also at the surface, tended to occupy deeper waters and to present deeper population maxima. The recorded hetero- and holococcolithophore life stages (see examples in Figs. 4 to 6) reproduced this pattern, although their distributions were often patchy. The preference of holococcolithophores for shallower depths than heterococcolithophores was reflected in the vertical distribution of the HOLL index, which decreased markedly with depth in the 2 cruises (Fig. 7), although its surface values in June were more than 3 times larger than in September.

## DISCUSSION

The ecological characteristics of the open NW Mediterranean marine ecosystem are intimately linked to the seasonal changes in environmental forcing and the annual cycles of mixing and stratification of the water column. Typically, phytoplankton biomass increases in late winter or spring, after winter mixing injects nutrients into the euphotic zone, and decreases towards the summer, when nutrients become depleted in the upper mixed layer. During the stratification period, maximum chl *a* concentrations, generally reflecting both higher phytoplankton biomass and enhanced chl *a* content per cell, are found at the bottom of the euphotic zone. In autumn, weakening of the pycnocline may produce a second phytoplankton peak (D'Ortenzio & Ribera d'Alcalà 2009). In the area of study, inshore of the Catalan Front, the winter-spring bloom is dominated by diatoms, but offshore of this front the dominant phytoplankton forms are haptophytes and dinoflagellates. As stratification increases, the contribution of these

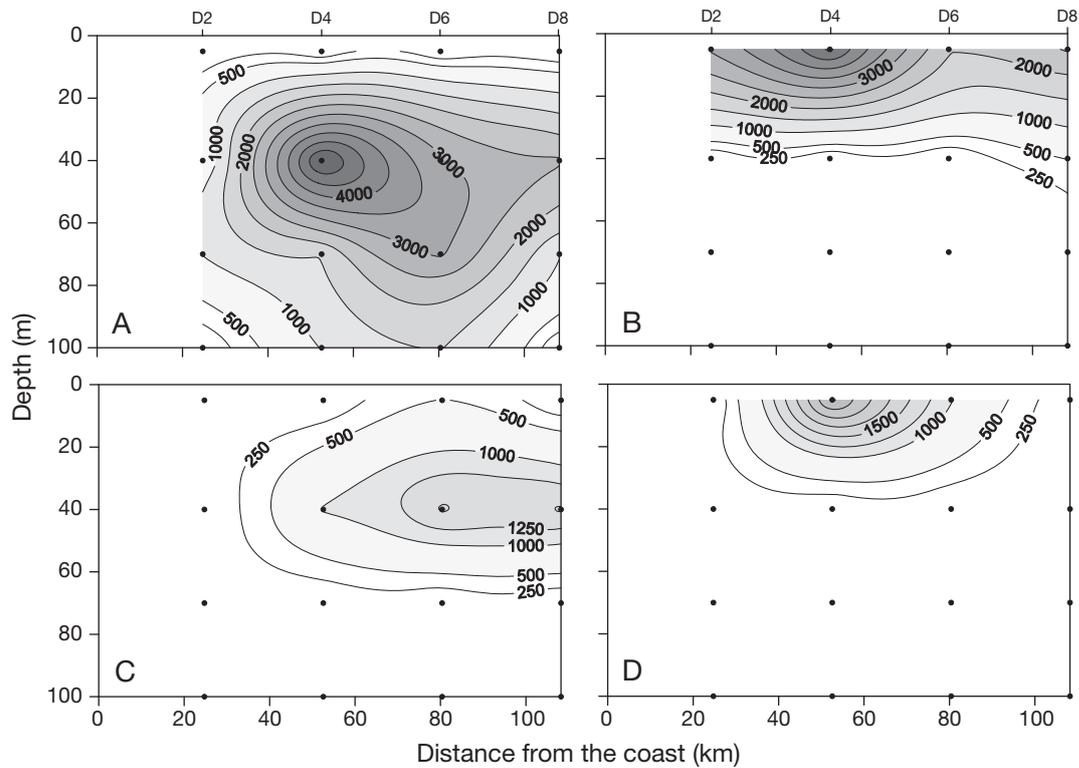


Fig. 4. Distribution of representative hetero-holococcolithophore pairs in transect D of MESO-96 (values in cells  $l^{-1}$ ). In (A) and (B), respectively: *Helicosphaera carteri* HET and *H. carteri* HOL, formerly *Syracolithus catilliferus*. In (C) and (D), respectively: *Syracosphaera halldalii* HET and *S. halldalii* HOL, formerly *Calyptrolithina divergens* var. *tuberosa*

