Calcareous Nannofossil Size and Abundance Response to the Messinian Salinity Crisis Onset and Paleoenvironmental Dynamics

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Abstract Dwarfism is a common feature affecting organisms across extreme events that characterized the Earth history, frequently referred as the result of “stressed conditions.” To date, no study addressed the morphological and biometric changes across the Messinian Salinity Crisis (MSC), one of the most recent and impacting event occurred in the Mediterranean Sea, historically interpreted as characterized by hypersaline conditions. Here we focus on morpho/biometric changes affecting calcareous nannofossils (CN) toward the MSC onset in order to better constrain the paleoenvironmental dynamics and the loosely defined “stressed conditions” characterizing this interval. Size characterization and absolute abundance of selected CN taxa were performed in the Perales (Spain, Western Mediterranean) and in the Banengo and Pollenzo sections (Italy, Northern Mediterranean). We also tested whether size changes and orbital cyclicity were related through analyzing size and calcite mass of Reticulofenestra minuta using an automated image analysis system of CN recognition (SYRACO). We recorded a significant size reduction affecting the CN taxa involved in the MSC onset bioevent, related to the restriction of the Mediterranean Basin that resulted in increased productivity and enhanced environmental variability, stimulating CN growth rate and decreasing their platelet sizes. Reticulofenestra minuta size and mass correlate with the orbital cyclicity with minimum values during periods of enhanced environmental variability, coinciding with the diatomite deposition in the Sorbas Basin. Our finding reveals that the size change recorded across the MSC onset coincided with the instauration of a productive and highly variable environment, linked to the restriction of the paleo Gibraltar Strait.

1. Introduction

Calcareous nannofossils (CN) are the remaining of calcifying marine algae (coccolithophores) and incertae sedis species producing small calcite platelets that are usually well preserved in the fossil record. The coccolithophores represent a powerful tool to document past environmental variability through the study of their assemblages (e.g., Negri et al., 1999), their morphologic features (e.g., Faucher et al., 2020; Grelaud et al., 2012), or the stable isotope/trace elements of their calcite (e.g., Bolton et al., 2016; Dedert et al., 2014; Stoll et al., 2019). Previous studies addressed the coccolithophores response to major environmental changes in the world ocean, both in the past (e.g., during Oceanic Anoxic Events) (Browning & Watkins, 2008; Erba et al., 2010; Lübbeke et al., 2015) and in the present (e.g., anthropogenic climate change) (Milner et al., 2016; Zondervan, 2007 and references therein), revealing that the coccolithophores responded to these perturbations by drastic changes in their specific diversity, their total abundances and the morphology and mass of their platelets (Meier et al., 2014). Sea temperature, salinity, nutrients content, light intensity, trace metals or carbonate chemistry (e.g., pCO$_2$) changes are proven to impact the size of coccolithophore cells and their calcitic platelets (Beaufort et al., 2011; Bollmann & Herrle, 2007; Paasche, 2002; Watabe & Wilbur, 1966; Zondervan, 2007). Of particular interest is the impact of past (e.g., Beaufort et al., 2011) and current (anthropogenic) (de Bodt et al., 2010; Engel et al., 2005; Langer et al., 2006; Riebesell et al., 2000, 2016; Zondervan, 2007) increase in pCO$_2$ on the coccolithophores affecting their major role on the oceanic carbon budget and carbonate pump (Rost & Riebesell, 2004; Salter et al., 2014).
Zondervan, 2007) highlight coccolithophores sensitivity to changes in abiotic parameters affecting their CN plate morphometry. Consequently, applying the outcome from modern studies, the CN morphological study can be used as a proxy to reconstruct past environmental conditions (Faucher et al., 2020; Gibbs et al., 2013 and references therein).

Marine biota frequently responded to extreme events such as greenhouse warming, eutrophication and volcanic events showing an overall size reduction (Erba et al., 2010; Ferreira et al., 2017; Keller & Abramovich, 2009; Lübke et al., 2015; Mattioli et al., 2009; Salaviale et al., 2018; Tremolada et al., 2008). Also, minor environmental changes such as the shallowing/shrinkage of the basin, were documented to impact the size of marine biota (Keller & Abramovich, 2009). Overall, the marine biota size decrease is related to variation in the oxygen, salinity and nutrients contents as well as change in temperature (Keller & Abramovich, 2009). This biotic response to such events is referred as “Lilliput effect” (Urbanek, 1993), and accompanied for instance the Oceanic Anoxic Events (Erba et al., 2010) and mass extinction episodes, but was also recorded in other time intervals characterized by environmental conditions loosely defined as “stressed condition” (Ferreira et al., 2017; Violanti et al., 2013). Although the CN morphometry and its relations with the environmental parameter was explored in modern environment and during extreme event characterizing the Earth history, no study addressed the impact of one of the most recent and rapid events affecting the Mediterranean: the Messinian Salinity Crisis (MSC).

During the Messinian, the Mediterranean Basin was affected by climatic and tectonic instability, resulted in the progressive restriction of the basin (Flecker et al., 2015) that culminated in the deposition of a huge amount of evaporite, started at 5.97 Ma (Manzi et al., 2013). The precipitation of the 6% of the late Miocene ocean NaCl (1 million of Km³; Blanc, 2006) testifies the dramatic nature of this event, that likely contributed to the decline of pCO₂ characterizing the late Miocene, through change in the Mediterranean-Atlantic exchanges and consequently on the world ocean circulation (Capella et al., 2019). Calcareous nanoplankton were sensitive to the changing environment characterizing the MSC onset; in fact, a peculiar fossil signal was recognized at the Mediterranean basin scale approximating the MSC onset (Lozar & Negri, 2019; Lozar et al., 2018; Mancini et al., 2020). This fossil signal was first identified by Lozar et al. (2018) and described as a succession of peak in abundance of the taxa Sphenolithus abies, followed by Helicosphaera carteri, Umbilicosphaera rotula, and Rhabdosphaera clavigera. This event, previously recognized in the Piedmont Basin, in the Northern Apennines and in the Eastern Mediterranean (Lozar & Negri, 2019), was recently recorded also in the Westernmost Messinian basin (i.e., the Sorbas Basin) and referred as MSC onset bioevent; it marks the last restriction step affecting the Mediterranean-Atlantic water exchange, resulting in an increase in the sensitivity of the Mediterranean basin to external forcing (Mancini et al., 2020).

