

Thank you for your constructive remarks. Please find our detailed responses to your comments, including expected modifications of the manuscript, below.

1. **COMMENT:** This study presents the distribution of omega saturation state as if this has not been published before. It is not clear if this is novel result of this study or the profiles were constructed based on data published before. If this is not clear, it is difficult to judge the suitability of carbonate chemistry data.

REPLY: The Ω_{ar} presented in this study is from each station at the time of the collection. This has been made more explicit in the Methods section. In the discussion, we use the aragonite saturation state data in the Mediterranean Sea, and elaborate on the gradient of West -East Ω_{ar} . Detailed Mediterranean carbonate chemistry data used in our study from the same oceanographic cruise and stations have being already published (e.g. D’Amario et al., 2017a, 2018; Gemayel et al., 2015; Hassoun et al., 2015a, 2015b; Mallo et al., 2017) and archived in an open access database (<https://doi.pangaea.de/10.1594/PANGAEA.841933>; Goyet et al., 2015).

Updated text:

The Ω_{ar} presented in this study is from data collected during the cruise at the time of plankton sampling.

2. **COMMENT:** By splitting the basin into W-E before analysing the station variability within sub-basin ignores inter- and intra-specific variability of each sub-basin. Based on the abundance data (Fig 5), there intra sub basin variability could be as large as the W-E comparison. The same pattern is true for species distribution. As such, the authors first need to reconcile the level of variability between the stations before they can attempt to make a W-E division.

3. **COMMENT:** Based on Fig 2 and Fig 5, W and E part of the basins have comparable abundances and species distribution, the difference is really in the transition zone between the E and W (station 9, 14, 15, 16). This is the real results

and not random W-E division. However, this makes a large portion of the discussion invalid and needs to be reconsidered and restructured.

REPLY: The Mediterranean Sea is separated into two large sub-basins by the relatively shallow (average depth of 330 m) Strait of Sicily. These basins have distinct environmental profiles, with the eastern basin characterised by warmer, more saline and oligotrophic conditions and the western basin characterised by cooler, less saline conditions. The division between the eastern and western Mediterranean Sea sub basins is recognised as an important biogeographical boundary (Dayan et al., 2015; Hassoun et al., 2015b; Rohling et al., 2009; Schneider et al., 2007; Uitz et al., 2012).

In our study, average abundance across all stations in the Mediterranean Sea was $1.17 \text{ ind. m}^{-3} \pm 1.52 \text{ (SD)}$, yet there were clear differences in abundance between the two sub-basins, with an average of $0.4 \text{ ind. m}^{-3} \pm 0.37 \text{ (SD)}$ for the western basin, and an average 5x higher of $1.96 \text{ ind. m}^{-3} \pm 1.8 \text{ (SD)}$ in the eastern basin. Figures 2 and 5 show consistently lower pteropod standing stocks in the Western Mediterranean than in the eastern Mediterranean. In the eastern basin, the overall abundance is higher, as is the variability in abundance. Figure 2 (Figure 1 here) of the manuscript has been re-done to better illustrate the difference in total abundance between the eastern and western basins.

