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Ocean Science Discussions
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Interactive comment on "Is coccolithophore distribution in the Mediterranean Sea related to seawater carbonate chemistry?" *by* A. M. Oviedo et al.

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The authors want to thank K.J. Sebastian Meier for these comments. The comments from the referee together with our answers are provided below:

General comments (1) Sampling has been carried out over a relatively short time in April 2011. I don't think that this is representative of a whole year, and phytoplankton populations may change during the year. In the study of Knappertsbusch (1993) who studied different seasons, this effect seems to be relatively large. I would like to see short discussion on how the assemblages from the study of Knappertsbusch compare to your results, and how representative the sampling in April 2011 may have been.

Also, a comparison with surface sediments may help.

Response: We agree with the reviewer on the importance of discussing seasonal variations. The Mediterranean is characterized by a strong seasonal cycle. The samples studied in our work were collected in a period of relatively high primary production during early spring. We are conscious of the time frame of the study and did not pretend to extrapolate these observations to other seasons. Seasonality be will be addressed in the discussion. We will compare our results with those obtained in other studies, during other seasons (e.g. Ignatiades et al., 2009 and Knappertsbusch, 1993). Ignatiades et al. show that during June 1999 coccolithophores dominated both, the eastern and western basins and that cell densities increased towards the western Mediterranean. These observations are similar to our results in April 2011. Concerning the coccolithophore community, the study of Knappertsbusch (1993) suggests the occurrence of interannual variability. His sampling was divided in two periods: February-March (1988) and September-October (1986). Knappertsbusch observed higher cell densities during late winter. In this period, coccolithophore cell density increased towards the eastern Mediterranean, reaching 230000 cell/l in the Levantine basin. During September-October this pattern was reversed. Our results resemble those corresponding to September-October in Knappertsbusch work, although with higher cell densities. We will further discuss the implications of these comparisons.

(2) The filter preparation and counting may cause a bias. Filtering and oven-drying will destroy most phytoplankton with an organic covering, so dinoflagellates will be underrepresented on the filters. Furthermore, counting at 3000x magnification a relatively small patch of the filter will lead to an underrepresentation of larger cells, e.g. diatoms and dinoflagellates. Therefore, the conclusions drawn on the dominance of coccolithophores may be wrong.

Response: We had counted only thecate and calcareous dinoflagellates. The low oven temperature used to dry the filters (40°C) apparently did not damage several dinoflagellates species that were quantified by SEM and were very well preserved. However, it

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could be that some delicate forms had been destroyed resulting in an underestimation of their cell density. Ignatiades et al. (2009) cell density of thecate and non-thecate dinoflagellates ranged between ≈2x103 to 4x104 cells/l. The lower and mid values in the latter study were in the same order of magnitude as our estimations (local maxima ≈3x103 - 1x10 4 cells/l). They registered a pick of ≈4x105 cells/l in the north western Mediterranean; such value is 1-2 orders of magnitude higher than our estimations. Lasternas et al. (2011) show a similar pick at the Tyrrhenian Sea, during late summer (4x105 cells/l including thecate and non thecate species). A closer inspection at their data reveals that, among thecate dinoflagellates, Scripsiella sp. displayed the maximum abundance (1.3x105 cells/l). The species belonging to the Scripsiella genus are"armoured" with delicate plates. We did not observe this species and cannot discard the possibility that this difference is due to the methodological constrains. Other species with "delicate"plates that were common during the study of Lasternas et al. (2011) were not in our counts. We will omit the data concerning dinoflagellate counts. It should be noticed that, even if dinoflagellate abundances were close to the maximum reported by previous studies, coccolithophores would still be dominant in relation to dinoflagellates and diatoms.

