Coastal zones as important habitats of coccolithophores: A study of species diversity, succession, and life-cycle phases

Jelena Godrijan [iD],[1,2]* Jeremy R. Young,[3] Daniela Marić Pfannkuchen,[2] Robert Precali,[2] Martin Pfannkuchen[2]

[1]Division for Marine and Environmental Research, Ruđer Bošković Institute, Zagreb, Croatia
[2]Centre for Marine Research, Ruđer Bošković Institute, Rovinj, Croatia
[3]Department of Earth Sciences, University College London, London, United Kingdom

Abstract

Coccolithophores are unicellular calcifying eukaryotes with a complex life-cycle; they are important primary producers, and major drivers of global biogeochemical cycles. The majority of research on coccolithophores has been focused on open ocean waters, while the knowledge of their roles in coastal ecosystems is limited. Early 20th century studies of the Adriatic coast produced major taxonomic papers describing half of the projected diversity of coccolithophores. Afterwards, there have been only scarce records of their diversity and ecology in the Adriatic, and coastal systems in general. We aimed to assess coccolithophore diversity and species succession, and closely examine their ecological preferences in the coastal area of the northern Adriatic. We recorded coccolithophores throughout the year at 100% frequency, documented 52 taxa, abundances as high as 2.4×10^5 cells L^{-1}, and noted the winter domination over the phytoplankton community. Out of 52 observed coccolithophore taxa, 31 were heterococcolithophorids and 21 holococcolithophorids. Moreover, seven pairs of heterococcolith and holococcolith phases were noted, and two strategies of temporal separation of life-cycle phases were observed. We report ecological preferences of those life-cycle pairs and give in situ evidence that holo- and heterococcolith phases are widening the specie's ecological niches. This expansion allows the species to exploit a wider range of ecological conditions and enables phenotypic plasticity, important in response to changes in the environment. With this study we emphasize that coastal areas are highly productive for coccolithophore studies. Our results indicate that a shift in research effort on coccolithophores in the coastal waters is needed.

*Correspondence: jelena.godrijan@irb.hr

Coccolithophores are unicellular, planktonic eukaryotes with cells enclosed by calcareous plates, coccoliths. They are ubiquitous in oceanic environments and play a major role in global biogeochemical cycles (Winter and Siesser 1994). Even though they are mostly cosmopolitan species, they do exhibit specific ecological preferences (Quinn et al. 2004). In consequence, they are used by palaeontologists as proxies of environmental conditions, especially of past global changes, via their ample fossil record (Baumann et al. 2005). Studies on living coccolithophores are invaluable in calibrating species-specific ecological tolerances which are widely used in paleoceanographic reconstructions (Young et al. 2005). In

addition, knowledge of their distribution and ecology helps us understand past marine ecosystems and predict how they may be affected by human activities, through climate change and eutrophication (Boyd et al. 2010; Milner et al. 2016).

Due to their complex life-cycle (Parke and Adams 1960) there have been many taxonomic ambiguities, and open questions still exist in the biology of many species. Coccolithophores in general have a heteromorphic life-cycle, with the haploid and diploid phases being characterized by the synthesis of different types of coccoliths (Billard 1994). The majority of species have a diploid phase with heterococcoliths, and a haploid phase with holococcoliths (Billard and Inouye 2004), and they were described as different taxa. Meticulous studies of environmental samples revealed rare combination coccospheres, enabling the linkage between diploid and haploid phases, and started the resolution of taxonomy (Cros et al. 2000; Kleijne and Cros 2009; Triantaphyllou et al. 2015). Experiments with cultured coccolithophores showed that nutrient, light, and temperature stress can be important in inducing life-cycle alterations (Billard and

Inouye 2004), indicating that changes between pelagic and coastal environments, together with changes in seasonal conditions, might be the main drivers of their biology (Nöel et al. 2004). Nevertheless, coccolithophores remain a phytoplankton group whose environmental controls are poorly understood (Boyd et al. 2010).

Coccolithophores are traditionally observed as open ocean plankton, and their diversity in coastal areas is perceived as rather low (Baumann et al. 2005). The coastal zone is a sensitive and unstable ecosystem under the influence of rivers, urban activities, meteorological elements, and various physical factors (circulation, morphology of the coast, and the relief of the seabed) (Ramesh et al. 2015). Recent studies indicate a potential importance of coastal zones for the coccolithophore evolution and ecology. The hypothesis was raised that adaptation to this varying environment is a significant factor in the evolutionary development, and calcareous plankton in general (Bown 2005). This was further developed by recovery of the living fossil *Tergestiella adriatica* from the coastal waters of Japan and Croatia (Hagino et al. 2015). *Tergestiella* is a direct descendant of *Cyclagelosphaera* (presumably extinct since Eocene ∼ 54 Ma), and survived due to adaptation to near shore environments (Hagino et al. 2015), thus explaining the lack of fossil recorded in open ocean sediments during last 54 myr. Hagino et al. (2015) pointed out that their findings support the hypothesis of selective survivorship of coastal coccolithophores during major extinction events, as coastal organisms are more likely to cope with more variability, and are thus more ready to withstand sudden environmental changes. But the exact cause of differential survival between coastal and oceanic taxa is still not known. Further, von Dassow et al. (2015) showed that coastal, highly productive, and seasonally cycling parts were populated with *Emiliania huxleyi* strains maintaining the biphasic life-cycle, while a loss of flagella and 1N phase occurred in some open ocean sub-populations of *E. huxleyi*. von Dassow et al. (2015) speculate that this loss of the haploid phase could be related to lower biotic pressure and low environmental variability of the ocean, as opposed to highly variable environmental conditions of neritic environments that favor sexuality (von Dassow et al. 2015). Moreover, a study form the coast of the eastern Adriatic revealed high diversity and overall dominance of families with a holococcolith-heterococcolith lifecycle in the Mediterranean Sea, indicating that alternating life-cycles likely represent an evolutionary adaptation to highly seasonal environments (Šupraha et al. 2016). These studies point toward an intricacy of information yet to be discovered by researching coastal coccolithophores.

We aimed to examine the coccolithophore community in a yearlong cycle in the coastal area of the Adriatic Sea. The northern Adriatic has a significant place in the history of coccolithophore taxonomy, since it was particularly thoroughly surveyed by plankton taxonomists during the early 20th
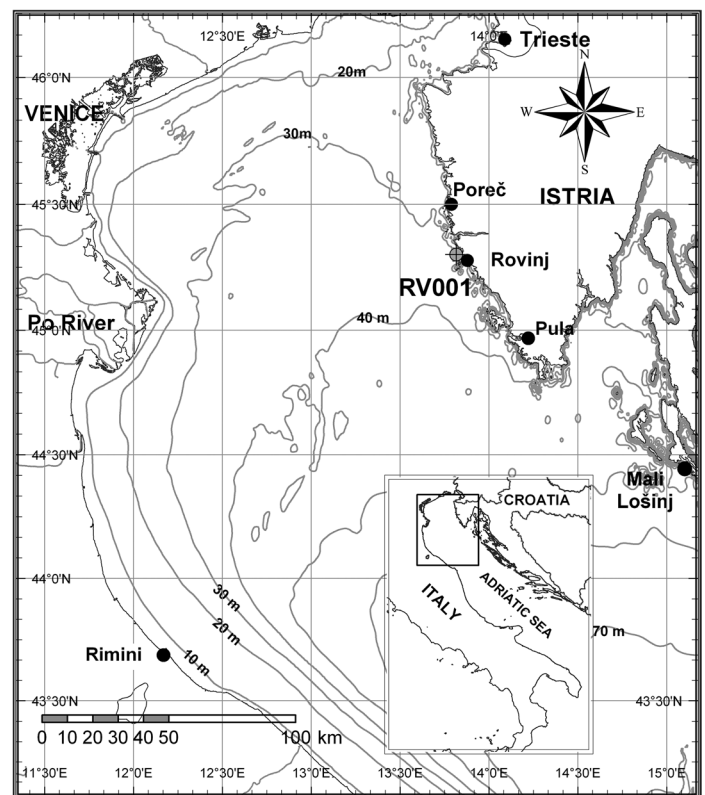

Fig. 1. Map of the investigated area.

century. Detailed descriptions of coccolithophorid species were given by Brunnthaler (1911), Schiller (1913, 1925), and Kamptner (1941), unfortunately without reference to their biological importance or annual cycle. After the initial period of taxonomic research, coccolithophores were only sporadically investigated in the Adriatic, integrated in studies of the whole phytoplankton community. Thus, our main goals were to (1) assess the diversity of coccolithophores in this coastal site, (2) investigate species-specific patterns and their succession, and (3) more closely examine ecological preferences in the light of new findings on paired life-cycle phases. What is more, we intended to illustrate that coccolithophore studies in coastal habitats are indeed imperative.

Materials and methods

Sampling

Sampling was performed in the northern Adriatic Sea (Supporting Information 1). Samples were taken twice a month, from September 2008 to December 2009, at a coastal station one nautical mile off Rovinj, Croatia (45°04.8′ N, 13°36.6′ E, depth = 29 m) (Fig. 1). Sampling was carried out by research vessels "Burin" and "Villa Velebita." Conductivity–Temperature–Depth (CTD) profiles were recorded with an SBE 25 Sealogger CTD probe (Sea-Bird Electronics, Bellevue, Washington, U.S.A.). Seawater samples for nutrients, chlorophyll *a* (Chl *a*), and phytoplankton/coccolithophore analysis
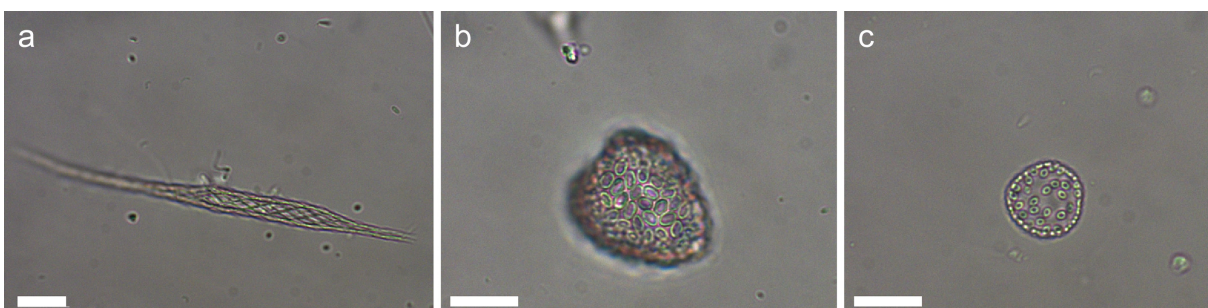
Fig. 2. Light micrographs: (**a**) *Anoplosolenia brasiliensis*, (**b**) *Syracosphaera pulchra* HOL *oblonga* type, and (**c**) miscellaneous taxon. Scale bars 10 μm.

were taken with 5 L Niskin bottles at standard oceanographic depths (surface, 5 m, 10 m, 20 m, and 2 m above bottom).

Analytical protocol

Nutrient analysis for nitrate (NO_3^-), nitrite (NO_2^-), orthophosphate (PO_4^{3-}), and orthosilicate (SiO_4) were made on a Shimadzu UV-Mini 1240 spectrophotometer with 10 cm cells following Parsons et al. (1984). Ammonium (NH_4^+) was analyzed by a modification of the indophenol method (Ivančič and Degobbis 1984). Total inorganic nitrogen (TIN) was obtained by summing nitrate, nitrite, and ammonium.

For estimation of phytoplankton/coccolithophore abundance we followed the standard phytoplankton counting method (Utermöhl 1958), regularly used in the Rovinj long-term phytoplankton time series (Marić et al. 2012). Phytoplankton abundance was estimated after 48 h sedimentation of 50 mL subsamples fixed with neutralized formaldehyde (2% final concentration) in Utermöhl chambers (Hydro-Bios, Kiel, Germany). Phytoplankton was counted on a transect at ×400 or ×200 magnification using an Axiovert 200 inverted light microscope (Zeiss GmbH, Oberkochen, Germany) and cells were identified following Tomas (1997). When necessary, coccolithophore cells were examined under ×1000 magnification and ones that could not be identified to the species level were classified as miscellaneous (Fig. 2).