The present study focuses on the impact of the MSC on CN morphometry through biometric analyses performed on selected CN taxa, with the aim to explore if CN morphometry changes accompanied this event and to constrain the paleoenvironmental meaning of these morphological variations. Samples were collected in the Sorbas Basin, which is one of the best constrained basins from a chronostratigraphic and paleoenvironmental perspective (Baggley, 2000; Clauzon et al., 2015; Filippelli et al., 2003; Flores et al., 2005; Martín et al., 2004; Mancini et al., 2020; Modestou et al., 2017; Pérez-Folgado et al., 2003; Rghizzi et al., 2017; Sierro et al., 2001, 2003; Troelstra et al., 1980). In order to demonstrate that the recorded size change does not represent a stochastic or local response, samples from the Piedmont Basin were analyzed and compared with Sorbas Basin samples.

We also discuss the main drivers of the recorded size fluctuation in order to better constrain the loosely defined environmental “stressed conditions” affecting the Mediterranean Sea before and during the MSC, often thought to be the result of hypersaline conditions in the water column.

We also test if the CN size fluctuations are related to orbital forcing, known to modulate the overall abundances of the microfossil assemblages and the deposition of lithological quadripartite cycle in the Sorbas basin (Mancini et al., 2020; Sierro et al., 2003), by analyzing the taxa Reticulofenestra minuta at high resolution using SYRACO, an automated system of coccoliths recognition and measurements (Beaufort & Dollfus, 2004).

The possible effects on the CN biometry of the decreasing pCO₂ trend characterizing the Messinian is also discussed.
2. Material and Methods

2.1. Studied Material and Age Model

Samples were collected in the Perales section (37° 05′ 49″N, 2° 03′ 19″W, Sorbas Basin, S-E Spain; Figures 1a and 1b) spanning a time interval from ≃6.23 to 5.97 Ma (11 precessional cycles) retrieved from ≃23 m of outcropping succession. At least six samples were collected for each precessional cycle, for a total of 116 samples. The pre-evaporitic Sorbas Basin deposits belong to the upper member of the Abad marls (UA) and consist of precession driven quadripartite sedimentary cycles, each consisting of an alternation of sapropel and diatomite layers that are intercalated between homogeneous white marls (Sierro et al., 2001, 2003).

The age model was built based on foraminifer bioevents (Mancini et al., 2020; Sierro et al., 2001) and on the recognition of the first gypsum layer, marking the MSC onset in the Sorbas Basin (Mancini et al., 2020; Manzi et al., 2013). In the Piedmont Basin, six sample were studied spanning the last five pre-evaporitic precessional cycle in the Banengo section (45° 04′21″N, 8° 03′23″E; Figures 1a and 1c) and nine samples spanning the last seven pre-evaporitic precessional cycle in the Pollenzo section (44° 41′06″N, 7° 55′29″E). In the Piedmont Basin, the precessional cyclicity in the pre-evaporitic successions is defined by the alternation of shale (sapropel equivalent) and white marls/carbonate rich layers (Dela Pierre et al., 2011; Lozar et al., 2018). The age model relies on foraminifer bioevents (Lozar et al., 2018; Violanti et al., 2013) and the recognition of the first branching selenite bed, that corresponds to the sixth precessional cycle of the first stage of the MSC (Dela Pierre et al., 2011; Lugli et al., 2010).

2.2. Absolute Abundance: Samples Preparation and Counting

In order to perform the absolute abundance analyses, samples were prepared following the protocol developed by Beaufort et al. (2014). Briefly, a small amount of dried sediment (≃2.0 mg) was disaggregated into a 50 ml tube using a micro spoon. The tube was filled with ELIX water until 45 ml, hand mixed and sonicated for few seconds (≃2 s). A constant volume of the suspension (7 ml) was extracted with a pipette, poured into a beaker and sonicated a second time (≃2 s). The solution was then transferred, the beaker being cleaned 3 times by spraying ELIX water, into a petri dish with a cover slide at the bottom and left to settle for 4 h. After the sedimentation of the material, the water was evaporated in an oven at 70°C. The obtained samples were permanently mounted on a slide with Norland Optical Adhesive 61 (“NOA61”) and analyzed through light microscopy.

In each sample, a minimum of 500 nannofossils were identified using Olympus BX51 polarized microscope and counted at 1250X magnification. The reworked specimens, whose stratigraphic range is older than that of the analyzed material, were not included in the counting but they were counted separately. The final results are expressed in number of nannofossils per gram of dried sediment (CN/g).
2.3. Biometric Measurements

One of the major issues in the biometric studies is the preservation of the specimens under exam, therefore we performed biometric measurements only on the well preserved specimens (see Supporting Information S1 for details); the broken, overgrown and etched specimens were excluded from the measurements following the common practice in this kind of study (Faucher, Erba, et al., 2017; Herrmann & Thierstein, 2012; Herrmann et al., 2012; Lübke & Mutterlose, 2016; Mattioli et al., 2004, 2009; Suchéras-Marx et al., 2010). For each selected sample, the morphological parameters (Figure 2) of 50 specimens were measured, that represents the common number used among the CN biometric studies (Faucher, Erba, et al., 2017; Lübke & Mutterlose, 2016; Tremolada et al., 2008). The number of analyzed samples depended on the abundance of the analyzed taxa and the overall preservation of the assemblage; measurements were performed in 24 samples for *S. abies*, in 34 samples for *H. carteri*, in 26 samples for *U. rotula* and in 32 for *Coccolithus pelagicus*.