For diatoms, counting at 3000X did not cause a bias towards smaller sizes in a subset of samples that we have counted by light microscope, at 1000X. As well as in SEM counts, diatom relative abundances were low in the optical microscopy counts. They comprised between 1.7 and 3.3% (SD= 0.85) of cells counted by optical microscopy and between 0.8 and 4.1% of cells by counted SEM. Even further, in some cases we counted fewer diatoms by optical microscopy than by SEM. It is important to notice that the size of phytoplankton in the Mediterranean Sea is thought to be very small, with a predominance of cells <10 μm during the spring period (Raimbault et al., 1988; Decembrini et al., 2009). Diatom cell densities in the relatively productive Tyrrhenian Sea have ranged from 0.2x103 to 0.8 x103 cell/l in surface and from 1 x103 up to 5 x103 cell/l below the thermocline in September-October 2004 (Lasternas et al., 2011). In July 2005, cell densities ranged between 0.8 x103 to 2 x104 and in December between

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0.7 x103 and 2.6 x103 (Decembrini et al., 2009). In a transept along the Mediterranean Sea (Ignatiades et al., 2009) cell densities varied from few individuals, in the Ionian Sea-surface to ≈ 3.5x104 in the Tyrrhenian (June 1999). Therefore, previous data on diatom densities are on the same order of magnitude as in our work, which also support the results of the revision performed for our counts.

(3) The outcome of the statistical analysis feels strange sometimes. When looking at Figure 2, CO32- and temperature look very similar (which they mostly do), but statistically CO32- is highly significant, while temperature seems to be not significant. Can you explain this? Furthermore, why are the species representing the Atlantic inflow, i.e. E. huxleyi type B/C and Gephyrocapsa species negatively correlated with CO32- in Table 3? When comparing Figures 2 and 6, they are found in the region with highest CO32- values. Also, the rho values in Table 1 are not so much lower for combinations of more than 3 variables. Therefore, there may be too much focus on the carbonate system parameters in the discussion.

Response: It should be noticed that while Figure 2 shows the distribution of environmental properties along the Mediterranean Sea, the BIOENV procedure relates the differences or similitudes in the phytoplankton community among the different stations, with the differences or similitudes in environmental parameters among stations. Therefore, neither total abundance data, nor species richness or diversity data will provide the same information. Comparing the latter data to the distribution of environmental parameters does not equal a comparison of the similarities or dissimilarities matrices derived from the biological and environmental data –a community analysis-. Nevertheless, it brings general information on the selected group and/or taxon as a whole. CO32- and temperature are, in fact, correlated (Figure 1 in this responses, rho: 0.562, $p<0.01$). Both variables showed a general W-E gradient, with highest temperature and CO32- in the eastern Mediterranean, as showed in Figure 2 in the manuscript. Nevertheless, the gradient in temperature was a feature of waters below 25m. Above this depth, surface temperature was relatively high along all the transect. CO32- was

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lower in the western basin (also in the first sampled depths) and increased towards the East. These differences could explain, at least partly, why the two variables do not contribute equally in the“best fit” analysis (BIOENV procedure) for heterococcolithophores. The fact that neither heterococcolithophore abundances nor species diversity correlated to temperature, while species diversity correlated to CO32- (rho: -0.437, p<0.01), could also explain why the latter environmental parameter appeared to be more important than temperature in explaining heterococcolithophore distribution patterns in the BIOENV routine. The species E. huxleyi type B/C and Gephyrocapsa species were actually more abundant in the regions with lower CO32-concentration (western basin). As mentioned above, CO32- showed a W-E increasing gradient (Figure 2). Figure A shows the relationship between both, CO32- and temperature and G. oceanica, a typical species of the cluster that we argue can be tracing Atlantic waters. A wider dispersion of the data can be observed in relation to temperature while, it is clear that higher cell densities are found at lower CO32- concentrations. Rho values in Table 1 describe how the differences or similitudes in the coccolithophore community (heterococcolith bearing cells) among stations relate to the differences or similitudes in environmental parameters. The combinations: 1) CO32- , pH, salinity, and PO43- and 2) CO32- and salinity were not so much lower than the “best fit” (CO32- , pH, salinity). It is worth noticing that while the only environmental parameter that correlated to heterococcolithophore abundance was pH (rho: 0.247, p<0.05), all of those chosen as the best 4 variable combination (CO32- , pH, salinity and PO43-) correlated to the species diversity (H‘) of coccolithophores in heterococcolith phase, but PO43- showed the weakest and least significant correlation (rho: 0.322, p<0.05). We will include the results of these correlations in Table 3 as well as a brief discussion on the relative importance of PO43- for heteroccolithophores.