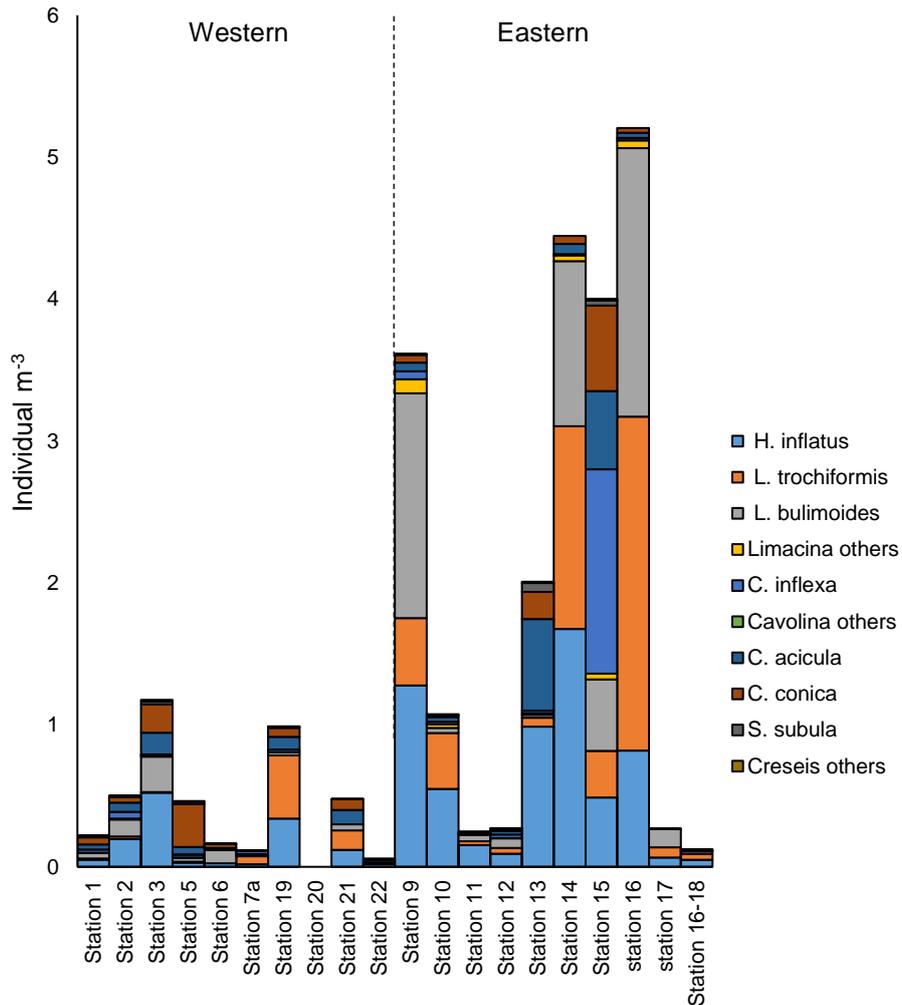


Figure 1. Absolute abundance of planktic pteropods from stations 1-22 on the MedSEA cruise, 2013. The category of ‘Others’ for each family includes specimens that were not a target species in this study or that were unidentifiable to the species level. Total pteropod abundance is greater and more variable in the eastern Mediterranean basin than in the western.

In order to analyse significant differences in total and individual species abundance considering both sub-basins, a Kruskal-Wallis Test was used between western and eastern stations, however this analysis has now been removed and we are use a more station-level approach with a parsimonious CCA and Pearson’s correlations. However, due to the substantial difference in environmental parameters between the eastern and western basin, as well as the difference in total pteropod abundance, we still highlight the differences in the Mediterranean in terms of the two major sub-basins.

A k-means cluster analysis was initially conducted on the species abundances, (IBM SPSS v23), incrementally increasing the amount of clusters until all variables significantly contributed to the cluster formation (3 clusters). The results show a uniform distribution with a cluster in the western Mediterranean, and more diverse communities in the eastern Mediterranean (Figure 2).

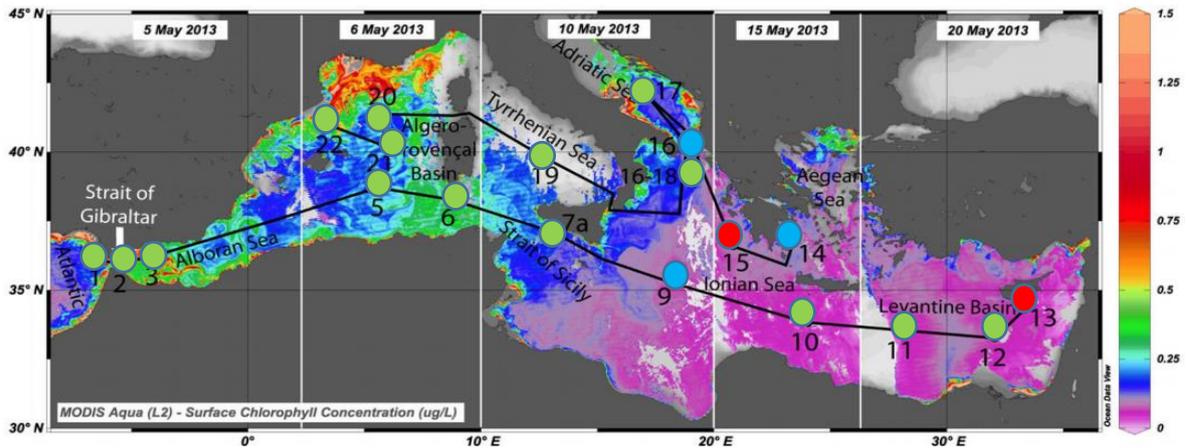


Figure 2. The results of a K-means cluster based on species abundances split the 20 stations within the Mediterranean.