Usually, only 16 coccolithophore species were documented under light microscopy in the northern Adriatic (Viličić et al. 2009). Thus, for detailed floristic study of coccolithophore species succession and biodiversity we further examined a subset of the samples with a scanning electron microscope (SEM). We selected samples from December 2008 to December 2009 from each sampled date ($N = 29$, Supporting Information Table 1). Depth of 5 m was chosen prior to Utermöhl counting. Additional samples were chosen at random: from 0 m for date 14th August 2009, from 10 m for 30th September and 29th October 2009, and from 20 m for 21st May 2009. For comparable results of cell counts from light microscopy and SEM, approximately the same volume ($\sim$ 50 mL) was filtered from the original fixed sample on a 25 mm diameter filter (Whatman, Maidstone, UK), chosen to achieve the suitable cell density for the analyses. Filters were rinsed with tap water

and dried in an oven at 50°C. Filter segments of circa 1 cm^2 were mounted on aluminium stubs and sputter coated with gold-palladium (208HR Cressington Scientific, Watford, UK) or gold (S150A Sputter coater, Edwards Ltd., Crawley, United Kingdom) for observations under SEM (Phillips XL30 FEG SEM and Philips 515 SEM, Amsterdam, The Netherlands). The whole area of the mounted filter was examined and cells counted. Taxonomic classification of the coccolithophores followed Young et al. (2003), Kleijne and Cros (2009), Young and Bown (2014) and the electronic guide to the biodiversity and taxonomy of coccolithophores Nannotax 3 (http://www.mikrotax.org/Nannotax3/index.html). Contributions of each species to the coccolithophore community within each sample were calculated.

Data analysis

Principal component analysis (PCA) was applied to investigate relationships between environmental factors, and diatom and coccolithophore abundances. The analysis was based on Bray–Curtis coefficients, computed from the $\log(x + 1)$ transformed data of diatom and coccolithophore light microscopy abundances, and physico-chemical data. The analysis included data from all sampled depths. Additionally, samples were ordinated on the plot and labeled according to their season: spring (March–May), summer (June–August), autumn (September–November), and winter (December–February), corresponding to a mixed winter and summer stratified water column, and the two mixed seasons (spring and autumn).

The SEM counts were used for calculations of diversity indices, SIMPER analysis, and Canonical correspondence analysis (CCA). These counts were used as relative abundance data representing percentages of each species in the coccolithophore community within each sample (Supporting Information Table 1). Margalef species richness (Margalef 1951) and Shannon Wiener (H') diversity index (Shannon 1948) were calculated in order to understand the structure of the coccolithophore community. Species principally contributing to differences in community structure in each season were investigated using the SIMPER analysis (Clarke and Warwick 2001). We used the relative abundance data to first
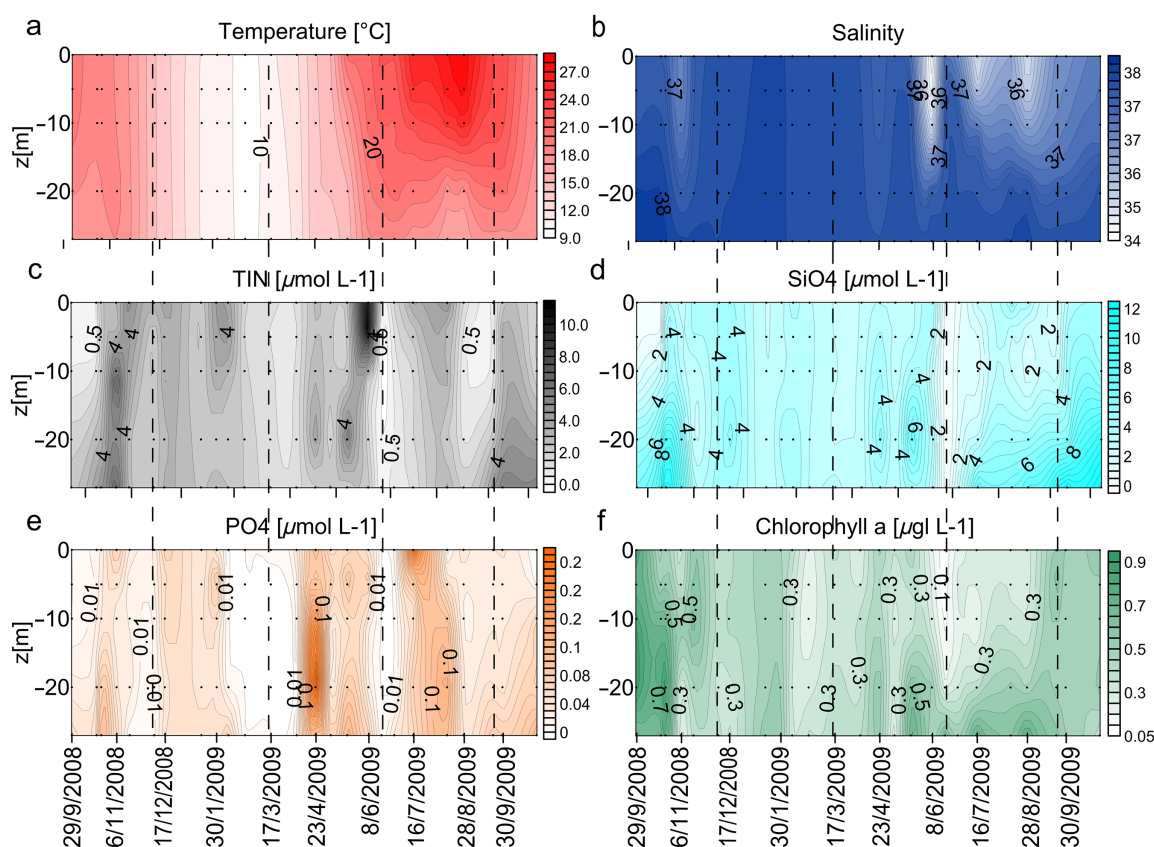
Fig. 3. Plots of temperature (**a**), salinity (**b**), total inorganic nitrogen (**c**), orthosilicate (**d**), orthophosphate (**e**), and Chl *a* (**f**) during the investigated period 2008–2009. Dashed lines are indicating seasons.

construct the Bray–Curtis similarity matrix, and afterwards calculate contributions of each separate species. Further, CCA (Braak and Šmilauer 2002) was used to investigate relationships between species composition and environmental factors. The species were selected on the basis of maximal contribution to the community of above 6% (in total 26 species) with addition of identified heterococcolithophore and holococcolithophore pairs. Relative abundance data and environmental data were $\log(x + 1)$ transformed to obtain normal distribution. Furthermore, a nonparametric statistical analysis, Spearman rank correlation, was made between environmental variables and selected species to further confirm their relations. All statistical analyses were performed with Statistica (StatSoft, Tulsa, U.S.A.), Primer (PIMER-E Ltd, Plymouth, UK), and Canoco (Biometris, Wageningen, The Netherlands) softwares. Graphical representations of data were done with Grapher and Surfer softwares (Golden software, Golden, U.S.A.).

Results

Environmental conditions and coccolithophore dynamics

During the investigated period, seawater temperature was the coldest in February (9.26°C) and warmest in August (27.48°C). The stratification period began in May and lasted until October (Fig. 3). Throughout winter and early spring salinity averaged 37.77 (maximum 38.18), while during summer and autumn it dropped to 36.91 (minimum 34.22) in upper layers. There were three marked decreases of salinity on 8th June (S 34.22), 20th July (S 34.77), and 28th August (S 35.04) (Fig. 3). Concentrations of nutrients ranged from: the detection limit to 0.26 μmol L^{-1} for PO$_4^{3-}$, from 0.15 μmol L^{-1} to 17.51 μmol L^{-1} for TIN, and between 0.19 μmol L^{-1} and 11.75 μmol L^{-1} for SiO$_4$. During the annual cycle the highest Chl *a* was recorded in September 2008 (850 μg L^{-1}) (Fig. 3).

Šilović et al. (2012), Godrijan et al. (2013), and Bosak et al. (2016) provided an overview of the environmental conditions and phytoplankton community along the northeastern Adriatic during 2008 and 2009, and noted that coccolithophores were, alongside diatoms, the most abundant group. Thus, here we focus only on abundances of coccolithophores, and the alternation of their dominance with diatoms (Fig. 4). Highest abundances of coccolithophores were recorded on 17th January 2009 (2.4 $\times$ 10^5 cells L^{-1}). Coccolithophore abundances were also high ($< 10^5$ cells L^{-1}) throughout the water column on 21st May 2009, and during winter on 8th December 2008, 17th December 2008, 17th
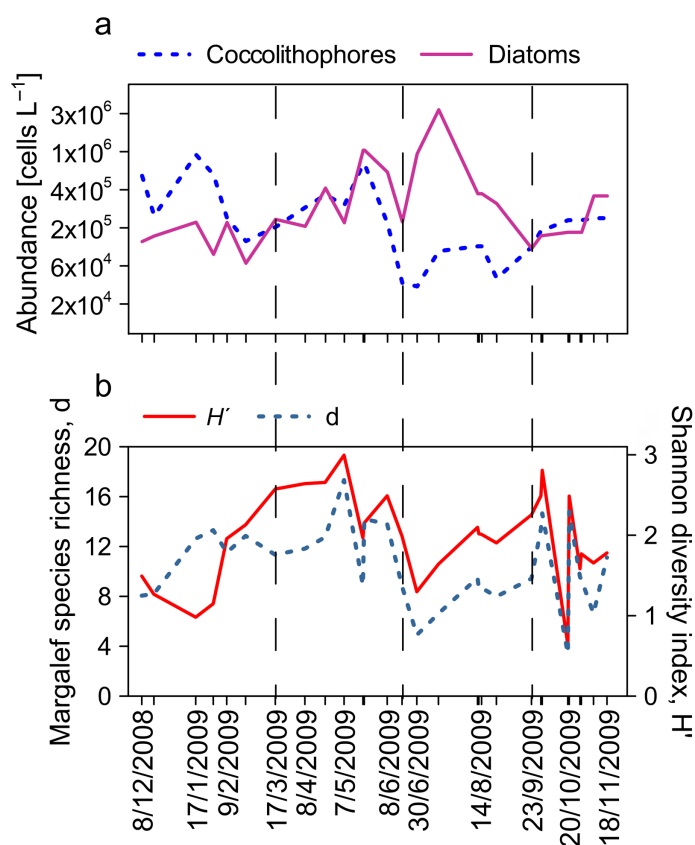
Fig. 4. (**a**) Coccolithophore and diatom abundances aggregated over depth, data used from light microscopy cell counts; (**b**) the distribution of species diversity as measured by the Margalef and Shannon indices and comparison, here the scanning electron microscopy floristic study was used for selected samples from each date. Dashed lines are indicating seasons.

Fig. 5. Representation of the PCA analysis of environmental factors and diatom and coccolithophore abundance. Symbols on the scatter plot represent each sampling point in the study. All dates, stations, and depths are included and grouped according to seasons.

January, and 30th January. Highest abundances of diatoms were recorded on 16th July 2009 at 5 m depth (1.4×10^6 cells L^{-1}), and at surface (1.1×10^6 cells L^{-1}). This high abundance during rather low Chl *a* concentrations (Fig. 3) was attributed to *Chaetoceros vixvisibilis* and discussed in Bosak et al. (2016). Elevated diatom abundances ($< 3.5 \times 10^5$ cells L^{-1}) were also recorded on 21st May 2009 in bottom layers, and in autumn throughout the water column on 29th September 2009, 20th October 2009, and 24th October 2009.

Physico-chemical data were analyzed in relation to Utermöhl counts of coccolithophores and diatoms by PCA (Fig. 5). The first two principal components (PC1 and PC2) accounted for 54.4% of the cumulative variation, with the first axis explaining 29.4% and the second axis 25.0%. PC1 was mostly positively related to nutrients, while PC2 was mostly positively related to temperature and diatoms, and negatively with salinity and coccolithophores. Winter was the most aggregated season and distribution of samples was governed by salinity and nitrite, while other seasons were more dispersed (Fig. 5). Summer samples were distributed
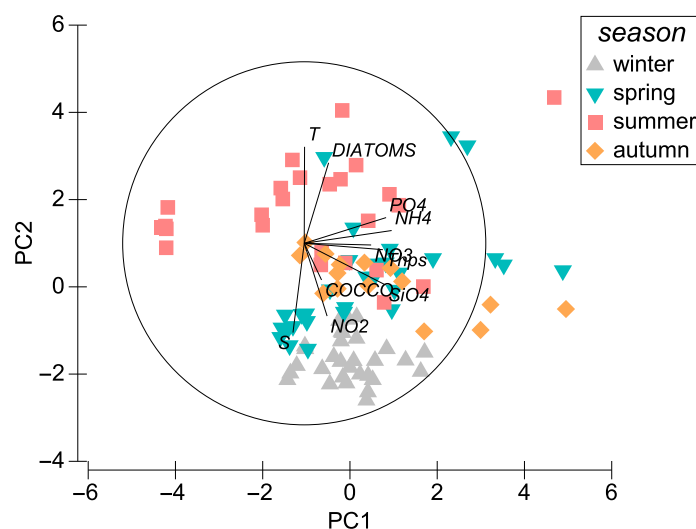
around the temperature arrow, while spring and autumn around nutrient arrows.