Minor comments (probably incomplete, please check for more typos and grammar mistakes)

Abstract

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P 614 L 5: as marine calcifying organisms L 6: systems parameters L 7: physicochemical

Response: L 5 – L 7 we will correct these mistakes and check carefully the complete text.

L 19: What do you mean by preferentially distributed? Higher abundance?

Response: For preferentially distributed, we meant with higher abundances. We will clarify it.

Introduction

It may be noteworthy, that there are observations of coccolith and calcareous dinoflagellate distributions in the sediment, that show some distinct W-E patterns (see Knappertsbusch 1993; Meier and Willems 2003)

Response: We will add this information. Regarding coccoliths, Knappertsbusch (1993) observed in both, water and sediments, a similar community composition to the one we have observed in water samples. In specific, Gephyrocapsa spp. in the western basin and R. clavigera, U. tenuis and D. tubifera in the eastern basin. G. oceanica was also observed in eastern sediment samples.

P 615 L 10: check grammar in “being calcification....”

Response: The grammar will be checked throughout the text.

L 15: what is PAR?

Response: We meat photosynthetically active radiation (PAR).

P616 L 17: above mentioned

Response: L17, It will be corrected

L 18: “Additionally, when a west to east transect was sampled (Knappertsbusch, 1993; Ignatiades et al., 2009) carbonate chemistry parameters were not.” This sentence

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sounds very strange to me. Perhaps something like “Carbonate chemistry parameters were not measured in older studies on the distribution of coccolithophores in the Mediterranean Sea (...)” could be better.

Response: We will change the sentence to: “Carbonate chemistry parameters were not available in older studies focussed on the distribution of coccolithophores in the Mediterranean Sea (...)”.

Material and methods

P 617 L 7: Rosette

Response: It will be corrected

L12: Filtering and especially oven drying will destroy most of the dinoflagellates, as they have cellulosic walls.

Response: As stated in the response to the second general comment: We had counted only thecate and calcareous dinoflagellates. The low oven temperature used to dry the filters (40°C) apparently did not damage several dinoflagellates species that were quantified by SEM and were very well preserved. However, it could be that some delicate forms had been destroyed resulting in an underestimation of their cell density. Ignatiades et al. (2009) cell density of thecate and non-thecate dinoflagellates ranged between ≈2x103 to 4x104 cells/l. The lower and mid values in the latter study were in the same order of magnitude as our estimations (local maxima ≈3x103 - 1x10 4 cells/l). They registered a pick of ≈4x105 cells/l in the north western Mediterranean; such value is 1-2 orders of magnitude higher than our estimations. Lasternas et al. (2011) show a similar pick at the Tyrrhenian Sea, during late summer (4x105 cells/l including thecate and non thecate species). A closer inspection at their data reveals that, among thecate dinoflagellates, Scripsiella sp. displayed the maximum abundance (1.3x105 cells/l). The species belonging to the Scripsiella genus are“armoured” with delicate plates. We did not observe this species and can not discard the possibility that this

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difference is due to the methodological constrains. Other species with “delicate”plates that were common during the study of Lasternas et al. (2011) were not in our counts. We will omit the data concerning dinoflagellate counts. It should be noticed that, even if dinoflagellate abundances were close to the maximum reported by previous studies, coccolithophores would still be dominant in relation to dinoflagellates and diatoms.