A k-means cluster analysis was also conducted using the environmental parameters (5 clusters). Please refer to Figure 3 for a visual representation of the clusters within the Mediterranean Sea. This figure shows a clear distinction between the eastern and western Mediterranean, with the west being more heterogeneous and the east more homogenous.

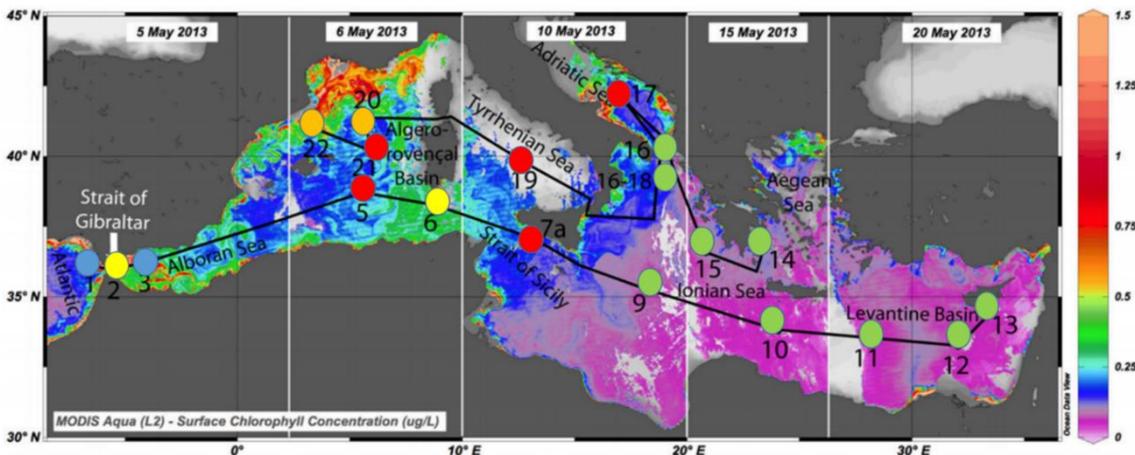


Figure 3. The results of a K-means cluster split the 20 stations within the Mediterranean into 5 clusters, grouped by their similarity in environmental variables. Each cluster has the same colour circle and indicate a heterogeneous western basin and a homogenous, oligotrophic eastern oligotrophic eastern basin. In this figure, there is a clear distinction between stations in the east of the Mediterranean (except station 17 in the Adriatic Sea) and stations in the west of the Mediterranean.

The k-means cluster analysis of the species and environmental parameters provide further evidence about the east and west Mediterranean as two distinct biogeographical regions. These figures and text will be added to the supplementary material.

4. COMMENT: Authors should analyse and interpret species distribution in greater depth based on the species habitat niches, maybe inter-specific species difference are driven by the differences in the vertical migration pattern (DVM)? How could sampling biased the results of species with deeper than 200m DVM? None of this is currently in the paper. e. Current interpretation does not hold up against the presented results and need significant restructuring. Based on the snap-shot spring distribution, the authors need to scale down the extrapolation in the discussion.

REPLY: Thank you for this comment which gives us the opportunity to clarify our methodology and improve our manuscript.

Sampling the upper 200 m water depth allowed us to capture most of the total pteropod community, though we are aware that we may have underestimated species with a deeper distribution (see below). The goal of this sampling strategy was to be able to perform a wide survey across the Mediterranean Sea during the one month sampling research cruise.