Pictures of the specimens were obtained from a Jenoptik camera (using Progres 5 software) mounted onto Olympus BX50 microscope with a magnification of 1250X. The pictures were analyzed with the software ProGres 5. At least 1–2 samples were analyzed for each precessional cycle (from UA23 to UA33), except in the last pre-evaporitic cycle (UA34) where all samples containing the selected CN taxa were analyzed. This has been done in order to reveal the biometric change accompanying the MSC onset bioevent and the MSC onset.

Regarding *S. abies*, the measures were taken with the specimens viewed at 45° to the polarizers in order to discern the apical spine length. For *H. carteri* and *C. pelagicus*, the ellipticity index (average length/average width) was calculated in order to highlight morphometric changes. In our study the ellipticity index is used to track eventual malformations affecting nannofossils, evaluating the deviation from the ellipticity average calculated in our data set. In the Perales section, for *U. rotula*, both the coccolith external and central area diameters (Figure 2) were measured. The ratio between central area diameter/total diameter lengths were calculated and values close to one indicate a large central area over the total diameter.

In order to demonstrate that the morphometric change accompanying the MSC onset bioevent were global or coeval, size analyses were performed in Banengo and Pollenzo sections (Piedmont basin, North Mediterranean) as well. The samples were analyzed following the procedure described above. In Banengo section six samples were analyzed (1 for each precessional cycle) for *S. abies* and *H. carteri*; in Pollenzo section nine samples were analyzed (1–2 for each precessional cycle) for *S. abies* and *H. carteri*. *Coccolithus pelagicus* and *U. rotula* were not analyzed in the Piedmont Basin because their lower abundance and poorer preservation.

2.4. SYRACO: Corrected Length and Calcification Index

SYRACO is an automated system of coccoliths recognition developed by Beaufort and Dollfus (2004). In our study, SYRACO was used for the recognition and the measurement of the length and the mass of *R. minuta* coccoliths; data collection was performed at the Universitat Autònoma de Barcelona (UAB) during spring 2018. This analysis was performed on *R. minuta*, the only taxon present in all the analyzed samples, from
the Perales section which is precession controlled and spans a long enough time interval. This makes possible the comparison of long term morphometry changes to the paleoenvironmental context associated with precessional fluctuation in the Perales section, which is well constrained by previous studies (Baggley, 2000; Clauzon et al., 2015; Filippelli et al., 2003; Flores et al., 2005; Mancini et al., 2020; Martín et al., 2004; Modestu et al., 2017; Pérez-Folgado et al., 2003; Reghizzi et al., 2017; Sierra et al., 2001, 2003; Troelstra et al., 1980). A certain amount of pictures (at least 40, depending on the abundance of *R. minuta*) were taken with a SPOT insight camera mounted on a LEICA 6000B microscope with a X1000 magnification. A total of 97 samples were processed and a minimum of 200 *R. minuta* coccoliths were measured by SYRACO for each sample. In order to test the reliability of the SYRACO measures, 10 samples were selected for comparing the SYRACO results with a classical manual measurement at the light microscope. In each sample 100 *R. minuta* were identified and measured using the software ProGres 5. The obtained average values were successively processed in order to obtain the equation of the linear regression between SYRACO and the manual measurements (Figure 3a). The linear regression equation was successively used to correct the SYRACO-derived length in all the remaining samples (Figure 2a). In order to have a comparable data set, we used only the SYRACO measurements for the doubled measured parameters.

*Reticulofenestra minuta* calcification index was obtained following the protocol developed by D’Amario et al. (2018); dividing the mass values obtained from SYRACO by the normalized mass (density of calcite × coccolith volume). To calculate the coccolith volume, we multiplied the cube of the corrected length by $K_s$ (estimated shape dependent constant). Unfortunately, the $K_s$ values for *R. minuta* was never calculated (Young & Ziveri, 2000), so we choose to use the $K_s$ calculated for *Gephyrocapsa oceanica* (0.05) that approximate the shape of *R. minuta*.

### 2.5. Statistical Analysis

All the statistic treatments were performed using the software Past 4.03 (Hammer et al., 2001) and Excel. For each selected species, the average of the morphometric parameters, the deviation from the mean average (expressed in %), as well as the standard deviation were calculated. One-way ANOVA (parametric ANalysis Of VAriance) test were performed in order to assess the degree of variance between the obtained means. The null hypothesis is that all the obtained means are the same and the encountered differences among the means are stochastic and not significant. In the case that ANOVA revealed significant differences ($p < 0.05$), a post-hoc Tukey HSD (Honestly Significant Difference) test was performed in order to identify which of the samples (or group of samples belonging to the same precessional cycle) differed. The correlation coefficient $R^2$ was calculated between the measured biometric parameters for all the analyzed taxa in the three analyzed sections. Finally, frequency histograms were performed for all the analyzed taxa in order to assess which size class is more common in our data set.

### 3. Results

Calcareous nanofossils are abundant and moderately/well preserved within the study interval and sections (for detail see Figure S1). The ANOVA test results recorded a significant size variation affecting our data set in all the section studied for all the taxa considered. The Tukey HSD test identified where are located the samples and cycles which statistically differed (for detail see Figures S2 and S3). The target taxa concentration and morphometric data are shown in Table 1 and Figures 3–8 with key trends noted below.