L 19: Counting at 3000x magnification for all groups may cause a bias towards smaller groups.

Response: As stated in the response to the second general comment: Counting at 3000X did not cause a bias towards smaller sizes in a subset of samples that we have counted by light microscope, at 1000X. As well as in SEM counts, diatom relative abundances were low in the optical microscopy counts. They comprised between 1.7 and 3.3% (SD= 0.85) of cells counted by optical microscopy and between 0.8 and 4.1% of cells by counted SEM. Even further, in some cases we counted fewer diatoms by optical microscopy than by SEM. It is important to notice that the size of phytoplankton in the Mediterranean Sea is thought to be very small, with a predominance of cells <10 μm during the spring period (Raimbault et al., 1988; Decembrini et al., 2009). Diatom cell densities in the relatively productive Tyrrhenian Sea have ranged from 0.2x103 to 0.8 x103 cell/l in surface and from 1 x103 up to 5 x103 cell/l below the thermocline in September-October 2004 (Lasternas et al., 2011). In July 2005, cell densities ranged between 0.8 x103 to 2 x104 and in December between 0.7 x103 and 2.6 x103 (Decembrini et al., 2009). In a transept along the Mediterranean Sea (Ignatiades et al., 2009) cell densities varied from few individuals, in the Ionian Sea-surface to $\approx$ 3.5x104 in the Tyrrhenian (June 1999). Therefore, previous data on diatom densities are on the same order of magnitude as in our work, which also support the results of the revision performed for our counts.

Results

P 620 L 24: Figure 3 is mentioned before Figure 2. P 622

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Response: It will be corrected

Discussion

P 623 C180 L 5: nutrients concentration

Response: It will be corrected

L 13: “Even though only reached at the Gibraltar Strait, the highest cell density of coccolithophores was 1–2 orders of magnitude higher than for the other phytoplankton groups.” This may be due o the preparation and counting bias mentioned above. Therefore this whole section should be discussed more carefully. If larger numbers of diatoms and dinoflagellates were present (but not recorded due to methodological restrictions), a discussion on competition between these groups could be necessary.

Response: As mentioned above, we have estimated no bias caused by the counting procedure. For dinoflagellates, however, we cannot estimate the possible loses caused by the filter preparation methods and thus, we will not use this data in the manuscript. Nevertheless, previous data on dinoflagellate abundances in the Mediterranean Sea (please see details in the response to P617 L12 and L19) are not higher than the cell densities reported for coccolithophores during April 2011. This might, however, change the relation diatoms/dinoflagellates to an extent we could not state one group dominated over the other.

P 624 L 16: Section 4.2 lacks clarity and structure. Especially the discussion about the holo- coccolithophores is unclear to me. I understand that the different stages of the same organism may have different ecological preferences. The data presented here seems to suggest that all holococcolith phases have similar preferences, while the heterococcolith phases form three groups. However, these groups comprise only 12 out of 70 species. What about the other 58 species? I assume that they do not show a clear regional distribution. Could it be, that the majority of the holococcolith phases are related to these species? It would be good to see the distribution of, e.g.

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the Syracosphaera pulchra HOL types or other HOL phases of the species that show a eastward distribution. Are they as well evenly distributed over the entire Mediterranean?

Response: The reviewer clearly stated the some of the main findings from this section. However, we will separate this section in two. The other 58 species showed a patchy distribution with no clear pattern. Could it be that holococcolithophore cells are related to the heteroccolithophore cells that that did not show a clear distribution? Apart from the Gibraltar strait, holococcolithophores were more frequent and abundant at the eastern basin while the heterococcolith bearing species, with a patchy distribution, cannot be clearly related to one of the two basins. Statistically, the BIOENV analysis takes into account these heterococcolith bearing species as it analyses the differences in species composition and abundances between stations. The reviewer brings a very interesting question we have left aside in the manuscript: Does a species that was found to be more abundant in the eastern Mediterranean show the same distribution in the two life stages? We have checked this issue for Syracosphaera pulchra. The holococcolithophore phase shows a slightly wider distribution range as it is present until the Algerian Sea, but maximum cell densities are lower. We will compare the distribution of the species were the two phases were reconigzed.