Coccolithophore floristic composition and diversity indices

During the study period we observed 52 taxa of coccolithophores (Table 1), out of which 31 were heterococcolithophorids and 21 holococcolithophorids. The most diverse was the order Syracosphaerales, and within this order the genus *Syracosphaera* with 19 recorded taxa (14 heterococcolith and five holococcolith phases).

The Margalef species richness and the Shannon diversity index for coccolithophores showed generally parallel trends during the investigation period (Fig. 4). Evident dissimilarity between two indices was recorded in January 2009 during the greatest abundance of coccolithophores (Fig. 4), when *Emiliania huxleyi* dominated the community. Both indices were the highest during spring, consistent with the elevated abundance of coccolithophores (Fig. 4). Coccolithophore abundance was the lowest in summer. During summer, Margalef species index was rather low, and Shannon diversity was in the middle range, indicating a well-balanced community of several species. In autumn the coccolithophore abundance was higher than in summer, as were diversity indices. The lowest values of diversity indices were recorded on 20th October 2009, when abundance of organic matter interfered with the coccolithophore identification (personal observation).

The most common species was *E. huxleyi* (only morphotype A) present in 93% of samples with maximum contribution to the coccolithophore community (96%) in January 2009 (Table 1; Fig. 6). In contrast, *Tergestiella adriatica* was recorded only once on the 28th August 2009, when it

Table 1. Taxonomic list of coccolithophores recorded at the station RV001 during the investigation period (2008–2009), number of samples 29. Max, the maximum proportion of taxa in one sample; *F*%, frequency of occurrence in the samples; HET, heterococcolithophore; HOL, holococcolithophore.

		Taxon	Max%	*F*%
1	HET	*Acanthoica quattrospina* Lohmann, 1903	3.1%	17.2%
2	HOL	*Acanthoica quattrospina* HOL Lohmann, 1903*	4.0%	24.1%
3	HET	*Algirosphaera robusta* (Lohmann 1902) Norris, 1984	12.5%	34.5%
4	HOL	*Algirosphaera robusta* HOL (Lohmann 1902) Norris, 1984[†]	14.2%	27.6%
5	HET	*Alisphaera extenta* Kleijne et al., 2002	0.6%	10.3%
6	HET	*Alisphaera gaudii* Kleijne et al., 2002	0.4%	3.5%
7	HOL	*Anthosphaera periperforata* type 2 Cros and Fortuño, 2002	24.8%	20.7%
8	HOL	*Calcidiscus leptoporus* ssp. *quadriperforatus* HOL (Kamptner 1937) Geisen et al., 2002[‡]	0.8%	3.5%
9	HET	*Calciopappus rigidus* Heimdal in Heimdal and Gaarder, 1981	19.1%	17.2%
10	HET	*Calciosolenia brasiliensis* (Lohmann 1919) Young in Young et al., 2003[§]	2.2%	17.2%
11	HET	*Calciosolenia murrayi* Gran, 1912	12.3%	20.7%
12	HOL	*Calicasphaera blokii* Kleijne, 1991	5.3%	10.3%
13	HOL	*Calyptrolithina divergens* var. *tuberosa* (Heimdal 1980) Jordan et al., 1993	7.7%	13.8%
14	HET	*Calyptrosphaera sphaeroidea* Schiller, 1913 HET[‖]	0.5%	3.5%
15	HOL	*Corisphaera gracilis* Kamptner, 1937	14.3%	48.3%
16	HOL	*Corisphaera strigilis* Gaarder, 1962	18.9%	34.5%
17	HET	*Cyrtosphaera aculeata* (Kamptner 1941) Kleijne, 1992	2.7%	6.9%
18	HET	*Cyrtosphaera lecaliae* Kleijne, 1992	3.1%	13.8%
19	HET	*Emiliania huxleyi* (Lohmann 1902) Hay and Mohler, in Hay et al., 1967	95.6%	93.1%
20	HET	*Helicosphaera carteri* (Wallich 1877) Kamptner, 1954	0.5%	3.5%
21	HOL	*Helicosphaera* HOL type Young et al., 2003[¶]	6.3%	3.5%
22	HOL	*Helicosphaera* HOL *catilliferus* type Young and Bown (2014)[#]	0.8%	3.5%
23	HOL	*Helicosphaera* HOL *dalmaticus* type Young and Bown (2014)**	6.8%	6.9%
24	HOL	*Helladosphaera cornifera* (Schiller 1913) Kamptner, 1937	4.3%	13.8%
25	HOL	*Holococcolithophora adenensis* (Kleijne 1991) Triantaphyllou and Dimiza 2008	3.9%	6.9%
26	HOL	*Holococcolithophora* sp. 1 Cros and Fortuño, 2002[††]	17.1%	6.9%
27	HOL	*Homozygosphaera spinosa* (Kamptner 1941) Deflandre, 1952	1.4%	6.9%
28	HOL	*Homozygosphaera vercellii* Borsetti and Cati, 1979	1.5%	6.9%
29	HET	*Ophiaster hydroideus* (Lohmann 1903) Lohmann, 1913	11.6%	44.8%
30	HET	*Palusphaera vandelii* Lecal, 1965	3.1%	3.5%
31	HET	*Rhabdosphaera clavigera* var. *stylifera* (Lohmann, 1902) Kleijne and Jordan, 1990	54.3%	55.2%
32	HET	*Rhabdosphaera xiphos* (Deflandre and Fert 1954) Norris, 1984	7.3%	6.9%
33	HET	*Syracosphaera ampliora* Okada and McIntyre, 1977	5.0%	13.8%
34	HET	*Syracosphaera arethusae* (Kamptner 1941) Triantaphyllou et al., 2015[‡‡]	16.2%	24.1%
35	HOL	*Syracosphaera arethusae* HOL *arethusae* type	2.5%	10.3%
36	HET	*Syracosphaera bannockii* (Borsetti and Cati 1976) Cros et al., 2000	3.6%	17.2%
37	HET	*Syracosphaera corolla* Lecal, 1966	16.4%	34.5%
38	HET	*Syracosphaera dilatata* Jordan et al., 1993	3.3%	20.7%
39	HET	*Syracosphaera hirsuta* Kleijne and Cros, 2009[§§]	0.9%	10.3%
40	HET	*Syracosphaera histrica* Kamptner, 1941	1.0%	10.3%
41	HOL	*Syracosphaera histrica* HOL *papillifera* type[‖‖]	6.8%	27.6%
42	HET	*Syracosphaera marginaporata* Knappertsbusch (1993)	1.0%	10.3%
43	HET	*Syrachosphaera mediterranea* Lohmann 1902	6.3%	17.2%
44	HOL	*Syrachosphaera mediterranea* HOL *wettsteinii* type[¶¶]	24.8%	31.0%
45	HET	*Syracosphaera molischii* Schiller 1925[##]	5.1%	10.3%
46	HOL	*Syracosphaera molischii* HOL *fragaria* type***	49.2%	20.7%
47	HET	*Syracosphaera nodosa* Kamptner, 1941	17.2%	10.3%

TABLE 1. Continued

		Taxon	Max%	F%
48	HET	*Syracosphaera ossa* type 2 Young et al. 2003	7.2%	58.6%
49	HET	*Syracosphaera protrudens* Okada and McIntyre, 1977	2.4%	13.8%
50	HET	*Syracosphaera pulchra* Lohmann, 1902	80.0%	86.2%
51	HOL	*Syracosphaera pulchra* HOL *oblonga* type Young et al., 2003	44.4%	24.1%
52	HET	*Tergestiella adriatica* Kamptner, 1941	15.9%	3.5%

Taxonomic remarks:

* HOL phase described in Cros et al. (2000), similar to *Sphaerocalyptra*, not separately described before.

† Dimiza et al. (2008): 10 unambiguous combination coccospheres of *A. robusta* and *Sphaerocalyptra quadridentata* described from natural populations (Kamptner (1941) Triantaphyllou and Dimiza 2003).

‡ HET phase is *C. leptoporus* (sensu lato) Geisen et al. 2002; combination coccosphere Geisen et al. (2002).

§ Species *C. corsellii* Malinverno 2004 was also recorded in samples, note that they were grouped together in cell counts.

‖ HOL phase described in Noel et al. (2004).

¶ Informal grouping *Helicosphaera* HOL *perforate* type.

\# Synonyms: *Syracolithus catilliferus* (Kamptner 1937) Deflandre 1952, *H. carteri* HOL solid.

** Synonyms: *Syracosphaera dalmatica* Kamptner 1927; *Syracolithus dalmaticus* (Kamptner 1927) Loeblich and Tappan 1966, combination coccosphere noted with *H. wallichii* HET - Geisen et al. (2002).

†† *Sphaerocalyptra* sp. 1 Cros and Fortuño (2002).

‡‡ Triantaphyllou et al. (2016) established that *S. didyma* Kliejne and Cros 2009 formed combination coccospheres with the *H. arethusae* Kamptner 1941 and so that the name *S. arethusae* has priority.

§§ *Syracosphaera borealis* type 2 Young et al. (2003).

‖‖‖ *Calyptrolithophora papillifera* (Halldal 1953) Heimdal, in Heimdal and Gaarder 1980, was linked as HOL phase of *S. histrica* in Cros et al. 2000 and Triantaphyllou et al. (2014).

¶¶ Zygosphaera wettsteinii Kamptner 1937 was associated to *S. mediterranea* as a HOL phase in Kamptner (1941) i Cros et al. (2000), and McGrane (2007).

\#\# *S. molischii* shows variable ornamentation, we recorded two types: 1 and 2 Young et al. (2003).

*** A mixed collapsed coccosphere of *S. molischii* type 2 sensu Young et al. 2003 with *A. fragaria* shown by Cros et al. (2003).

achieved 16% of relative abundance in the coccolithophorid community (Table 1; Fig. 6). The species *Syracosphaera pulchra* was observed in 86% of samples and contributed the most to the coccolithophore community in autumn (up to 80%, Table 1). The species *Rhabdosphaera clavigera* var. *stylifera* was present in 55% of the samples and dominated the community during the summer period (Table 1; Fig. 6). *Syracosphaera ossa* type 2 was also very common (observed in 59% of the samples) mainly in summer and autumn (Table 1; Fig. 6). The most frequent holococcolithophorid species was *Corisphaera gracilis*, which considerably contributed to the coccolithophore community with 48% during the stratification period (Table 1; Fig. 6).

Coccolithophore succession

SIMPER analysis facilitated an overview of the most important species in the coccolithophore community and their characteristic season of dominance (Table 2). *E. huxleyi* was the principal species in most seasons, except in summer when the central species was *Rhabdosphaera clavigera* var. *stylifera*. *S. pulchra* was the second important species, playing key roles in almost all seasons. Winter was the most uniform season, with an average similarity within the season of 73.05 (Table 2). During winter, important species alongside *E. huxleyi* and *S. pulchra* were *Algirosphaera robusta* and *Ophiaster hydroideus*. Spring was the most diverse season, and significance of

holococcolithophorids as well as the genus *Syracosphaera* was noted (Table 2). In summer *R. clavigera* var. *stylifera* and *S. pulchra* were the key species, together with *C. gracilis*, *S. molischii* HOL *fragaria*, and *Calciopappus rigidus*. During autumn, apart from the three key species (*E. huxleyi*, *S. pulchra*, and *R.clavigera*), *S. ossa* type 2, *Algirosphaera robusta*, and *Anthosphaera periperforata* type 2 were significant (Table 2).