#### 3.1. *Helicosphaera carteri*

In the Perales section, *H. carteri* absolute abundances never exceed $4.0 \times 10^6$ CN/g, except in the cycles UA23, UA32, and UA34, where absolutes abundances from $0.55 \times 10^7$ to $1.35 \times 10^7$ CN/g were recorded (Figure 4). *Helicosphaera carteri* length and width range from a minimum of 4.7 and 3.4 μm respectively, to a maximum of 11.0 and 7.9 μm (Figure 3c) respectively. The average size of *H. carteri* shows maxima values in a cluster of samples in the cycle UA29; minima values are reached in the cycle UA34, where a statistically significant decrease from the mean average was recorded (Figures 4 and S2). The ellipticity index show higher values preferentially when the *H. carteri* width is below the average size. Highest ellipticity index values were recorded in the cycles UA23, UA24, UA26, and UA34 (Figure 4). In the Banengo
Figure 3.
section, *H. carteri* length and width span from 6.1 to 12.1 μm and from 4.2 to 7.3 μm respectively (Figure 5). The minimum average *H. carteri* size values are recorded during the MSC onset bioevent in concomitance with *H. carteri* peak in relative abundance (Figure 6); the ellipticity index shows highest values in the cycles Bm2 and Bm5. In the Pollenzo section *H. carteri* length and width span from 4.27 to 11.7 μm and from 2.5 and 6.5 μm respectively (Figure 5). The minimum *H. carteri* size value is recorded during the MSC onset bioevent in concomitance with *H. carteri* peak in relative abundance (Figure 6), although sizes below the mean average were documented also in the 2 cycles before (UA32 and UA33) the MSC onset bioevent. In the Pollenzo section, after the MSC onset bioevent, *H. carteri* partially recovered its mean size which remains below the average mean size calculated in this site. The ellipticity index in the Pollenzo section shows an opposite trend compared to the other section, with minimum values during the MSC onset bioevent. Some differences emerged comparing the correlation coefficient (i.e., $R^2$), between the measured length, width and the ellipticity index (Figures 3, 5, and S4). The correlation coefficient between length and width was higher in Perales section ($R^2 = 0.9154$) than in the Banengo ($R^2 = 0.5608$) and Pollenzo ($R^2 = 0.6731$) sections. These differences could be the results of the different amount of sample analyzed and specimens measured. Furthermore, the analyzed time interval in the two sections of the Piedmont Basin contain few precessional cycle before the MSC onset, which were characterized by unstable environmental conditions, likely resulting in a more variability of the measured biometric parameters.

### 3.2. *Sphenolithus abies*

In the Perales section, *S. abies* absolute abundance never exceeds the $0.5 \times 10^9$ CN/g, except in the cycle UA34 where the highest abundances are recorded (from $2.06 \times 10^9$ to $3.45 \times 10^9$ CN/g). As already observed for *H. carteri*, the 2 measured parameters (base and length) tend to increase together although this relation seems to be less marked for *S. abies* ($R^2 = 0.5601$). *Sphenolithus abies* length and base span from 2.3 to 6.9 μm and 1.0–5.7 μm, respectively (Figure 3e). The averages of *S. abies* lengths and base in the cycle UA34 show statistically significant decrease (Figures 4, S2, S3, and S5). More generally, a decreasing trend of the measured parameters is discernible from the cycle UA29 (where the maxima values are recorded) upward (Figure 4).

In the Banengo section *S. abies* base and length span from 1.8 to 5.9 μm and 2.5–6.2 μm, respectively (Figure 5). It shows a decreasing trend of the average basal width from the bottom to the top (Figure 6). The minimum *S. abies* average value is recorded during the MSC onset bioevent, matching the *S. abies* peak in relative abundance (Figure 6).

In the Pollenzo section *S. abies* length and base span from 1.5 to 6.5 μm and 1.7–6.5 μm, respectively (Figure 5); the minimum average value is recorded during the MSC onset bioevent, matching the *S. abies* peak...
Figure 4. *Helicosphaera carteri* and *S. abies* biometric measurements and absolute abundances in the Perales section compared with the change in orbital parameters (precession and eccentricity from Laskar et al. [2004]). The error bars represent the standard deviation. The dashed line represents the mean average, the lower axis shows the variation expressed in % from the mean average. The red rectangle indicates the position of the Messinian Salinity Crisis onset bioevent (Mancini et al., 2020).
3.3. *Umbilicosphaera rotula*

Due to the rarity and poor preservation of *U. rotula* in the Piedmont Basin, it has only been possible to measure it consistently throughout the Perales section.

In the Perales section, *U. rotula* absolute abundance never exceed the $0.2 \times 10^9$ CN/g, except in the cycle UA24 ($0.37 \times 10^9$ CN/g) and in the cycle UA34, where its maximum abundance was reached ($1.12 \times 10^9$ CN/g) (Figure 7). *Umbilicosphaera rotula* diameter and central area size span from a minimum 3.2 and 1.2 μm, to a maximum of 8.3 and 4.1 μm respectively (Figure 3g). We found a large morphometric variability in *U. rotula*, with specimens showing heterogeneous central area/diameter index (Figure 7). High *U. rotula* diameter size is recorded in the cycles UA23, UA28 and UA29; the lowest *U. rotula* diameter size is recorded in the cycle UA34, where a statistically significant decrease from the average recorded (Figures 7 and S2).

Likewise the *S. abies* trend, a general decreasing trend of the *U. rotula* diameter is recognizable from UA29 onward with the exception of UA32 where an increase in the diameter is observed. *Umbilicosphaera rotula* central area measurements show high values in the cycle UA23 and UA28, whereas minimum values are recorded in the cycle UA34.