Based on the study by Bednaršek et al. (2012) investigating the global distribution of pteropods, which utilised a very large dataset (25939 data points) that included 41 scientific studies (Bednaršek et al. 2012), it was found that most of the species

live in the photic zone, although some pteropod species could be found at depths >500 m (Figure 4 from Bednaršek et al. 2012).

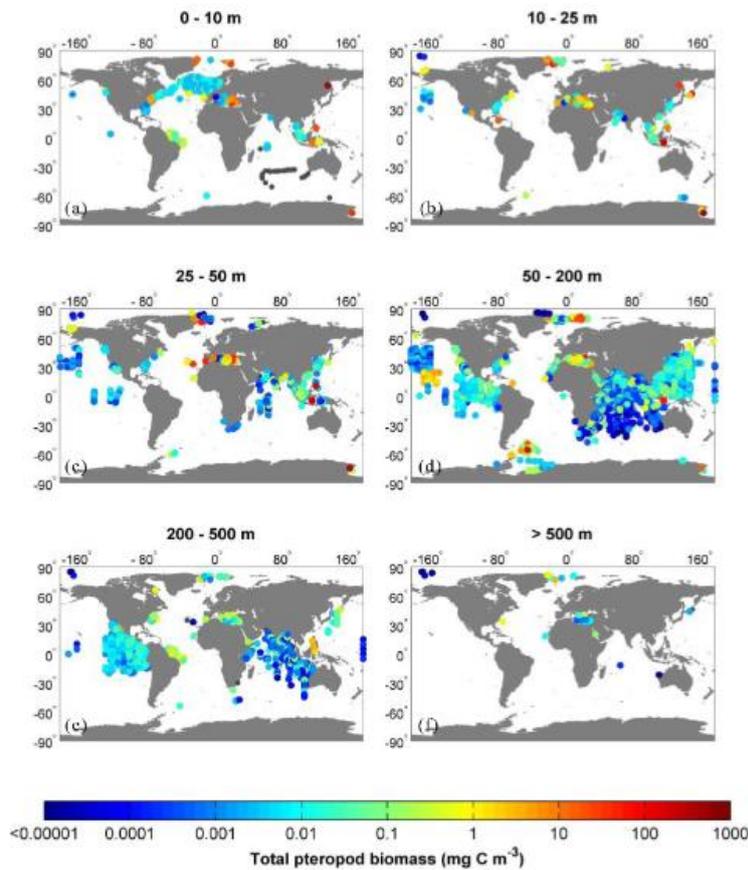


Figure 7. Pteropod carbon biomass (mg C m^{-3}) for six depth intervals: (a) surface (0–10 m), (b) 10–25 m, (c) 25–50 m, (d) 50–200 m, (e) 200–500 m, (f) ≥ 500 m.

Figure 4. From Bednaršek et al. 2012.

From the same dataset of Bednaršek et al. (2012) in PANGAEA (doi:10.1594/PANGAEA.777387), we generated the graph in Figure 5A to show the distribution of ind. m^{-3} of pteropods in the upper 850 m water depth of the Mediterranean Sea. The figure only shows values from stations where the total pteropod community have been collected from below and above 200 m, to allow for a comparison between depth distributions within the water column (see map generated in Figure 5B with the station locations). These data are comprised of sampling conducted during the day and night and does not differentiate between the two. On the basis of this dataset, 93% of pteropods (individual standing stocks) are distributed in the upper 200 m (Table 1).