CCA was used to detect relationships of species and environmental factors (Fig. 7). Total variation was 2.59 and explanatory variables accounted for 62%, with the first two axes significantly explaining constrains ($p = 0.001$). The first two components of the CCA together explained 30% of the species-environment relation. The first component explained 16% of variation with an eigenvalue of 0.41, while the second component of CCA explained 14% and had an eigenvalue of 0.37. Analysis showed that temperature and salinity have a great impact, as their arrows were the longest. They were followed by nitrite and silicate, while phosphorus and ammonium showed very little relation. Winter and autumn arrows (representing median position of the samples) were positioned in the general direction of nutrient arrows, indicating they were nutrient rich seasons, and summer arrow was next to the temperature arrow. *E. huxleyi* positioned closest to the winter arrow and as such was distinguished as a winter species (Fig. 7). *E. huxleyi* was negatively related to temperature and positively with salinity nitrate and
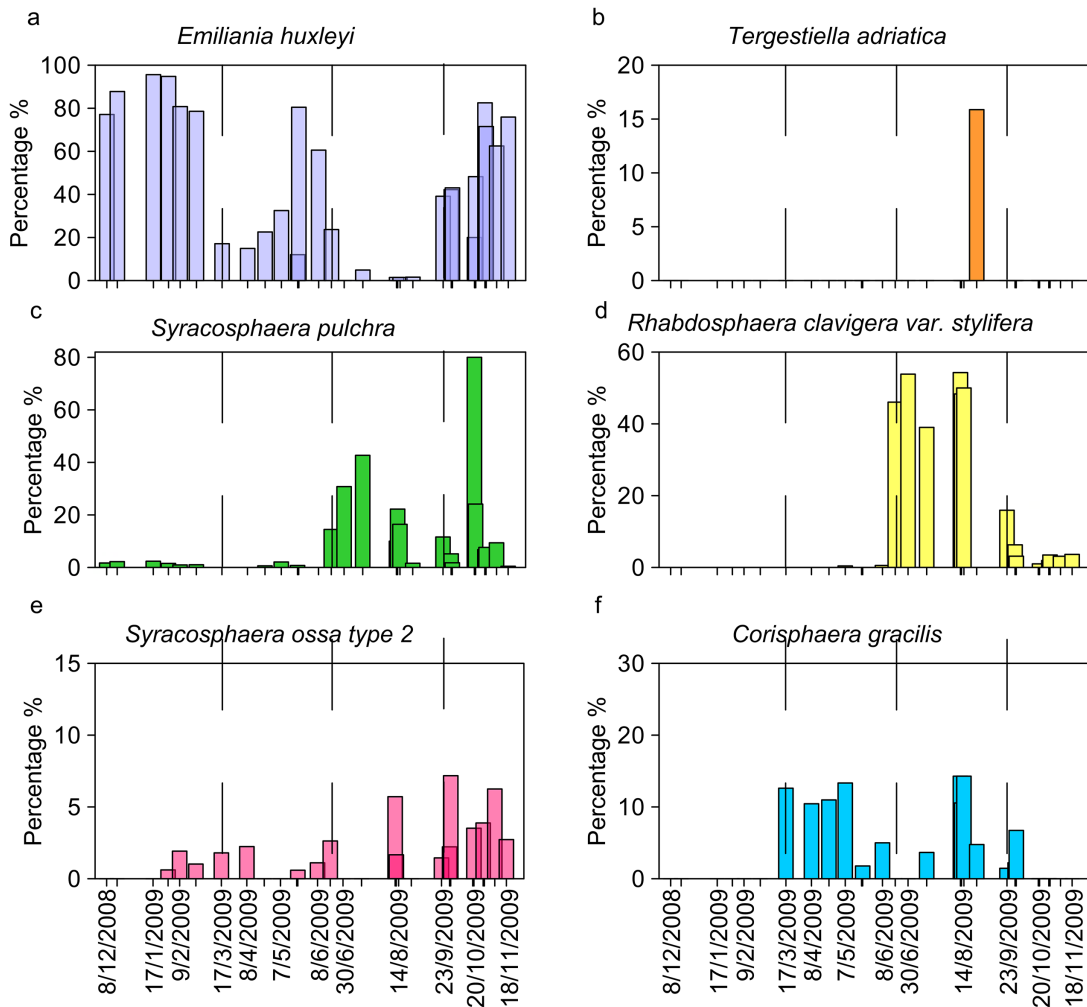
Fig. 6. The relative abundance in the samples analyzed by scanning electron microscopy of selected species of coccolithophores, *Emiliania huxleyi* (a), *Tergestiella adriatica* (b), *Syracosphaera pulchra* (c), *Rhabdosphaera clavigera var. stylifera* (d), *Syracosphaera ossa* type 2 (e), *Corisphaera gracilis* (f). Dashed lines are indicating seasons.

orthosilicate (Supporting Information Table 2). *A. robusta*, *O. hydroideus*, and *S. molischii* were also near the winter arrow and all negatively related to temperature (Fig. 7; Supporting Information Table 2). The largest number of species was positioned around the spring arrow, confirming the greatest diversity in this period (Fig. 7). Spring was abundant with holococcolithophorid species, including *Syracosphaera mediterranea* HOL *wettsteinii* type, *Corisphaera gracilis*, *S. pulchra* HOL *oblonga* type, *Corisphaera strigilis*, and *Algirosphaera robusta* HOL. Spring species (Table 2) *Syracosphaera arethusae*, *Syracosphaera nodosa*, *Acanthoica quattrospina* HOL, and *Syracosphaera histrica* HOL *papillifera* did not relate to investigated environmental factors (Supporting Information Table 2). From the other significant spring species: *Syracosphaera corolla* was related to low temperatures and high salinity, *C. gracilis* and *Syrachosphaera mediterranea* HOL *wettsteinii* were negatively related to nitrite, and *S. pulchra* HOL *oblonga* related

negatively to nitrite and positively to ammonium (Supporting Information Table 2). Summer species that positively related to the temperature and negatively with salinity were *R. clavigera* var. *stylifera*, *C. strigilis*, *S. molischii* HOL *fragaria*, while *C. rigidus* only related positively to temperature (Supporting Information Table 2). One more species of the genus *Rhabdosphaera* was recorded in summer, *R. xiphos*, but it did not relate to investigated environmental factors (Supporting Information Table 2). *S. pulchra* was related positively to temperature and negatively to salinity (Supporting Information Table 2), but was positioned near the autumn arrow (Fig. 7). *S. ossa* type 2, *Calciosolenia murrayi*, and *Syracosphera mediterana* were also grouped near the autumn arrow (Fig. 7), and *S. ossa* type 2 was related positively with silicate, while *C. murrayi* and *S. mediterana* were related positively with silicate and nitrite, and were positioned between the autumn and winter arrows (Supporting Information Table 2; Fig. 7).

Table 2. List of coccolithophore species responsible for differences among seasons, calculated by similarity percentage analyses (SIMPER) of species percentage data and Bray–Curtis similarity measure ($\hat{S}$ = average similarity within the season).

Species	Code	AvgA	AvgS	S	Con%	Cum%
Winter. Ŝ: 73.05						
Emiliania huxleyi	E.huxl	0.93	50.63	6.19	69.31	69.31
Algirosphaera robusta	A.robu	0.16	6.99	3.65	9.57	78.89
Syracosphaera pulchra	S.pulc	0.13	6.37	3.66	8.72	87.6
Ophiaster hydroideus	O.hydr	0.13	4.12	1.12	5.63	93.24
Spring. Ŝ: 42.42						
Emiliania huxleyi	E.huxl	0.55	13.71	3.3	32.32	32.32
Syrachosphaera mediterranea HOL *wettsteinii*	S.medH	0.23	5.6	3.23	13.2	45.52
Corisphaera gracilis	C.graH	0.25	5	1.37	11.79	57.31
Syracosphaera pulchra HOL *oblonga*	S.pulH	0.25	4.17	1.27	9.82	67.13
Syracosphaera corolla	S.coro	0.17	2.44	1	5.76	72.89
Syracosphaera arethusae	S.aret	0.14	1.55	0.65	3.66	76.56
Corisphaera strigilis	C.strH	0.13	1.33	0.58	3.13	79.69
Algirosphaera robusta HOL	S.quaH	0.12	0.94	0.4	2.22	81.91
Ophiaster hydroideus	O.hydr	0.07	0.89	0.6	2.1	84.02
Syracosphaera ossa type 2	S.ossa	0.07	0.88	0.61	2.08	86.1
Syracosphaera nodosa	S.nodo	0.13	0.76	0.3	1.79	87.89
Acanthoica quattrospina HOL	A.quaH	0.06	0.71	0.58	1.68	89.57
Syracosphaera histrica HOL *papillifera*	S.hisH	0.08	0.69	0.38	1.62	91.19
Summer. Ŝ: 49.05						
Rhabdosphaera clavigera var. *stylifera*	R.clav	0.6	20.54	1.52	41.87	41.87
Syracosphaera pulchra	S.pulc	0.42	13.5	2.13	27.51	69.39
Corisphaera gracilis	C.graH	0.21	4.76	0.88	9.7	79.09
Emiliania huxleyi	E.huxl	0.15	2.59	0.88	5.28	84.37
Syracosphaera molischii HOL *fragaria*	S.molH	0.18	1.88	0.61	3.83	88.2
Calciopappus rigidus	C.rigi	0.14	1.8	0.57	3.67	91.87
Autumn. Ŝ: 50.54						
Emiliania huxleyi	E.huxl	0.72	26.6	3.86	52.62	52.62
Syracosphaera pulchra	S.pulc	0.33	8.78	1.69	17.37	69.99
Rhabdosphaera clavigera var. *stylifera*	R.clav	0.18	4.74	1.59	9.37	79.36
Syracosphaera ossa type 2	S.ossa	0.15	3.58	1.08	7.08	86.43
Anthosphaera periperforata type 2	A.perH	0.14	1.25	0.52	2.48	88.91
Algirosphaera robusta	A.robu	0.11	1.24	0.37	2.46	91.37

Pairs of life-cycle phases

Seven pairs of heterococcolith (diploid) and holococcolith (haploid) phases were noted (Figs. 8, 9). For the first group of pairs, a clear temporal separation of life-cycle phases was observed. *S. mediterranea* was present in autumn and *S. mediterranea* HOL *wettsteinii* type in spring (Fig. 8). *S. molischii* was recorded winter and *S. molischii* HOL *fragaria* type in summer (Fig. 8). The second group exhibited rather short succession of life-cycle phases, as the phases appeared one after the other. *S. pulchra* was most abundant in summer and autumn, while *S. pulchra* HOL *oblonga* type in spring (Fig. 8). *S. arethusae* was most abundant in early spring and *arethusae* HOL *arethusae* type appeared in late spring (Fig. 8). The diploid phase of *S. histrica* was present in winter and haploid throughout the rest of the year (Fig. 8). We recorded *A.*

robusta in autumn and winter, and *A. robusta* HOL in spring (Fig. 8). For those species their life-cycle phases were closely linked, while phases of *Acanthoica quattrospina* were in alternation throughout the year (Fig. 8). The CCA analysis for the first group showed an evident separation of diploid and haploid phases in opposite quadrants (Fig. 7), indicating the different environmental preferences. The second group of species were positioned more closely, but still positioned in different quadrants confirming different requirements for the life-cycle phases. The exception is *S. arethusae*, with both phases positioned in the same quadrant (Fig. 7). In general, holococcolithophores preferred oligotrophic periods (spring/summer) while heterococcolithophores preferred the nutrient rich periods (autumn/winter). In particular, *S. mediterranea* was positively related to nitrite and silicate, and its
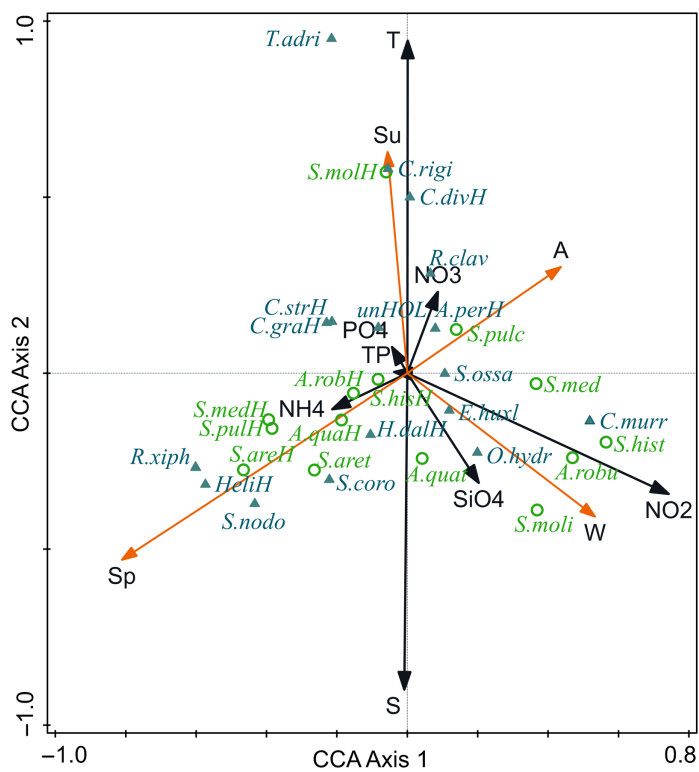
Fig. 7. Representation of the CCA analysis of environmental factors and the percentage of the most important species in the coccolithophore community. Triangles and circles represent the position of the Coccolithophore species (circles—species with both life-cycle phases). The arrows show environmental factors. Length and direction of the arrows of environmental parameters indicates their importance in terms of impact on the community. Abbreviations: *Acanthoica quattrospina - A.quat, Acanthoica quattrospina* HOL - *A.quaH, Algirosphaera robusta - A.robu, Algirosphaera robusta* HOL - *A.robH, Anthosphaera periperforata* type 2 - *A.perH, Calciopapus rigidus - C.rigi, Calciosolenia murrayi - C.murr, Calyptrolithina divergens* var. *tuberosa - C.divH, Corisphaera gracilis - C.graH, Corisphaera strigilis - C.strH, Emiliania huxleyi - E.huxl, Helicosphaera carteri* HOL - *HeliH, Helicosphaera* HOL *dalmaticus* type - *H.dalH, Ophiaster hdroideus - O.hydr, Rhabdosphaera clavigera* var. *stylifera - R.clav, Rhabdosphaera xipos - R.xiph, Syracosphaera arethusae - S.aret, Syracosphaera arethusae* HOL *arethusae - S.aretH, Syracosphaera corolla - S.coro, Syracosphaera histrica - S.hist, Syracosphaera histrica* HOL *papillifera* type - *S.hisH, Syracosphaera mediterranea - S.med, Syracosphaera mediterranea* HOL *wettsteini - S.medH, Syracosphaera molischii - S.moli, Syracosphaera molischii* HOL *fragaria* type - *S.molH, Syracosphaera nodosa - S.nodo, Syracosphaera ossa* type 2 - *S.ossa, Syracosphaera pulchra - S.pulc, Syracosphaera pulchra* HOL *oblonga - S.pulH, Tergestiella adriatica - T.adri,* unidentified holococco - unHOL.