3.4. *Coccolithus pelagicus*

In the Perales section, *C. pelagicus* absolute abundance is generally low (below $2.5 \times 10^8$ CN/g), with the exception of cycle UA34 where it increases up to $8.72 \times 10^8$ CN/g (Figure 7). *Coccolithus pelagicus* length and width span from a minimum of 4.3 and 3.8 μm respectively, to a maximum of 14.3 and 13.4 μm respectively (Figure 3i). The ellipticity index does not mark any significant variation, and slightly fluctuates close to the mean average (Figure 7). The length and the width trends are well correlated ($R^2 = 0.7412$, Figure 3i),

in relative abundance (Figure 6). Above the MSC onset bioevent, *S. abies* shows size values below the average calculated in this site.
showing maxima values from the bottom up to the cycle UA26. A slight increase in *C. pelagicus* length and width is also recognizable in the cycle UA34 (Figure 7). Differently from the others analyzed taxa, *C. pelagicus* does not show a size reduction in the UA34 but it retains size values that fluctuate around the mean average.

**Figure 6.** *Helicosphaera carteri* and *S. abies* biometric measurements in the Banengo and Pollenzo sections (Piedmont Basin) compared with the relative abundance of the species involved in the Messinian Salinity Crisis onset bioevent. The error bars represent the standard deviation. The dashed line represents the mean average, the lower axis shows the variation expressed in % from the mean average.
Figure 7. *Umbilicosphaera rotula* and *C. pelagicus* biometric measurements and absolute abundances in the Perales section compared with the change in orbital parameters (precession and eccentricity from Laskar et al. [2004]). The error bars represent the standard deviation. The dashed line represents the mean average, the lower axis shows the variation expressed in % from the mean average. The red rectangle indicates the position of the Messinian Salinity Crisis onset bioevent (Mancini et al., 2020). Legend as in Figure 4.
3.5. *Reticulofenestra minuta*

*Reticulofenestra minuta* is the main contributor to the total coccolith concentration in the Perales section as revealed by the calcareous nannofossil absolute abundance (Figure 8). High absolute abundance of this taxon is recorded preferentially in the lower part of the studied section (UA23 and UA24) and in the last pre-evaporitic cycle (UA34), where *R. minuta* exceed the $1.5 \times 10^9$ CN/g.

The average length of the *R. minuta* spans from 2.1 to 2.6 μm, with the lowest size and mass values recorded generally in the diatomite and the highest in the sapropel layers. The *R. minuta* length and mass are positively correlated ($R^2 = 0.6417$, Figure 3b) and show a similar trend (Figure 8). Differently, *R. minuta* calcification index is not correlated with the length and mass ($R^2 = 0.0265$ and $R^2 = 0.0276$, Figure S4). *Reticulofenestra minuta* mass shows a decreasing trend from UA32 toward the MSC onset, with lowest value in the cycle UA34 where a statistically significant size decrease was recorded (Figures 7 and S2).

4. Discussion

4.1. Size Decrease During MSC Onset Bioevent and Productivity Change

As revealed by the ANOVA and Tukey HSD test (Figures S2 and S3) a statistically relevant size decrease affected *H. carteri, S. abies,* and *U. rotula* (*U. rotula* was tested in the Perales sections only), concomitant with an increase in CN absolute abundance recorded in the cycle UA34 (Figures 4, 6, and 7). Calcareous nannofossils absolute abundance in the sedimentary record is extensively used to track changes in CN
productivity through the geological past (Browning & Watkins, 2008; Flores et al., 1997; Gibbs et al., 2018; López-Otalvaro et al., 2008). We excluded that the recorded increase in CN absolute abundance was due to a concentration factor as a result of a slowdown of the sedimentation rate, because the precessional cycle bearing the MSC onset bioevent in the Perales section is roughly twice as thick as compared with the other UA cycles. It can be inferred that the increased CN absolute abundance concomitant with a size reduction is not related to the slowdown of the sedimentation rate but represents a primary signal, thus indicating an increase in the calcareous nannoplankton productivity.

In our study, the recorded size reduction took place during a very peculiar event characterized by a distinct fossil signal approximating the MSC onset in several Mediterranean successions (Faneromeni, Pissouri, Polemi Basin, Tokhni, Falconara, Fanantello, Lemme, Pollenzo, Banengo, Moncalvo, and Sorbas; Gennari et al., 2018; Lozar & Negri, 2019; Lozar et al., 2018; Mancini et al., 2020). This event was first identified by Lozar et al. (2018) and described as a succession of CN peak in abundance of the species S. abies, H. carteri, U. rotula, and R. clavigera. This event was recently referred as MSC onset bioevent (Mancini et al., 2020), and marks the last restriction pulse affecting the Mediterranean Basin and triggering the MSC onset (Mancini et al., 2020). The decreased water exchange between the Mediterranean Sea and the Atlantic Ocean increased the sensitivity of the Mediterranean Sea to continental runoff and associated nutrient supply into the basin, ultimately resulting in enhanced marine productivity (Mancini et al., 2020). A further outcome of this study is the possibility to use the CN size reduction as a biostratigraphic signal in those sections in which it is masked by the cyclical peaks of the species involved in the MSC onset bioevent (e.g., Tokhni section, Gennari et al., 2018; Perales section, Mancini et al., 2020). However, several samples encompassing several precessional cycles needed to be analyzed in order to discern the size variation during the MSC onset bioevent, since the recorded absolute values of the CN sizes is not comparable between the 3 sections studied.