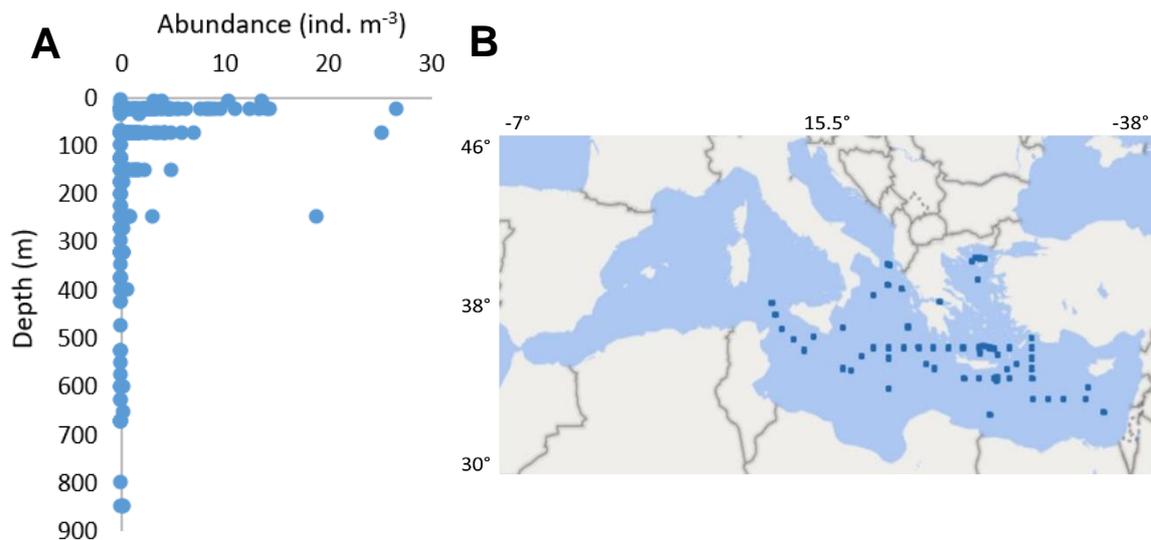


Figure 5. **A** Abundance of pteropods (expressed as ind. m⁻³) in the Mediterranean Sea from the dataset in Bednaršek et al. (2012) only including sites where pteropods have been collected below and above 200 m; **B** Location of stations within the Mediterranean Sea where data were collected above and below 200 m water depth. Unfortunately, there are a paucity of datasets in the western Mediterranean that allow us to differentiate the depth distribution of pteropods above and below 200 m (Bednaršek et al. (2012); see Figure 1 from this document, (c) depth 200-500 m and (d) below 500m for the western Mediterranean).

Table 1. Maximum, average, and percentage of total abundance of pteropods within the Mediterranean. A list of datasets used in this study can be found at the end of this document.

Depth range (m)	Max abundance (ind. m ⁻³)	Number of observations	Avg. abundance (ind. m ⁻³)	% Abundance (ind. m ⁻³)
0-200	26.67	455	0.98	93.37
201-850	19	502	0.07	6.63

An additional study performed in the Mediterranean South Adriatic Sea demonstrated that during a yearlong survey of the vertical distribution of gelatinous zooplankton, the large majority of pteropod species (78%) were found in the upper 50 m and 88% in the upper 200 m (Batistić et al., 2004).

According to the thesis of Rampal (1975), which was the first study investigating the pteropods distribution and ecology across the Mediterranean Sea, *Limacina bulimoides*, *L. trochiformis*, *Creseis acicula* and *C. conica* are classified as species living in the upper 200 m water depth (epipelagic depth preference). It was suggested that *H. inflatus* and *Styliola subula* can be found at depths below 200 m (mesopelagic depth preference) (Rampal, 1975), however, recent studies show that *Heliconoides inflatus* primarily occurs in the upper water column (Batistić et al., 2004; Granata et al., 2020; Juranek et al., 2003) while *S. subula* shows greater abundance below 150 m (Andersen et al., 1998). *Cavolina inflexa* is classified by Rampal (1975) as a species with a bathypelagic preference, and this preference for deeper water has been corroborated by more recent studies in the Ligurian Sea focusing on this species (Granata et al., 2020; Sardou et al., 1996; Tarling et al., 2001).

On the basis of the information above, we will include in the Methods section of the revised manuscript the paragraph below, highlighting that in this study we are characterising pteropod abundance, and that those abundances may be slightly underestimated as some species live below the sampling depth.

Added to methods section 2.2:

Sampling the upper 200 m water depth will probably not capture entirely the pteropod community at each location, however, it allows to quantify most of the total pteropod abundance. Based on a study investigating the global distribution of pteropods, which utilised a very large dataset (25939 data points) that included 41 scientific studies (Bednaršek et al. 2012), it was found that most of the species live in the photic zone and that in the Mediterranean Sea, specifically, pteropod abundance below 200 m is more than one order of magnitude lower (mean 0.07 ± 0.89 ind. m^{-3}) than the abundance in the upper 200 m (mean 1.04 ± 2.77 ind. m^{-3}) with 93% and 7% of pteropods distributed above and below the 200 m, respectively (Table 1 here and added as a supplementary table in the revised manuscript).