holococcolithophore phase was negatively related to nitrite (Supporting Information Table 2). *S. molischii* was negatively related to temperature, while *S. molischii* HOL positively related to temperature, and negatively to salinity (Supporting Information Table 2). Of the second group, *S. pulchra* was, as stated above, positively related to temperature and negatively to salinity, while *S. pulchra* HOL negatively related to nitrite and positively to ammonium (Supporting

Information Table 2). Life-cycle phases of *S. arethusae* were not related to any of the investigated parameters (Supporting Information Table 2). Diploid phase of *S. histrica* was positively related to nitrite, and haploid phase was not relating to investigated environmental condition (Supporting Information Table 2). *A. robusta* was negatively related to temperature, and positively to salinity and nitrite, while *A. robusta* HOL did not exhibit any relations (Supporting Information Table 2). Heterococcolithophore phase of *A. quattrospina* was negatively related to orthophosphate, while holococcolithophore phase did not relate to investigated parameters (Supporting Information Table 2).

Discussion

Coccolithophores in coastal zones

Coccolithophores inhabit both open oceans and coastal regions. In the latter they are exposed to wider ranges of environmental factors (Raven and Crawfurd 2012), like temperature, salinity, and pH. And, scenarios of global climate change predict increase in the nutrient input in coastal areas, which could foster greater productivity of coastal ecosystems (Seitzinger and Kroeze 1998; Bakun et al. 2010; García-Reyes et al. 2015). Open ocean was the focus of the most of the biology, ecology, and biochemistry studies of coccolithophores to this date (Taylor et al. 2016; Balch 2018). And, as we previously stated, there are valid indications that coastal zones could offer new hypothesis toward many of the still open questions in biology and ecology of this important phytoplankton group. Moreover, coccolithophores can indeed dominate continental shelves and river influenced coastal areas, where their distribution is governed by rapid changes in hydrodynamics, freshwater input and anthropogenic eutrophication (Balestra et al. 2008; Silva et al. 2008a; Bonomo et al. 2014). Thus, it is of great importance to reveal the role of coccolithophores in ecological and biogeochemical functions of variable coastal habitats.

The studies that did touch upon the coastal zone are challenging to compare regarding the information on coccolithophore abundance and species diversity (Table 3). Several difficulties could be identified: (1) variety in temporal nature of studies, from snapshots to seasonal surveys, (2) different scales of investigated areas, (3) range of data collection strategies, from sampling (depths and miles from the shore) to microscopy (LM/SEM) (Table 3). Nevertheless, some conclusions can be drawn: (1) *Emiliania huxleyi* is the most abundant species in coastal areas, followed by *Gephyrocapsa* spp., *Syracosphaera pulchra, Algirosphaera robusta,* and *Rhabdosphaera clavigera,* (2) the highest abundance of coastal coccolithophores was noted in eutrophic area (4000×10^3 cells L^{-1}), while in oligo-eutrophic and oligotrophic areas abundances ranged between 15 and 600×10^3 cells L^{-1}. The highest abundance in the eutrophic area is probably due to the opportunistic nature of r-selected species *E. huxleyi.* (Young 1994), (3)
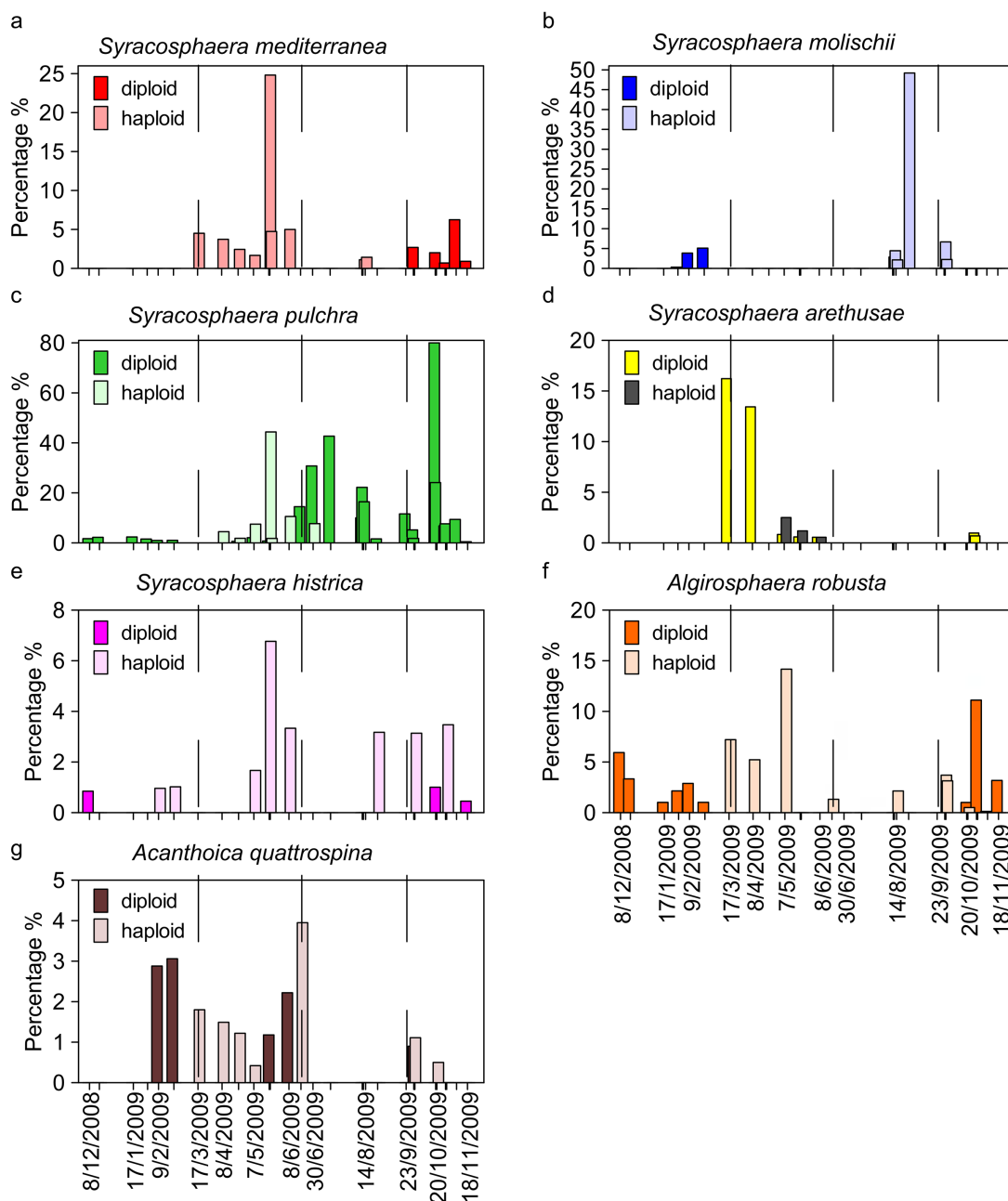
Fig. 8. The relative abundance of the diploid and haploid phase of species with both life-cycles recorded in the samples analyzed by scanning electron microscopy, *Syracosphaera mediterranea* (a), *S. molischii* (b), *S. pulchra* (c), *S. arethusae* (d), *S. histrica* (e), *Algirosphaera robusta* (f), *Acanthoica quattrospina* (g). Dashed lines are indicating seasons.

general impression is that Mediterranean is more thoroughly investigated, compared to the rest of the world, (4) more than half of the studies were snapshots, and (5) SEM studies resulted in higher diversity of the area (Table 3). From all this, we could conclude several recommendation for new coccolithophore survey, i.e., using the SEM analysis, putting grater effort into under-investigated areas, and rising the temporal frequency of sampling. These recommendations could be

rather costly, though, but might be well justified by the answers they should produce.

In this study, we surveyed the coccolithophore community at a Mediterranean coastal site over a 1-yr period. The maximal abundance we report here is in the range of those reported for the oceans (Charalampopoulou et al. 2016). We also recorded high relative coccolithophore abundance combined with high diversity in this rich and complex coastal
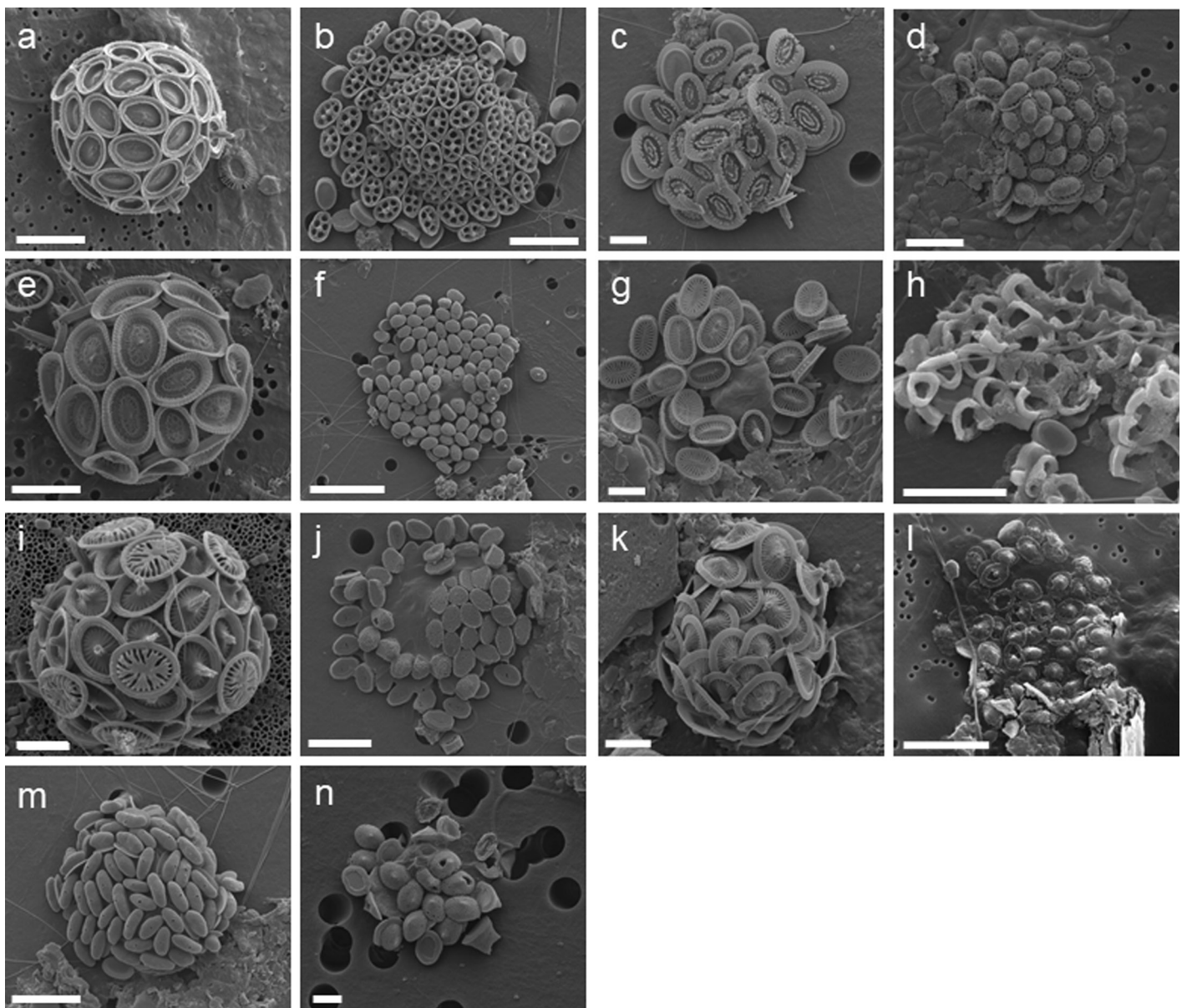
Fig. 9. Scanning electron micrographs *Syracosphaera mediterranea* (**a**) diploid Scale bar (SB): 5 μm, (**b**) haploid SB: 10 μm; *Syracosphaera molischii* (**c**) diploid SB: 2 μm, (**d**) haploid SB: 2 μm; *Syracosphaera pulchra* (**e**) diploid SB: 5 μm, (**f**) haploid SB: L 5 μm; Syracosphaera *arethusae*, (**g**) diploid SB: 2 μm, (**h**) haploid SB: 4 μm *Syracosphaera histrica*, (**i**) diploid SB: 2 μm, (**j**) haploid SB: 5 μm; *Acanthoica quattrospina* (**k**) diploid, SB 2 μm, (**l**) haploid SB: 5 μm; *Algirosphaera robusta* (**m**) diploid SB: 5 μm, (**n**) haploid SB: 2 μm.