High CN absolute abundances are also recorded in the cycles UA23 and UA24 (4.64 × 10^9 CN/g and 3.54 × 10^9 CN/g, respectively (Figure 8)), but not associated with noticeable size decreases. This suggests either that the CN growth rate alone was likely not high enough for driving any relevant size change, or the mechanisms leading to size reduction are likely associated with the triggering factors inducing the enhanced CN productivity. Therefore, we hypothesized that the CN size decrease concomitant with high CN abundance recorded during the MSC onset bioevent could be linked to the enhanced freshwater influence delivering nutrients and the associated changes in the water column structure (Mancini et al., 2020). Differently, in the lower UA23 and UA24 cycles, indication of important freshwater input was not recorded and the CN high abundance encountered in these cycles could be explained as a result of a diminished sedimentation rate resulting in highest concentration of CN in the bulk sediment. From this observation, it could be inferred that the enhanced freshwater input and the associated changes in the water column structure, turbidity and nutrients delivery are the primary candidates for explain the CN size reduction accompanying the MSC onset. Since the increase in water turbidity cannot explain high CN absolute abundance, we infer that the nutrient delivery may have had the primary control on the CN size reduction. In the Eastern Mediterranean, Karakitsios et al. (2017) show a sharp freshwater input occurring between 6.45 and 6.121 Ma evidenced by H. carteri, C. leptoporus, U. jafari, R. haqii, and Reticulofenestra minutula; this event was probably local since no evidence of such freshening has been detected further West in the Sorbas basin.

The relationship between the nutrient regime and coccolithophores size was highlighted by some culture experiments, in which the taxa C. leptoporus, C. quadriperforatus, H. carteri, and Emiliania huxleyi show that during the exponential growth phase, characterized by a rapid cell division rate, small cells (characterized by a low number of interlocking coccoliths) are produced (Gibbs et al., 2006, 2013; Sheward et al., 2017; Šupraha et al., 2015). Conversely, larger cells (with higher number of coccoliths) are produced during slow cell division (Gibbs et al., 2013; Sheward et al., 2017). Taking into account that a correlation could exist between the coccosphere size, the number of coccoliths covering the cell and the coccoliths size (Aloisi, 2015; Gibbs et al., 2013; Henderiks & Rickaby, 2007), an assessment of the cell division rate can be inferred from morphometrical changes measured on the coccoliths, as already performed by Gibbs et al. (2013, 2018) in the sediments straddling the Paleocene-Eocene thermal maximum (PETM). The size reduction affecting H. carteri, S. abies, and U. rotula, and the highest CN productivity recorded, could be the results of rapid cell division, occurred during period of excess nutrient availability and favorable water column structure (e.g.,
it was reconstructed that S. abies exploited nutrients in the deep photic zone when thermal stratification occurred, Flores et al., 2005; Mancini et al., 2020). In our study, similar morphometric changes were not observed in C. pelagicus, which is in line with the culture experiments that showed no significant coccolith size change related to experimental conditions (i.e., stationary growth phase vs. exponential growth phase; Gibbs et al., 2013; Sheward et al., 2017). However, a parallel between the fossil record and the laboratory experiments could be rather speculative, since some taxa considered in our study are extinct and the coccolithophores response to the environmental variables could be strain-specific, as demonstrated for C. pelagicus (Gibbs et al., 2013; Henderiks & Rickaby, 2007; Sheward et al., 2017). Differently from other coccolithophore taxa, C. pelagicus morphology was not affected also during the PETM, likely because of its insensitivity to the broad $p$CO$_2$ variation that characterized this event (Henderiks & Rickaby, 2007).

Therefore, we infer that during the MSC CN bioevent, S. abies, H. carteri, and U. rotula shifted to more r-selected strategy, by increasing their cell division rate to exploit nutrients during seasonal favorable conditions. This resulted in a size decrease and in an increase in CN absolute abundance (Figure 8).

In other geological case histories, the CN size reduction is often reported to be related to “stressed” conditions (Faucher, Erba, et al., 2017) and the CN size increase with stable conditions (Ferreira et al., 2017). However, our results show that the CN size reduction occurred during period characterized by high CN productivity, thus indicating optimal environmental condition for calcareous nannoplankton to growth rather than stressed environment, at least during the MSC onset bioevent. Relevant size decreases were recorded during Oceanic Anoxic Events (Erba et al., 2010; Faucher, Erba, et al., 2017; Lübke & Mutterlose, 2016) in relation with abrupt increase in CO$_2$ emissions (and the consequent pH decrease in surface water) (Erba et al., 2010), toxic metal increase (Faucher, Hoffmann, et al., 2017), or with an increase in continental runoff and the associated increase in water turbidity (Lübke & Mutterlose, 2016). Generally, the amplitude of the CN size reduction during the OAEs is comparable to that recorded during the MSC CN bioevent (Erba et al., 2019).

After the MSC onset, the CN productivity gradually decreased and the CN size (H. carteri, S. abies, and U. rotula) oscillates without reaching the previous size average. The basin restriction occurring during the MSC CN bioevent amplified the sensitivity of the Mediterranean Basin to external forces, such as temperature change, freshwater input, and nutrient delivery (Mancini et al., 2020). This ultimately may resulted in an increased environmental variability, which could fall within the definition of “stressed” conditions. An attempt of comparison between the paleoenvironment straddling the MSC with actual analog could be provided by modern lagoon environments (e.g., in the Términos Lagoon, SE Gulf of Mexico), in which strong changes in salinity, productivity and turbidity occurred in response mainly to seasonal change in the freshwater input (Poot-Delgado et al., 2015; Yáñez-Arancibia & Day, 2005). Coccolithophores were recorded in some lagoon environments (Poot-Delgado et al., 2015; Sakka et al., 1999) showing distribution pattern according mainly to seasonal variations (Poot-Delgado et al., 2015). Therefore, it could be inferred that after the MSC onset bioevent, calcareous nannoplankton were likely affected by strong environmental variability because of the increased basin restriction; they were forced to increase the cell division rate to face and survive the rapid development of hostile environmental conditions, such as rapid and extreme changes in salinity and nutrient delivery.