L 20: were only recently sampled

Response: It will be corrected

L 24: plausible

Response: It will be corrected

P 625 L 1: The carbonate species that is used for calcification is more likely HCO3- (see Bach et al. 2012).

Response: CO32- has been mistakenly used, as we refer later in L20. It will be corrected and the paragraph reformulated to avoid repeating the same information.

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P 626 L 1: What is the reference for E. huxleyi blooms in the Baltic Sea? Or do you mean the blooms recorded in the Skagerrak (which is more the North Sea)?

Response: The sentence is incomplete. It will be changed to: "Tyrell et al. (2008) observed that Emiliania huxleyi blooms in the Baltic Sea coincide with periods of high [CO32-]."

P 627 L 25: The statement "Interestingly, during this haploid life stage the different species seem to behave as a homogeneous group, exploiting a similar ecological niche." is difficult to understand.

Response: The sentence will be simplified to: "Holococccolithophores seem to behave as a homogeneous group, exploiting a similar ecological niche."

Table 1

I assume that temperature is not shown in the first row, as it has a rho value less than 0.2. This seems strange, as the temperature and CO32- usually covary, and also when looking at the maps in Figure 2, they show a very similar distribution.

Response: The assumption is correct. As previously mentioned: CO32- and temperature are, in fact, correlated (Figure A in this answer, rho: 0.562, $p<0.01$). Both variables showed a general W-E gradient, with highest temperature and CO32- in the eastern Mediterranean, as showed in Figure 2 in the manuscript. Nevertheless, the gradient in temperature was a feature of waters below 25m. Above this depth, surface temperature was relatively high along all the transect. CO32- was lower in the western basin (also in the first sampled depths) and increased towards the East. These differences could explain, at least partly, why the two variables do not contribute equally in the"best fit" analysis (BIOENV procedure) for heterococcolithophores. The fact that neither heterococcolithophore abundances nor species diversity correlated to temperature, while species diversity correlated to CO32- (rho: -0.437, $p<0.01$), could also explain why the latter environmental parameter appeared to be more important than temperature in

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explaining heterococcolithophore distribution patterns in the BIOENV routine.

Table 3

Why are the species representing the Atlantic inflow, i.e. E. huxleyi type B/C and Gephyrocapsa species negatively correlated with CO32-? When looking at Figure 2, they are found in the region with highest CO32- values.

Response: As mentioned in the response to the third general comment: It should be noticed that while Figure 2 shows the distribution of environmental properties along the Mediterranean Sea, the BIOENV procedure relates the differences or similitudes in the phytoplankton community among the different stations, with the differences or similitudes in environmental parameters among stations. Therefore, neither total abundance data, nor species richness or diversity data will provide the same information. Comparing the latter data to the distribution of environmental parameters does not equal a comparison of the similarities or dissimilarities matrices derived from the biological and environmental data –a community analysis-. Nevertheless, it brings general information on the selected group and/or taxon as a whole. CO32- and temperature are, in fact, correlated (Figure 1 in this responses, rho: 0.562, $p<0.01$). Both variables showed a general W-E gradient, with highest temperature and CO32- in the eastern Mediterranean, as showed in Figure 2 in the manuscript. Nevertheless, the gradient in temperature was a feature of waters below 25m. Above this depth, surface temperature was relatively high along all the transect. CO32- was lower in the western basin, including its surface, and increased towards the East, almost without signs of vertical stratification. These differences could explain, at least partly, why the two variables do not contribute equally in the“best fit” analysis (BIOENV procedure) for heterococcolithophores. The fact that neither heterococcolithophore abundances nor species diversity correlated to temperature, while species diversity correlated to CO32- (rho: -0.437, $p<0.01$), could also explain why the latter environmental parameter appeared to be more important than temperature in explaining heterococcolithophore distribution patterns in the BIOENV routine.