environment of the northern Adriatic. Our results corresponded to the similar findings in the Mediterranean area. However, Schiebel et al. (2004) recorded 83 coccolithophore species in the Arabian Sea by SEM analysis, and indicated that a great diversity of species could exists in other areas too.

Diversity and succession of coccolithophores

Looking at the whole phytoplankton community, we noted the dominance over the phytoplankton community of coccolithophores during winter and diatoms during summer.

In the north-eastern Black Sea, coccolithophores and diatoms successively dominated the phytoplankton community, and these two phytoplankton groups were controlled by different nutrients (Mikaelyan et al. 2015). Mikaelyan et al. (2015) concluded that high biomass of diatoms corresponded to the increased ammonium, while high phosphorus determined the proliferation of coccolithophores. In our study, we did not relate diatoms and coccolithophores to aforementioned conditions, although we must note that our concentrations of P were much lower than those reported by Mikaelyan et al. (2015). In our study coccolithophores were

Table 3. Literature overview of coastal coccolithophores surveys.

Literature	Survey period	Area	Trophic state	Method	Taxa	10^3 cells L^{-1}	Dominant taxa
Godrijan et al. This study	2008–2009	Mediterranean, North Adriatic	Oligo-mesotrophic	LM/SEM	52	520	*Emiliania huxleyi, Syracosphaera pulchra, Rhabdosphaera clavigera, Corisphaera gracilis*
Cerino et al. (2017)	2011–2013	Mediterranean, Gulf of Trieste	Oligo-mesotrophic	pLM/SEM	17	350	*E. huxleyi, R. clavigera, Syracosphaera* spp.
Bonomo et al. (2017)	2009/July	Mediterranean, southern Tyr-rhenian Sea	Oligotrophic	pLM	40	68	*E. huxleyi, Gephyrocapsa* spp., *Calciosolenia* spp.
Šupraha et al. (2016)	2013/February, July	Mediterranean, Krka estuary	Oligo-eutrophic	SEM	82	540	*E. huxleyi, S. pulchra, A. quattro-spina, S. mediterranea*
Dimiza et al. (2016)	2001–2014	Mediterranean, Aegean Sea	Oligo-mesotrophic	SEM	99	396	*E. huxleyi, A. robusta, G. oceanica, R. clavigera*
Bonomo et al. (2014)	2012/June	Mediterranean, southern Tyr-rhenian Sea	Oligotrophic	LM	24	15	*E. huxleyi, R. xiphos, S. pulchra, holococcolithophores*
Bonomo et al. (2012)	2008/June, July	Mediterranean, Gulf of Sirte	Oligotrophic	pLM	44	18	*U. tenuis, E. huxleyi, F. profunda, S. pulchra* HOL, *Rhabdosphaera* spp.
Balestra et al. (2008)	2000/October	Mediterranean, Gulf of Manfredonia	Oligo-mesotrophic	SEM	48	40	*E. huxleyi, Syracosphaera* spp., *Rhabdosphaera* spp., *Coronosphaera* spp., *U. tenuis*
Dimiza et al. (2008)	2001–2004	Mediterranean, Andros Island	Oligotrophic	SEM	102	35	*E. huxleyi, R. clavigera, Syracos-phaera* spp., *A. robusta, H. cornifera*
Giraudeau et al. (2016)	2014	Barents Sea	Eutrophic	pLM/SEM	15	4000	*E. huxleyi, A. robusta, A. quatro-spina, C. pelagicus, S. molischii*
Guerreiro et al. (2014)	2010/March	Nazaré Canyon, Portugal	Oligo-mesotrophic	pLM/SEM	35	600	*E. huxleyi, Syracosphaera* spp., *Gephyrocapsa* spp., *S. mediterranea*
Silva et al. (2013)	2008/2009	Azores, Atlantic	Oligotrophic	LM	16	17	*E. huxleyi, Syracosphaera, D. tubifera, C. leptoporus, C. quadriperforatus*
Silva et al. (2008b)	2001–2005	Lisbon Bay, Portugal	Oligo-mesotrophic	LM	7	100	*E. huxleyi, Gephyrocapsa* spp.
Hernandez-Becerril et al. (2001)	1997/March	Baja California, Mexico	–	LM/SEM	24	–	*E. huxleyi, Gephyrocapsa oceanica*
Luan et al. (2016)	2010/October	Yellow and East China Seas	Oligo-eutrophic	SEM	32	252	*E. huxleyi, Gephyrocapsa* spp., *Syracosphaera* spp., *A. robusta*
Jin et al. (2015)	2012/May, November	Bohai Sea and Yellow Sea	Oligo-eutrophic	LM	14	216	*E. huxleyi, G. oceanica, H. carteri, C. leptoporus.*
Sun et al. (2014)	2011–2012	Yellow Sea and East China Sea	Oligo-eutrophic	LM	13	176	*G. oceanica, E. huxleyi, H. carteri, A. robusta, C. leptoporus*
Bai et al. (2014)	2011/October, November	Yellow Sea and East China Sea	Oligo-eutrophic	LM	18	119	*G. oceanica, E. huxleyi, H. carteri, A. robusta*
Schiebel et al. (2004)	1995, 1997	Arabian Sea	Oligo-eutrophic	SEM	83	112	*E. huxleyi, G. oceanica, F. profunda, O. antillarum*

more abundant in waters of high salinity, while diatoms were more abundant in high temperature waters. Besides, during spring, coccolithophores were abundant alongside diatoms, rather than just succeeding them. This might indicate that the group dynamics might not be as straightforward as depending on just two nutrients. Moreover, the relation might be further complicated by silicate and its role in calcification of some coccolithophores (Durak et al. 2016). Different requirements for silicate might also have a significant influence on the competitive interactions between the two groups.

Next, we looked at the richness of species, and we documented 52 taxa of coccolithophores. Apart from the old taxonomic surveys (Supporting Information 2) and a recent study by Šupraha et al. (2016) which revealed 82 coccolithophore species, data on ∼ 20 species of coccolithophores were regularly recorded in the Adriatic (Viličić et al. 2009; Cerino et al. 2017). That rather low species number was due to the fact that coccolithophores were analyzed with light microscopy. In our 1-yr study, we determined the floristic composition of coccolithophores with SEM, which facilitated the higher taxonomic resolution. One of the main goals of this study was to assess the diversity of coccolithophore community in the coastal waters of northern Adriatic. This also facilitated the overview of succession of species within this community. We found that winter was characterized by high coccolithophore abundances, and rather low value of Shannon diversity index compared to Margalef species richness, this combination indicated a dominance of one species, *Emiliania huxleyi*. The recorded high abundances, high values of dominance, and reduced diversity are all characteristic of the initial stage of succession (Margalef 1978). *E. huxleyi* dominated the community in the midst of winter, which is in contrast to bloom forming occurrences of *E. huxleyi* in the north Atlantic, where it blooms in warm, stratified, low P waters (Tyrrell and Merico 2004). In general, *E. huxleyi* is an opportunistic and r-selected species (Young 1994). However, Dimiza et al. (2008) characterized the winter coccolithophore community of the Aegean Sea as an *E. huxleyi* Group. The group was dominated by *E. huxleyi*, but the co-occurring species differed from ours. Our co-occurring species *A. robusta*, *O. hydroideus*, and *S. molischii* were all related to low temperatures. During winter, mixing of the water column is the strongest, and the resulting environmental conditions were the most favorable for the r-selected *E. huxleyi*, while other species were interchangeable during this period. Offshore the Terceira Island, Azores, Narciso et al. (2016) reported the dominance of *E. huxleyi* from autumn to spring, and attributed this to low temperatures, moderate nutrients concentrations, and mixing. Their sampling station was 2.6 nautical miles offshore, and situated in similar temperate region. This indicates there may be specific dynamics characteristic of the winter coccolithophore communities in the temperate regions.

In spring we noted an increase in both indices of diversity, with the peak in diversity in May coinciding with another high in coccolithophore abundances. The fact that almost half of the spring species, among them many holococcolithophores and small *Syracosphaera* species, did not relate to any investigated environmental factor could be associated to great environmental differences this transition period exhibits. On the other hand, in the northern Adriatic the influence of the East Adriatic Current (EAC) is greater during winter and spring than during the rest of the year (Poulain and Cushman-Roisin 2001). This current consists of two distinct water masses: Ionian Surface Water (ISW) close to the surface, and Levantine Intermediate Water (LIW) at intermediate depths (Poulain and Cushman-Roisin 2001). These waters are very rich in holococcolithophores (Kleijne 1991) as well as small *Syracosphaera* species (Kleijne and Cros 2009). Hence, the inflow of this EAC might be the main driver of growth of these two groups of species in the northern Adriatic coastal area.

In the summer, the coccolithophore community was rather uniform. During this period, one of the most frequently observed species was *Rhabdosphaera clavigera* var. *stylifera*. This species characterizes the coccolithophore community in warm, oligotrophic environments (Dimiza et al. 2008). During summer fresh water from the Po River tends to spill across the northern Adriatic, and the fast response of the phytoplankton communities along the west coast depletes nutrients from this water (Ivančić et al. 2012). Therefore, waters with decreased salinity and low nutrient concentrations come to the eastern coast of the northern Adriatic. *R. clavigera* is able to thrive in the low salinity waters of northern Adriatic, which are very low in nutrients during summer. We can also characterize *C. strigilis* and *S. molischii* HOL *fragaria* as warm, oligotrophic, summer species, together with *C. rigidus* that develops in even more oligotrophic conditions of late summer. *Tergestiela adriatica* was in this study recorded only in one sample in late August. In general, it has a patchy coastal distribution and a narrow period of occurrence (5–15 d) (Hagino et al. 2015). This species is a living fossil (fossil genus *Cyclagelosphaera*) and a survivor of the K/Pg extinction together with *Braarudosphaera bigelowii* and *Cruciplacolithus neohelis* (Hagino et al. 2015). In coastal waters of Tomari, Japan, *T. adriatica* was present in mid-June together with the *B. bigelowii* (Hagino et al. 2015). Hagino et al. (2015) also noted that other coccolithophores were more abundant offshore and were typically recorded for longer periods of time compared to short appearance of the coastal *T. adriatica*. Records of coastal coccolithophores are rare in general and this could be facilitated by their strong seasonality and short occurrence period. All this is stressing the need for more detail, temporal and spatial, studies of coastal zones for collection of such invaluable data.