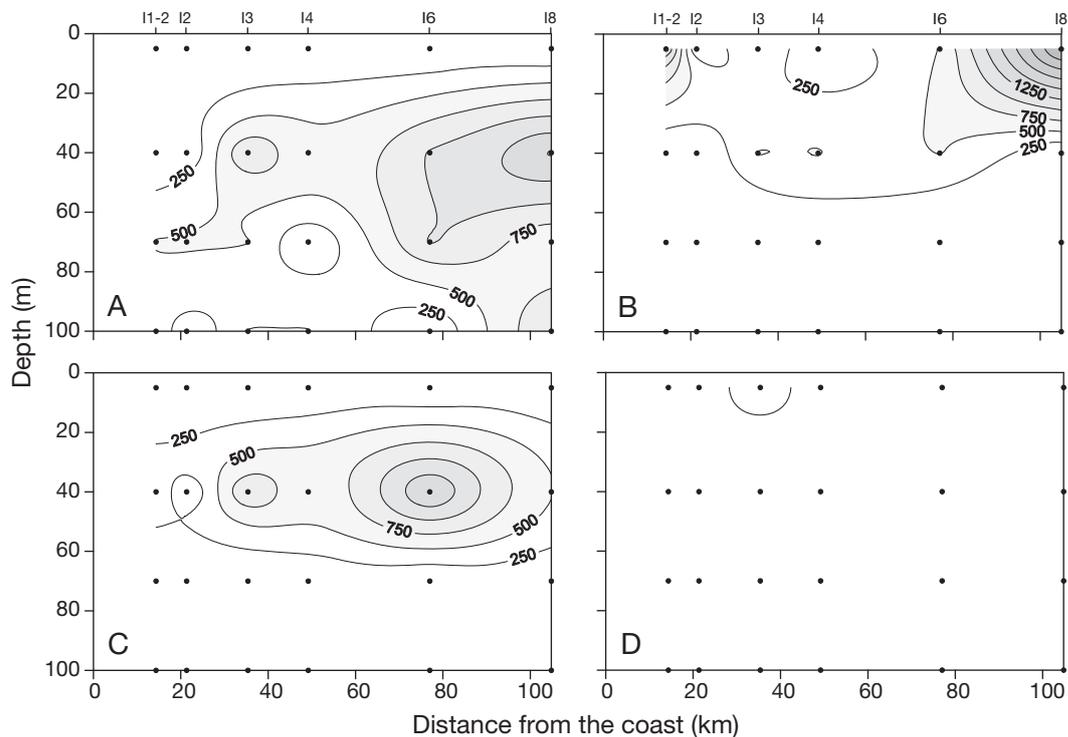


Fig. 5. Distribution of representative hetero-holococcolithophore pairs in transect I of MESO-96 (values in cells  $l^{-1}$ ). In (A) and (B), respectively: *Helicosphaera carteri* HET and *H. carteri* HOL, formerly *Syracolithus catilliferus*. In (C) and (D), respectively: *Syracosphaera halldalii* HET and *S. halldalii* HOL, formerly *Calyptrolithina divergens* var. *tuberosa*