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Figures

The figures nicely present the data and analyses. The resolution (dpi) should be improved on the transects for better viewing.

Response: We will increase the resolution of the mentioned Figures.

References

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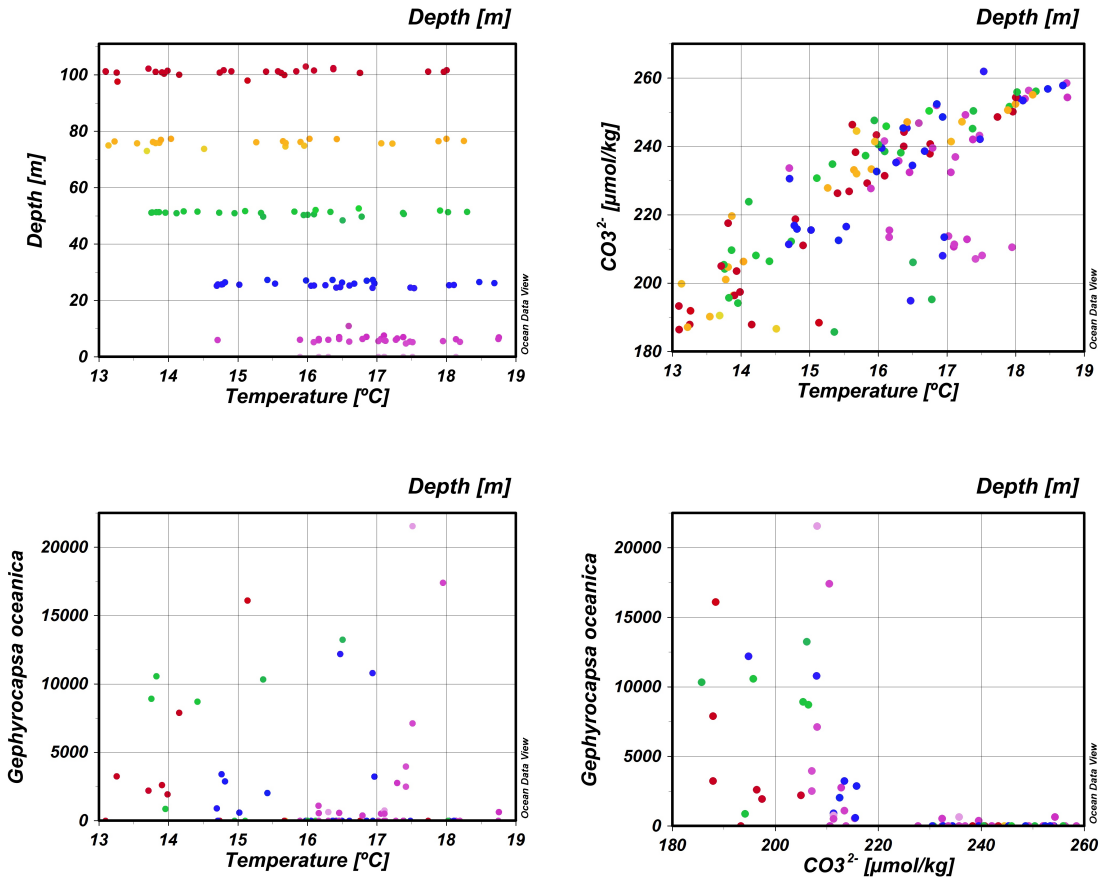

Fig. 1. Figure 1. Temperature against [CO32-] (upper-right) and G. oceanica cell density (cells/l) against [CO32-] and temperature (down). Colors indicate the depth of the sample as in the upper-left panel.

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