Throughout autumn, high diversity and prevalence of K-selected species were noted. The K-selected taxa, or

equilibrium species, develop during the later stage of the succession (Margalef 1978). Dimiza et al. (2008) characterized *S. ossa* type 2 as an eutrophic coastal species, and in our study it was present during the entire investigation period, with the highest abundance recorded in autumn. We also found that *S. ossa* type 2 was positively related to silicate. As requirement for silica during calcification in coccolithophores is species specific (Durak et al. 2016), our finding might indicate that *S. ossa* type 2 is one of the species that needs silicate for calcification. The autumn group relating positively to nutrients, especially silicate, develops independent of the diatom bloom and includes the above mentioned species. The onset of mixing and breakdown of stratification, which allows the upward flux of nutrients from the bottom, coincided with a new decline in abundances and diversity of coccolithophores, suggesting reduced activity of the coccolithophore community in the late autumn.

Life-cycle parings: Heterococcolithophores and holococcolithophores

During this study we recorded both life-cycle phases for seven species, while Koebrich et al. (2016) found six species in both their holo- and heterococcolith-bearing phases using sediment traps offshore NW Africa. D'Amario et al. (2017) reported seven species from two cruises in April 2011 and May 2013 in the southern basins of the Mediterranean Sea, and Šupraha et al. (2016) found 14 species in the Krka estuary. Okada and Mcintyre (1979) sampled five stations in Western North Atlantic, and first noted the seasonal separation for life-cycle phases of the species *Coccolithus pelagicus*. They found that motile, holococcolith-bearing phase could be found during spring and late summer, whereas the non-motile phase was blooming during mid-summer, when the motile phase almost disappeared (Okada and Mcintyre 1979). Additionally, depth separation of life-cycle phases has been repeatedly reported, as well. Okada and Mcintyre (1979) reported that non-motile *C. pelagicus* at 100 m depth dominated over the motile HOL phase. And, Cros et al. (2000) stressed that holococcolithophore *S. catilliferus* prevailed near the surface while heterococcolithophore *H. carteri* occupied deeper waters. Giraudeau (1992) noted that *H. carteri* is related with low salinity and nutrient rich waters found deeper. There is an overall tendency of holococcolithophorids prevailing near the surface, and heterococcolithophorids in middle depths (Cros et al. 2000). Thus, the two phases would appear to occupy different ecological niches.

In our study, the maximum depth of the sampling station was 27 m, which is why we did not record the depth zonation of the two life-cycle phases, but rather nicely isolated the seasonal signal. With this we noted two types of temporal distribution patterns. For the first type, we can postulate a strong separation of life-cycle phases. This is substantiated by their relatively high abundances for both phases that are separated wide apart temporally, indicating the possibility of

both phases to thrive in different ecological conditions. For the second type, we assume a rather direct succession of life-cycle phases. This might be when, after a "bloom" of one life-cycle phase, unfavorable conditions induce a transition to a different life-cycle phase, indicating the extension of presence into less favorable conditions. We can hypothesize that species undertake two different strategies in their phenotypic plasticity. First is a clear temporal separation of life-cycle phases, and the second is fast transition between the phases. Recorded separation of the two phases could to be attributed to environmental preferences.

In this study, the heterococcolith phase of *S. mediterranea* was present in the autumn-winter period and was positively related with nitrite and silicate, while the holococcolith phase was recorded in the late spring and negatively related to nitrite. Silva et al. (2008*b*) described the *S. mediterranea* heterococcolith phase as a species that has a very wide range of tolerance to temperature and salinity. Moreover, in the light of these findings we might hypothesize that this species utilizes the phenotypic plasticity to cope with environmental variation in the nitrogen cycle.

On the other hand, *S. arethusae* had both life-cycle phases developing in spring. Nevertheless, a temporal separation was evident, the heterococcolith phase developed in early spring, while the haploid phase in late spring. The more vibrant species, with the phases shifting throughout the year in our study was *A. quattrospina*. We found both phases of *A. quattrospina* with a patchy temporal distribution with coexistence during autumn, but a difference in nutrient preferences was noted for each phase. Moreover, Šupraha et al. (2014) found an abundant assemblage of *Acanthoica quattrospina* in all life-cycle phases (coccospheres with hetero-, holococolith and combination of both) in the Krka River estuary. They conclude that high tolerance to low salinity allowed it to outcompete other coccolithophores in the unstable environment of the halocline. The presence of all phases could indicate the start of a shift toward the dominance of one of the phases.

Conclusions

Our study confirmed that both diatoms and coccolithophores are important phytoplankton in the E. Adriatic coastal ecosystem, with coccolithophore diversity indicating the importance of this region for further studies on coccolithophore environmental physiology. The data are also generally consistent with previous studies, suggesting that holococcolithophores occupy more oligotrophic waters and diploid heterococcolithophores inhabit relatively nutrient rich waters. Patterns of ecological partitioning were observed among the two life-cycle phases of several species of coccolithophores, supporting the hypothesis that phase transitions are an important ecological strategy that allow for an ecological success in waters under dynamic change of ecological factors. Thus, investigations of coastal areas, which undergo more variations of

ecological conditions, would facilitate more insights into the different strategies single species undertake. And finally, our analyses indicate that a change is needed in the studying coccolithophores. The noted strategies of life-cycle phases would not be discernible without the frequent temporal sampling in ecologically dynamic environment, and this should be the next direction for the in situ life-cycle studies.

References

Bai, J., X. Gu, Y. Feng, W. Jiang, H. Jin, J. Chen, and J. Sun. 2014. Autumn living coccolithophores in the Yellow Sea and the East China Sea. Acta Oceanologica Sinica **33**: 83–94. doi:10.1007/s13131-014-0481-y

Bakun, A., D. B. Field, A. N. A. Redondo-Rodriguez, and S. J. Weeks. 2010. Greenhouse gas, upwelling-favorable winds, and the future of coastal ocean upwelling ecosystems. Glob. Chang. Biol. **16**: 1213–1228. doi:10.1111/j.1365-2486.2009.02094.x

Balch, W. M. 2018. The ecology, biogeochemistry, and optical properties of coccolithophores. Ann. Rev. Mar. Sci. **10**: 71–98. doi:10.1146/annurev-marine-121916-063319

Balestra, B., M. Marino, S. Monechi, C. Marano, and F. Locaiono. 2008. Coccolithophore communities in the Gulf of Manfredonia (Southern Adriatic Sea): Data from water and surface sediments. Micropaleontology **54**: 377–396. www.jstor.org/stable/30130915

Baumann, K.-H., H. Andruleit, B. Boeckel, M. Geisen, and H. Kinkel. 2005. The significance of extant coccolithophores as indicators of ocean water masses, surface water temperature, and paleoproductivity: A review. Paläontol. Z. **79**: 93–112. doi:10.1007/BF03021756

Billard, C. 1994. Life cycles, p. 167–186. *In* J. C. Green and B. S. C. Leadbeater [eds.], The haptophyte algae. Systematics Association Special Vol. No. 51. Clarendon Press.

Billard, C., and I. Inouye. 2004. What is new in coccolithophore biology?, p. 1–30. *In* H. R. Thierstein and J. R. Young [eds.], Coccolithophores: From molecular processes to global impact. Springer-Verlag.

Bonomo, S., and others. 2012. Living Coccolithophores from the Gulf of Sirte (Southern Mediterranean Sea) during the summer of 2008. Micropaleontology **58**: 487–503. doi: www.jstor.org/stable/24413307

Bonomo, S., A. Cascella, I. Alberico, L. Ferraro, L. Giordano, F. Lirer, M. Vallefuoco, and E. Marsella. 2014. Coccolithophores from near the Volturno estuary (central Tyrrhenian Sea). Mar. Micropaleontol. **111**: 26–37. doi:10.1016/j.marmicro.2014.06.001

Bonomo, S., F. Placenti, E. M. Quinci, A. Cuttitta, S. Genovese, S. Mazzola, and A. Bonanno. 2017. Living coccolithophores community from Southern Tyrrhenian Sea (Central Mediterranean — Summer 2009) Mar. Micropaleontology **131**: 10–24. doi:10.1016/j.marmicro.2017.02.002

Bosak, S., J. Godrijan, and T. Šilović. 2016. Dynamics of the marine planktonic diatom family Chaetocerotaceae in a Mediterranean coastal zone. Estuar. Coast. Shelf Sci. **180**: 69–81. doi:10.1016/j.ecss.2016.06.026

Bown, P. 2005. Selective calcareous nannoplankton survivorship at the Cretaceous-Tertiary boundary. Geology **33**: 653–656. doi:10.1130/G21566.1

Boyd, P. W., R. Strzepek, F. Fu, and D. A. Hutchins. 2010. Environmental control of open-ocean phytoplankton groups: Now and in the future. Limnol. Oceanogr. **55**: 1353–1376. doi:10.4319/lo.2010.55.3.1353

Braak, C. J. F., and P. Šmilauer. 2002. CANOCO reference manual and CanoDraw for Windows user's guide: Software for canonical community ordination (version 4.5). Section on permutation methods. Microcomputer Power.

Brunnthaler, J. 1911. Coccolithophoriden aus der Adria. Int. Revue Ges. Hydrobiol. Hydrogr. **3**: 545–547. doi:10.1002/iroh.19110030507

Cerino, F., E. Malinverno, D. Fornasaro, M. Kralj, and M. Cabrini. 2017. Coccolithophore diversity and dynamics at a coastal site in the Gulf of Trieste (northern Adriatic Sea). Estuar. Coast. Shelf Sci. **196**: 331–345. doi:10.1016/j.ecss.2017.07.013

Charalampopoulou, A., A. J. Poulton, D. C. E. Bakker, M. I. Lucas, M. C. Stinchcombe, and T. Tyrrell. 2016. Environmental drivers of coccolithophore abundance and calcification across Drake Passage (Southern Ocean). Biogeosciences **13**: 5917–5935. doi:10.5194/bg-13-5917-2016

Clarke, K. R., and R. M. Warwick. 2001. Changes in marine communities: An approach to statistical analysis and interpretation, 2nd ed. Primer-E.

Cros, L., A. Kleijne, A. Zeltner, C. Billard, and J. R. Young. 2000. New examples of holococcolith–heterococcolith combination coccospheres and their implications for coccolithophorid biology. Mar. Micropaleontol. **39**: 1–34. doi:10.1016/S0377-8398(00)00010-4

D'Amario, B., P. Ziveri, M. Grelaud, A. Oviedo, and M. Kralj. 2017. Coccolithophore haploid and diploid distribution patterns in the Mediterranean Sea: Can a haplo-diploid life cycle be advantageous under climate change? J. Plankton Res. **39**: 781–794. doi:10.1093/plankt/fbx044

Dimiza, M. D., M. V. Triantaphyllou, and M. D. Dermitzakis. 2008. Seasonality and ecology of living coccolithophores in Eastern Mediterranean coastal environments (Andros Island, Middle Aegean Sea). Micropaleontology **54**: 159–175. www.jstor.org/stable/30130910

Dimiza, M. D., O. Koukousioura, M. V. Triantaphyllou, and M. D. Dermitzakis. 2016. Live and dead benthic foraminiferal assemblages from coastal environments of the Aegean Sea (Greece): Distribution and diversity. Revue de Micropaleontologie **59**: 19–32. doi:10.1016/j.revmic.2015.10.002

Durak, G. M., and others. 2016. A role for diatom-like silicon transporters in calcifying coccolithophores. Nat. Commun. **7**: 10543. doi:10.1038/ncomms10543

García-Reyes, M., W. J. Sydeman, D. S. Schoeman, R. R. Rykaczewski, B. A. Black, A. J. Smit, and S. J. Bograd. 2015. Under pressure: Climate change, upwelling, and eastern boundary upwelling ecosystems. Front. Mar. Sci. **2**: 1–10. doi:10.3389/fmars.2015.00109

Giraudeau, J. 1992. Distribution of Recent nannofossils beneath the Benguela system: Southwest African continental margin. Mar. Geol. **108**: 219–237. doi:10.1016/0025-3227(92)90174-G

Giraudeau, J., V. Hulot, V. Hanquiez, L. Devaux, H. Howa, and T. Garlan. 2016. A survey of the summer coccolithophore community in the western Barents Sea. J. Mar. Syst. **158**: 93–105. doi:10.1016/j.jmarsys.2016.02.012

Godrijan, J., D. Marić, I. Tomažić, R. Precali, and M. Pfannkuchen. 2013. Seasonal phytoplankton dynamics in the coastal waters of the north-eastern Adriatic Sea. J. Sea Res. **77**: 32–44. doi:10.1016/j.seares.2012.09.009