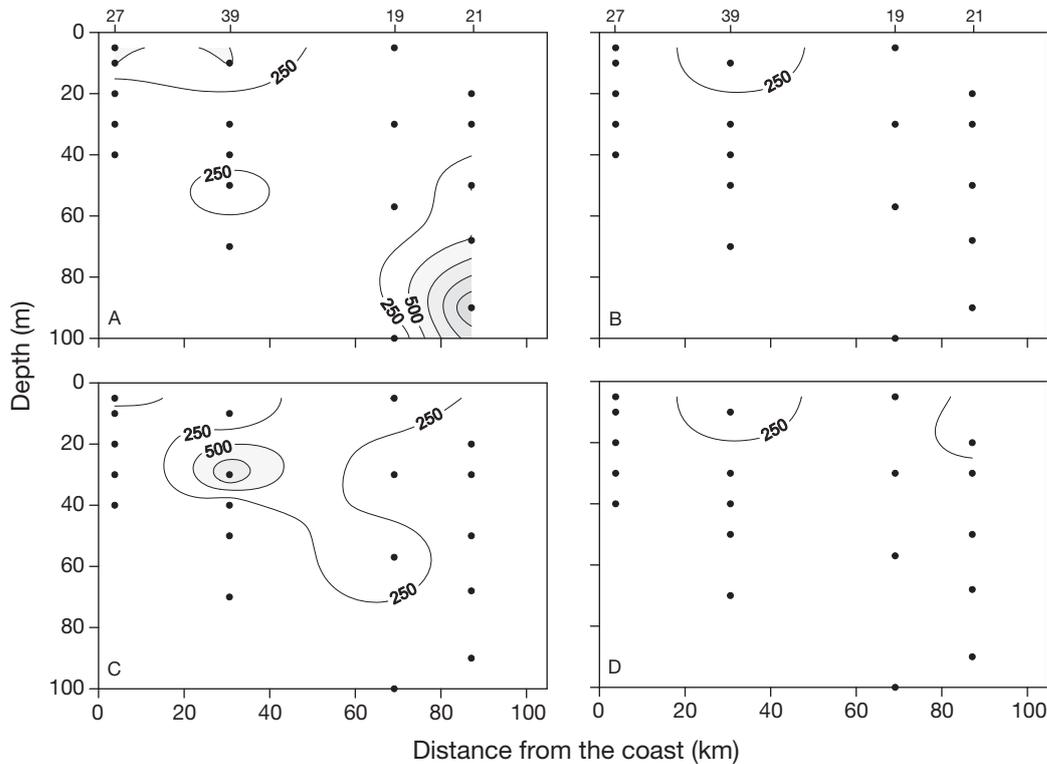


Fig. 6. Distribution of representative hetero-holococcolithophore pairs in FRONTS-96 (values in cells  $l^{-1}$ ). In (A) and (B), respectively: *Helicosphaera carteri* HET and *H. carteri* HOL, formerly *Syracolithus catilliferus*. In (C) and (D), respectively: *Coronosphaera mediterranea* HET and *C. mediterranea* HOL, formerly *Calyptrolithina wettsteinii*

groups becomes more important, while diatom concentrations decrease. MESO-96 (June) and FRONTS-96 (September) were carried out, respectively, about one month before and one month after the period of maximum vertical stratification, which tends to occur between late July and end of August (Segura-Noguera et al. 2011), and presented similar stratification conditions (as measured by estimations of the Brunt-Vaisälä frequency; data not shown). Both

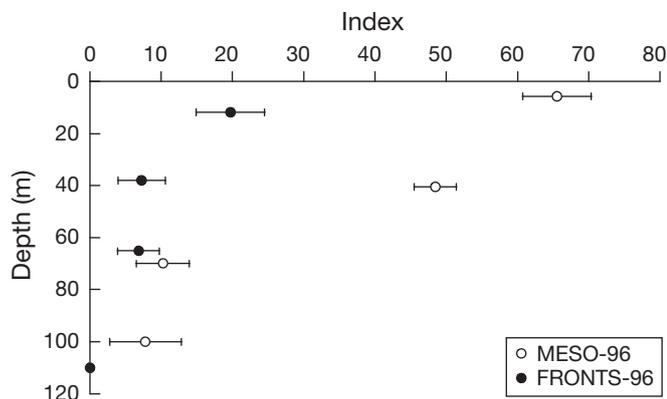


Fig. 7. Vertical distribution of the mean ( $\pm$  SE) holococcolithophore prevalence index for different depth intervals in the cruises MESO-96 and FRONTS-96

cruises showed fairly similar distributions of physico-chemical variables, but the deep chlorophyll maximum, a typical feature of the stratification period, was more intense (Figs. 2 & 3) in MESO-96 (June and July) than in FRONTS-96 (September). Holococcolithophores presented higher population densities and deeper distributions in June than in September (Figs. 2 to 6). In addition, in June and July, the coccolithophore diversity was higher and the dominance of *Emiliania huxleyi* and *Gephyrocapsa ericsonii* lower than in September (Tables 2 & 3). These differences could indicate a relatively higher nutrient availability in September, probably related to the incipient weakening of the pycnocline. Our findings agree with those of Dimiza et al. (2008b), who reported a higher diversity of holococcolithophores in the Aegean Sea during the more oligotrophic summer period. A temporal change between the relative abundance of the motile (holococcolithophore) and non-motile (heterococcolithophore) phases of *Coccolithus pelagicus*, with a high contribution of the motile form preceding that of the non-motile one had been noted by Okada & McIntyre (1979), who also found that population peaks of *E. huxleyi* occurred at different times of the year than those of both stages of *C. pelagicus*.