In our studied sections, the MSC onset bioevent is often accompanied by the disappearance of normal size planktic foraminifers (>125 μm; Lozar et al., 2018; Violanti et al., 2013); the assemblage is dominated by dwarf foraminifers in the 43–125 μm size fraction (Figure 9). The record of dwarf planktic foraminifers during the MSC interval was already reported in cores and land sections (Braga et al., 2006; Cita et al., 1978; Fortuin & Krijgsman, 2003; Hsi et al., 1978; Iaccarino & Bossio, 1999; Riding et al., 1998; van de Poel, 1992), and interpreted as the result of “stressed” or “anomalous” water column condition (Corbi et al., 2016), although some authors suggested that dwarf planktonic foraminifers were symptomatic of high nutrients input (Kaiho et al., 2006; van de Poel, 1992). We suggest that the anomalous or stressed conditions were likely related to the restriction of the Mediterranean Basin, that resulted in an increase in the environmental variability (e.g., increase in the freshwater input and the associated nutrients delivery) which forced also the foraminifers to faster complete their life cycle and hence, to reduce their size (Figure 9).
4.2. *Reticulofenestra Minuta* Size as a Tracer for Precessional Variability

Very little is known about how orbital forcing affects climate and environmental change and how this is linked to coccolithophore size. Suchéras-Marx et al. (2010) highlight the relevance of orbital-controlled environmental factors influencing CN morphometric parameters. To test this hypothesis, our analysis was restricted to *R. minuta*, which dominated the assemblage throughout the studied interval in the Perales section.

Our results reveal a good correlation between *R. minuta* size and mass and the lithological changes of the sedimentary quadripartite cycle (Figure 3b), deposited under precessional control (Sierro et al., 2003). *Reticulofenestra minuta* shows minimum in the average length and mass during diatomite deposition (Figures 3b and 8). The diatomites were usually characterized by a mixture of CN and foraminifers with palaeoecological affinity for either warm/oligotrophic, cold/eutrophic (i.e., warm-oligotrophic/cold-eutrophic index of Sierro et al. [2003]; i.e., *C. pelagicus*, Mancini et al., 2020), and/or salinity decrease (i.e., *H. carteri*, Mancini et al., 2020). Despite the diatoms-based information are available only for the lower cycle of the UA (i.e., UA5 – UA8; Pérez-Folgado et al., 2003), a strong seasonality is suggested to characterize these lithologies because of the presence of *Thalassionema* and *Rhizosolenia*. The former is generally associated with the cold season and enhanced surface primary productivity (McKenzie et al., 1979; Moissette & Saint Martin, 1992), while the latter is generally associated to the warm season and to a Deep Chlorophyll Maximum development (Kemp & Villareal, 2013; Margalef, 1978). Based on these evidence, it was inferred that the diatomite were deposited in an environment characterized by strong seasonality, with enhanced runoff inducing stratification during warm/humid phases and vigorous vertical mixing during cold/drier phases (Mancini et al., 2020).

On the opposite, *R. minuta* size and mass show higher value in the upper part of the sapropel partitioning (Figures 3b and 8), where the available proxies point to a stable and strongly stratified water column (Filippelli et al., 2003; Flores et al., 2005; Mancini et al., 2020; Pérez-Folgado et al., 2003; Reghizzi et al., 2017; Sierro et al., 2003).

From cycle UA31 toward the MSC onset, the mass of *R. minuta* shows higher fluctuation amplitude with the tendency toward lower values (Figure 8), likely reflecting an increase in the environmental variability at the precessional scale, that could be attributed to the climate sensitivity amplification induced by the restriction step occurred at ∼6.03 Ma and recorded in the nearby Bajo Segura Basin (Corbí et al., 2020) and in the whole Mediterranean approximately at the same time (Gennari et al., 2018; Kouwenhoven et al., 2006). The same response was recorded in the foraminiferal diversity index and in the benthic foraminiferal absolute abundance in the Perales section (Sierro et al., 2003), marking an increase in the environmental instability toward the MSC onset. In this framework, it is reasonable inferring that the drivers of the *R. minuta* size decrease are related to unstable environmental condition that occurred within an annual cycle, leading *R.
minuta to reproduce faster to face the unstable environmental condition during the diatomite deposition and toward the MSC onset.

4.3. The Possible Role of $pCO_2$ on Calcareous Nannoplankton Size

In recent years, many studies focused on the calcareous nannoplankton response to the ongoing ocean acidification and warming (Iglesias-Rodriguez et al., 2008; Langer et al., 2006, 2009; Meyer & Riebesell, 2015; Zondervan, 2007), caused by substantial accumulation of anthropogenic CO$_2$ emissions in the atmosphere, and the consequent impact on the biological carbon pump (Rost & Riebesell, 2004; Ziveri et al., 2007). The geological record offers many opportunities to study rapid environmental perturbations and their impact on the biota. For instances the Paleocene-Eocene Thermal Maximum (PETM) is often compared to the current anthropogenic climate change, although the rate of CO$_2$ emission is several times lower compared to the present scenarios (Zeebe et al., 2016).