Guerreiro, C., and others. 2014. Influence of the Nazaré Canyon, central Portuguese margin, on late winter coccolithophore assemblages. Deep-Sea Research Part II: Topical Studies in Oceanography **104**: 335–358. doi:10.1016/j.dsr2.2013.09.011

Hagino, K., J. R. Young, P. R. Bown, J. Godrijan, D. K. Kulhanek, K. Kogane, and T. Horiguchi. 2015. Re-discovery of a "living fossil" coccolithophore from the coastal waters of Japan and Croatia. Mar. Micropaleontol. **116**: 28–37. doi:10.1016/j.marmicro.2015.01.002

Hernández-Becerril, D. U., E. Bravo-Sierra, and Y. Ramírez-Valdéz. 2001. Coccolithophorids from the west coast of Baja California, Mexico. Hydrobiologia **452**: 31–45. doi:10.1023/A:1011961325093

Ivančič, I., and D. Degobbis. 1984. An optimal manual procedure for ammonia analysis in natural waters by the indophenol blue method. Water Res. **18**: 1143–1147. doi:10.1016/0043-1354(84)90230-6

Ivančič, I., G. Jelena, P. Martin, M. Daniela, G. Blazčenka, D. Tamara, and N. Mirjana. 2012. Survival mechanisms of phytoplankton in conditions of stratification-induced deprivation of orthophosphate: Northern Adriatic case study. Limnol. Oceanogr. **57**: 1721–1731. doi:10.4319/lo.2012.57.6.1721

Jin, H., Y. Feng, X. Li, W. Zhai, and J. Sun. 2015. Spring and autumn living coccolithophores in the Bohai Sea and Yellow Sea, China. Acta Oceanologica Sinica **34**: 132–146. doi:10.1007/s13131-015-0712-x

Kamptner, E. 1941. Die Coccolithineen der Sudwestkuste von Istrien. Ann. Naturhist Mus Wien **51**: 54–149. www.jstor.org/stable/41768414

Kleijne, A. 1991. Holococcolithophorids from the Indian Ocean, Red Sea, Mediterranean Sea and North Atlantic Ocean. Mar. Micropaleontol. **17**: 1–76. doi:10.1016/0377-8398(91)90023-Y

Kleijne, A., and L. Cros. 2009. Ten new extant species of the coccolithophore *Syracosphaera* and a revised classification scheme for the genus. Micropaleontology **55**: 425–462. www.jstor.org/stable/20627989

Koebrich, M. I., K. H. Baumann, and G. Fischer. 2016. Seasonal and inter-annual dynamics of coccolithophore fluxes from the upwelling region off Cape Blanc, NW Africa. J. Micropalaeontol. **35**: 103–116. doi:10.1144/jmpaleo2014-024

Luan, Q., S. Liu, F. Zhou, and J. Wang. 2016. Living coccolithophore assemblages in the Yellow and East China Seas in response to physical processes during fall 2013. Marine Micropaleontology **123**: 29–40. doi:10.1016/j.marmicro.2015.12.004

Margalef, R. 1951. Diversidad de especies en las comunitades naturales. Publ. Inst. Biol. Apl. **6**: 9–72.

Margalef, R. 1978. Life-forms of phytoplankton as survival alternatives in an unstable environment. Oceanol. Acta **1**: 493–509.

Marić, D., R. Kraus, J. Godrijan, N. Supić, T. Djakovac, and R. Precali. 2012. Phytoplankton response to climatic and anthropogenic influences in the north-eastern Adriatic during the last four decades. Estuar. Coast. Shelf Sci. **115**: 98–112. doi:10.1016/j.ecss.2012.02.003

Mikaelyan, A. S., L. A. Pautova, V. K. Chasovnikov, S. A. Mosharov, and V. A. Silkin. 2015. Alternation of diatoms and coccolithophores in the north-eastern Black Sea: A response to nutrient changes. Hydrobiologia **755**: 89–105. doi:10.1007/s10750-015-2219-z

Milner, S., G. Langer, M. Grelaud, and P. Ziveri. 2016. Ocean warming modulates the effects of acidification on *Emiliania huxleyi* calcification and sinking. Limnol. Oceanogr. **61**: 1322–1336. doi:10.1002/lno.10292

Narciso, Á., F. Gallo, A. Valente, M. Cachão, L. Cros, E. B. Azevedo, and J. Barcelos e Ramos. 2016. Seasonal and interannual variations in coccolithophore abundance off Terceira Island, Azores (Central North Atlantic). Cont. Shelf Res. **117**: 43–56. doi:10.1016/j.csr.2016.01.019

Nöel, M.-H., M. Kawachi, and I. Inouye. 2004. Induced dimorphic life cycle of a coccolithophorid, Calyptrosphaera sphaeroidea (Prymnesiophyceae, Haptophyta). J. Phycol. **40**: 112–129. doi:10.1046/j.1529-8817.2004.03053.x

Okada, H., and A. Mcintyre. 1979. Seasonal distribution of modern Coccolithophores in the Western North Atlantic Ocean. Mar. Biol. **54**: 319–328. doi:10.1007/BF00395438

Parke, M., and I. Adams. 1960. The motile (*Crystallolithus hyalinus* Gaarder & Markali) and non-motile phases in the life history of *Coccolithus pelagicus* (Wallich) Schiller. J. Mar. Biol. Assoc. UK **39**: 263–274. doi:10.1017/S002531540001331X

Parsons, T. R., Y. Maita, and C. M. Lalli. 1984. A manual of chemical and biological methods for seawater analysis. Pergamon Press.

Poulain, P.-M., and B. Cushman-Roisin. 2001. Circulation, p. 67–109. *In* B. Cushman-Roisin, M. Gačić, P.-M. Poulain,

and A. Artegiani [eds.], Physical oceanography of the Adriatic Sea: Past, present and future. Kluwer Academic Publishers.

Quinn, P. S., A. G. Sáez, K.-H. Baumann, B. A. Steel, C. Sprengel, and L. K. Medlin. 2004. Coccolithophorid biodiversity: Evidence from the cosmopolitan species *Calcidiscus leptoporus*, p. 299–326. *In* H. R. Thierstein and J. R. Young [eds.], Coccolithophores: From molecular processes to global impact. Springer Berlin Heidelberg.

Ramesh, R., and others. 2015. Land–ocean interactions in the coastal zone: Past, present & future. Anthropocene **12**: 85–98. doi:10.1016/j.ancene.2016.01.005

Raven, J. A., and K. Crawfurd. 2012. Environmental controls on coccolithophore calcification. Mar. Ecol. Prog. Ser. **470**: 137–166. doi:10.3354/meps09993

Schiebel, R., A. Zeltnerb, U. F. Treppkea, J. J. Waniekc, J. Bollmannd, T. Rixene, and C. Hemleben. 2004. Distribution of diatoms, coccolithophores and planktic foraminifers along a trophic gradient during SW monsoon in the Arabian Sea. Mar. Micropaleontol. **51**: 345–371. doi:10.1016/j.marmicro.2004.02.001

Schiller, J. 1913. Vorlauflge Ergebnisse der Phytoplankton-untersuchungen auf den Fahrten S. M. S. "Naiade" in der Adria. Aus Den Sitzungsberichten Der Kaisserl Akademie Der Wissenschaften in Wien, Mathem-Naturw **122**: 1–11.

Schiller, J. 1925. Die plantonischen Vegetationen des adriatischen Meeres. B. Chrysomonadina, Heterokontae, Cryptomonadina, Eugleninae, Volvocales. 1. Systematischer Teil. Arch. Protistenkunde **53**: 59–123.

Seitzinger, S. P., and C. Kroeze. 1998. Global distribution of nitrous oxide production and N inputs in freshwater and coastal marine ecosystems. Global Biogeochem. Cycles **12**: 93–113. doi:10.1029/97GB03657

Shannon, C. E. 1948. A mathematical theory of communication. Bell Syst. Tech. J. **27**: 379–423. doi:10.1002/j.1538-7305.1948.tb01338.x

Šilović, T., V. Balagué, S. Orlić, and C. Pedrós-Alió. 2012. Picoplankton seasonal variation and community structure in the northeast Adriatic coastal zone. FEMS Microbiol. Ecol. **82**: 678–691. doi:10.1111/j.1574-6941.2012.01438.x

Silva, A., C. R. Mendes, S. Palma, and V. Brotas. 2008*a*. Short-time scale variation of phytoplankton succession in Lisbon bay (Portugal) as revealed by microscopy cell counts and HPLC pigment analysis. Estuar. Coast. Shelf Sci. **79**: 230–238. doi:10.1016/j.ecss.2008.04.004

Silva, A., S. Palma, and M. T. Moita. 2008*b*. Coccolithophores in the upwelling waters of Portugal: Four years of weekly distribution in Lisbon bay. Cont. Shelf Res. **28**: 2601–2613. doi:10.1016/j.csr.2008.07.009

Silva, A., and others. 2013. Coccolithophore species as indicators of surface oceanographic conditions in the vicinity of Azores islands. Estuarine, Coastal and Shelf Science **118**: 50–59. doi:10.1016/j.ecss.2012.12.010

Sun, J., X. Y. Gu, Y. Y. Feng, S. F. Jin, W. S. Jiang, H. Y. Jin, and J. F. Chen. 2014. Summer and winter living coccolithophores in the Yellow Sea and the East China Sea. Biogeosciences **11**: 779–806. doi:10.5194/bg-11-779-2014

Šupraha, L., Z. Ljubešić, H. Mihanović, and J. Henderiks. 2014. Observations on the life cycle and ecology of *Acanthoica quattrospina* Lohmann from a Mediterranean estuary. J. Nannoplankton Res. **34–56**: 49.

Šupraha, L., Z. Ljubešić, H. Mihanović, and J. Henderiks. 2016. Coccolithophore life-cycle dynamics in a coastal Mediterranean ecosystem: Seasonality and species-specific patterns. J. Plankton Res. **38**: 1178–1193. doi:10.1093/plankt/fbw061

Taylor, A. R., C. Brownlee, and G. Wheeler. 2016. Coccolithophore cell biology: Chalking up progress. Ann. Rev. Mar. Sci. **9**: 18.1–18.28. doi:10.1146/annurev-marine-122414-034032

Tomas, C. R. 1997. Identifying marine phytoplankton. Academic Press.

Triantaphyllou, M. V., B.-T. Karatsolis, M. D. Dimiza, E. Malinverno, F. Cerino, S. Psarra, R. W. Jordan, and J. R. Young. 2015. Coccolithophore combination coccospheres from the NE Mediterranean Sea: New evidence and taxonomic revisions. Micropaleontology **61**: 457–472.

Tyrrell, T., and A. Merico. 2004. Emiliania huxleyi: Bloom observations and the conditions that induce them, p. 75–97. *In* H. R. Thierstein and J. R. Young [eds.], Coccolithophores: From molecular processes to global impact. Springer Berlin Heidelberg.

Utermöhl, H. 1958. Zur Vervollkommnung der quantitativen Phytoplankton-Methodik. Mitteilungen Des Internationale Vereinigung Für Theoretische Und Angewandte Limnologie, v. **9**, p. 1–38. Schweizerbart.

Viličić, D., T. Djakovac, Z. Burić, and S. Bosak. 2009. Composition and annual cycle of phytoplankton assemblages in the northeastern Adriatic Sea. Bot. Mar. **52**: 291–305. doi:10.1515/BOT.2009.004

von Dassow, P., and others. 2015. Life-cycle modification in open oceans accounts for genome variability in a cosmopolitan phytoplankton. ISME J. **9**: 1365–1377. doi:10.1038/ismej.2014.221

Winter, A., and W. G. Siesser. 1994. Coccolithophores. Cambridge Univ. Press.

Young, J. R. 1994. Variation in *Emiliania huxley*i coccolith morphology in samples from the Norwegian EHUX experiment, 1992. Sarsia **79**: 417–425. doi:10.1080/00364827.1994.10413573

Young, J. R., M. Geisen, L. Cros, A. Kleijne, C. Sprengel, I. Probert, and J. Østergaard. 2003. A guide to extant coccolithophore taxonomy. J. Nannoplankton Res. **1**: 124.

Young, J. R., M. Geisen, and I. Probert. 2005. A review of selected aspects of coccolithophore biology with implications for paleobiodiversity estimation. Micropaleontology **51**: 267–288. doi:10.2113/gsmicropal.51.4.267

Young, J. R., and P. R. Bown. 2014. Some emendments to calcareous nannoplankton taxonomy. J. Nannoplankton Res. **33**: 39–46.

Conflict of Interest

None declared.