The distribution of life-cycle stages and the HOLP index indicate a prevalence of the holococcolithophore phase in shallow layers and a preferential location of the heterococcolithophore phase in deeper waters (Figs. 2 to 7), in combination with temporal changes in the occurrence of the 2 phases. This segregation could be linked to a differentiation of ecological niches, with the haploid holococcolithophores inhabiting the more oligotrophic surface layers and the diploid heterococcolithophores occupying relatively richer waters below the mixed layer. This interpretation is supported by the experimental work of Houdan et al. (2006), who considered the conceptual model of Margalef (1978) and proposed that the diploid heterococcolithophores and the haploid holococcolithophores might be exploiting a range of trophic environmental situations, with ecological niches closer to those of diatoms for heterococcolithophores and to those of dinoflagellates for holococcolithophores. A holococcolithophore strategy of inhabiting the oligotrophic surface waters would agree with the 'nutrient scarcity' hypothesis of Lewis (1985), who concluded that factors favouring small cell size, such as low nutrient availability, should favour haploidy in single-celled organisms (in which lower DNA content is often associated with smaller cell sizes than those of the diploid stage). Although in general the examined holo-heterococcolithophore pairs did not present a substantially different cell size (Cros 2002, Cros & Fortuño 2002, Young et al. 2003), it could still be argued that DNA replication requires more nutrients (such as P or N) per cell for diploid than for haploid cells. Another factor that could help to explain the niche separation is the existence of mixotrophy in the haploid stage, as shown for *Coccolithus braarudii* HOL, which could supplement its diet by preying on small bacteria (Houdan et al. 2006). This ability could help holococcolithophores to supplement their diets in the nutrient-poor shallower waters. Whereas 'nutrient scarcity' and related hypotheses could provide an explanation for holo-heterococcolithophore segregation, there might be other relevant factors, such as UV radiation. Quintero-Torres et al. (2006) used a dipolar multiscattering model to conclude that the crystalline photonic structure of certain holococcoliths enhanced UV backscattering and suggested that this property could represent an advantage for these holococcolithophores, by improving their ability to live higher in the water column than other coccolithophores.

The ecological differentiation between haploid and diploid phases in coccolithophores could also have ecological implications for taxa from non-HHLC

families, such as the ubiquitous *Emiliania huxleyi*. The life cycle of this coccolithophore comprises a diploid, non-motile, coccolith-bearing phase, able to form extensive blooms, and a haploid flagellated phase with a cell covering of non-mineralized organic scales, which is difficult to recognize by standard microscopical methods and has been mostly overlooked. While *E. huxleyi* blooms are often terminated by viral infection, the haploid phase of *E. huxleyi* appears to be resistant to the viruses that kill the diploid phase (Frada et al. 2008). These authors show also that viral exposure may induce transition from the diploid to the haploid phase and conclude that this 'Cheshire cat' escape strategy releases host evolution from pathogen pressure. The mechanisms underlying the resistance of haploid *E. huxleyi* could include a protective effect of the organic scale covering or the absence of molecules recognized by the viruses (Frada et al. 2008), but there is no conclusive evidence and it is not known whether viral infection could have a relationship with life stage transitions in coccolithophores with calcified haploid phases. Rokitta et al. (2011) found that the haploid and diploid life-cycle stages of *E. huxleyi* presented different gene expression patterns and that both haploid and diploid individuals of *E. huxleyi* in late stationary culture stage could engulf particles of 0.5  $\mu\text{m}$  diameter by phagocytosis. According to these authors, the smaller genome and more basic transcriptome of the haploid stage could minimize cellular energetic and material needs (Rokitta et al. 2011). Surveys of calcified (diploid) and non-calcified (haploid) cells of *E. huxleyi* using molecular techniques suggested a scenario in which both life stages coexisted in the same water body, following parallel population dynamics (Frada et al. 2012), with calcified cells representing the most abundant phase and non-calcified cells increasing their contribution at specific time windows, such as the end of the exponential phase, coinciding with bursts of *E. huxleyi* viruses. Thus, the different morphology and marked physiological differentiation among haploid and diploid stages strongly strengthens the view that diploid and haploid cells may exploit different spatio-temporal niches, displaying distinct roles in nature (Rokitta et al. 2011, Frada et al. 2012). The scarcity of data on the distribution of the haploid phase of *E. huxleyi* hinders the consideration of other ecological aspects of life stage differentiation in this species; however, this situation could improve soon with the implementation of molecular techniques (Frada et al. 2006).

The preference of holococcolithophores for shallower levels of the water column than those occupied

by heterococcolithophores (Fig. 7), which may be related to drivers such as gradients in nutrient availability or UV irradiance, can be seen as a strategy for a more efficient exploitation of available ecological niches (Valero et al. 1992, Hughes & Otto 1999). Further research on the biphasic life cycle of coccolithophores, in particular regarding the distribution of the holococcolithophore stage, would help not only to understand the population dynamics of these biogeochemically important organisms, but would also provide insight into the general significance of haploid-diploid life cycle strategies.

**Acknowledgements.** We thank J. Salat for providing unpublished physico-chemical data and the teams of MESO-96 and FRONTS-96 for sample collection and chlorophyll analyses. We are very grateful to J.-M. Fortuño for assistance with the scanning electron microscope and to L. Arin for help with the figures. This research was supported by the CSIC, the Grup de Recerca Consolidat 2009 SGR 588 of the Generalitat of Catalonia and the projects AMB94-083 (CICYT) and CODENET (FRMX-ET97-0113).

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Editorial responsibility: Graham Savidge,  
Portaferry, UK

Submitted: January 18, 2013; Accepted: July 6, 2013  
Proofs received from author(s): October 11, 2013