The late Miocene was characterized by a rapid decline in the global sea surface temperature (6°C between 30° and 60°N within 7.5–5.5 Ma; Herbert et al., 2016), likely related to the decrease in $pCO_2$ inferred from the stable isotopes of coccolithophores and changes in the global vegetation covering (Bolton & Stoll, 2013; Bolton et al., 2016; Herbert et al., 2016). Recent studies underline the relevance of the MSC on the decline in CO$_2$ content occurring during the late Miocene, as the result of limited water exchange between the Mediterranean Sea and the Atlantic Ocean (Capella et al., 2019). Interestingly, we noted that the late Miocene cooling trend (Herbert et al., 2016) fits with the Coccolithophore Assemblage Size (CAS, Herrmann & Theirstein, 2012) and with the onset of the MSC (Figure 10). Henderiks and Pagani (2008) speculate that the shift toward smaller size of the reticulofenestrids during the Oligocene reflects an adaptive response to a decrease in $pCO_2$. However, this physiological adaptation to $pCO_2$ fluctuations is species specific and for instance did not affected C. pelagicus (Gibbs et al., 2013; Henderiks & Rickaby, 2007). The size reduction affecting some selected species during the MSC CN bioevent occurred abruptly, does not fit the gradual decrease in temperature (and $pCO_2$) inferred from late Miocene records (Herbert et al., 2016). Moreover the calculated sea surface temperature in the East Mediterranean during the Messinian (from 6.45 to 5.97 Ma) did not reveals any cooling trend toward the MSC onset (Vasiliev et al., 2019). Consequently, the size reduction during the MSC onset bioevent was likely not directly related to the ongoing global decrease in temperature and $pCO_2$. Differently, the cyclical R. minuta size and mass fluctuation could be related with precessional-induced $pCO_2$ variability, but this inference remains speculative, because of the scarcity of knowledge regarding the $pCO_2$ trend and its variability at the precessional scale throughout the late Miocene.

Although the trigger for the CN size reduction during MSC CN bioevent was likely related to local environmental changes, further efforts are necessary to better understand the sparse but striking data regarding the correlation within CN size, $pCO_2$ and MSC onset.

5. Conclusion

Calcareous nannofossil biometry and absolute abundance analyses were performed in the Sorbas Basin (Perales section) and in the Piedmont Basin (Banengo and Pollenzo sections) in order to highlight the impact of the MSC onset on marine organisms and ultimately decipher the paleoenvironmental dynamics spanning this time interval.

An increase in the CN absolute abundance, concomitant with a size decrease of H. carteri, S. abies, and U. rotula was recorded at the time of the MSC onset bioevent. These morphometric changes are in the same order of magnitude of those reported during major environmental perturbations during Earth history. These size reductions were likely related to an increase in calcareous nannoplankton productivity that led to selected calcareous nannoplankton taxa to increase the overall growth rate and decreasing their size, as supported by laboratory culture experiments. Calcareous nannoplankton productivity was triggered by a restriction pulse affecting the Atlantic-Mediterranean gateway that increased the continental runoff and the associated nutrient delivery influence in the Mediterranean Sea approximately at the MSC onset. After the MSC onset bioevent, the analyzed taxa did not recover their normal size, and their mean size always fluctuated below the mean average, in parallel with a drastic reduction of CN absolute abundance. In this
case, dwarf CN are symptomatic of a highly variable environment, comparable with actual lagoonal system in which the environmental parameters are strongly modulated by the freshwater input.

Our analysis also reveals that \textit{R. minuta} size and mass cyclically fluctuate mimicking the precession curve, revealing that such size decrease occurs during time interval characterized by strong seasonal variability.

\textbf{Figure 10}. Sea surface temperature evolution based on alkenone record over the late Miocene and Pliocene (Herbert et al., 2016; Vasiliev et al., 2019) compared with the 90th percentile of Coccoliths Assemblage Size (CAS-90) (Herrmann & Thierstein, 2012) and the \textit{Noëlaerhabdaceae} coccolith length (Bolton et al., 2016). The gray rectangle marks the period of the Mediterranean Basin restriction and of the Messinian Salinity Crisis (Capella et al., 2019). The blue line represents the temperature evolution at high latitudes (>50°N) in the North Hemisphere; the green line represents the temperature evolution at mid latitudes (30°–50°S) in the South Hemisphere; the black line represents the temperature evolution at mid latitudes (30°–50°N) in the North Hemisphere; the red line represents the temperature evolution at the tropics (Herbert et al., 2016). The violet rectangle represents the East Mediterranean sea surface temperature range (Vasiliev et al., 2019). The cyan and the gray lines represent the means of CAS-90 evolution at the low latitudes (<45°) and at high latitudes (>45°). The orange and the violet lines represent the \textit{Noëlaerhabdaceae} coccolith length measured at the site NGHP 01-01A (Indian Ocean) and at the ODP site 925 (Atlantic Ocean), respectively. Data from Herbert et al. (2016), Herrmann and Thierstein (2012), and Bolton et al. (2016); redrawn after Herbert et al. (2016) and Bolton et al. (2016).
corresponding to the diatomite layer in the Perales section. The \( R. \ minuta \) size and mass fluctuation amplitude increases from \(-6.03 \) toward the MSC onset, where it shows the lowest values. Also in this case, smaller CN were related to unstable water column conditions, which forced CN (and foraminifers, toward the MSC onset) to reproduce faster and decrease their size to face the unstable environmental conditions.

The influence of the gradual global decline of \( p\text{CO}_2 \), characterizing the Messinian, was not responsible for the size reduction recorded during the MSC onset bioevent, although a possible role governing the size variability of \( R. \ minuta \) at the precessional scale cannot completely be discarded.

Conflict of Interest

The authors declare no conflicts of interest relevant to this study.

Data Availability Statement

The generated data set is stored on Zenodo repository (https://zenodo.org/record/4569513#.YDz5k2hKg2w).

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