We will also be more conservative in our approach and not incorporate *S. subula* and *C. inflexa* (which may present strong seasonal variations in their depth distribution habitat) into the discussion of pteropod ecology and into our statistical analysis. We will focus on the species that mainly live in the upper 200 m. As these species formed a very small portion of the total abundance, when we compare the original Canonical Correspondence Analysis (CCA; Figure 3C in the original manuscript) against the new CCA (Figure 8 here with some variables were removed to avoid collinearity), the alignment of species with the significant environmental parameters (temperature and Ω_{ar}) is unchanged. The results and significance for the Pearson's correlations are also unchanged (please see response to comments 16, 17, 18 and 19 for details on the updated statistical analyses).

We have added the following paragraph in the revised Methods section to clarify our approach:

We are aware that vertical distribution of pteropods is species-specific. Limacina bulimoides, L. trochiformis, C. acicula and C. conica are classified as surface and subsurface species (Rampal, 1975). Heliconoides inflatus and Styliola subula can be found at depths below 200 m and are classified as mesopelagic by Rampal (1975), however recent studies show that H. inflatus primarily occurs in the upper water column (Batistić et al., 2004; Granata et al., 2020; Juranek et al., 2003) while S. subula shows the greatest abundance below 150 m (Andersen et al., 1998). Cavolina inflexa is classified by Rampal (1975) as a bathypelagic species with a distribution extending below 1000 m, and this preference for deeper water has been corroborated by more recent studies in the Ligurian Sea focusing on this species (Granata et al., 2020; Sardou et al., 1996; Tarling et al., 2001). Due to the strong diel and seasonal variations in their depth distribution habitat (Andersen et al., 1998; Rampal, 1975; Tarling et al., 2001), we have decided not to incorporate S. subula and C. inflexa into our statistical analyses of distribution in relation to the environmental parameters.

In regards to diurnal vertical migration (DVM), the collection of day and night samples at each location was not anticipated in our research as the aim was to

obtain an overall upper 200 m integrated distribution of pteropods. We considered DVM in our initial data exploration (visual interpretation, ANOVAs using a categorical approach by dividing stations between day and night) and found no significant difference in total or species abundances when comparing results between day and night sampling (Table 2). We also sampled 10 stations during the day and 10 during the night, spread across the Mediterranean. For a multivariate approach, we conducted a CCA which indicated that PAR (photosynthetically active radiation) did not heavily affect pteropod community composition within the Mediterranean Sea (Figure 6).

As stated in the original manuscript – “After an initial analysis, PAR (photosynthetically active radiation) was removed as it did not significantly contribute to the variation of environmental parameters.” We acknowledge that species do undertake DVM and we have made a point in the revised manuscript that the time of day for collection did not appear to be a driving factor affecting abundances in the upper 200 m integrated samples of this study.

Updated text:

Many pteropod species undertake diurnal vertical migration, and after an initial analysis, PAR was removed as it did not appear to be a driving factor affecting pteropod’s abundances in the upper 200 m integrated samples of this study (Supplementary Figure – CCA and ANOVA using day/night stations).

Table 2. ANOVA table using night/day as the dependent variable against total and species abundances.

		Sum of Squares	df	Mean Square	F	Sig.
total abundance	Between Groups	1.988	1	1.988	.713	.409
	Within Groups	50.182	18	2.788		
	Total	52.170	19			

<i>H. inflatus</i>	Between Groups	.000	1	.000	.000	.987
	Within Groups	4.317	18	.240		
	Total	4.317	19			
<i>L. trochiformis</i>	Between Groups	.354	1	.354	1.029	.324
	Within Groups	6.198	18	.344		
	Total	6.552	19			
<i>L. bulimoides</i>	Between Groups	.251	1	.251	.789	.386
	Within Groups	5.726	18	.318		
	Total	5.977	19			
<i>C. acicula</i>	Between Groups	.002	1	.002	.056	.816
	Within Groups	.590	18	.033		
	Total	.592	19			
<i>C. conica</i>	Between Groups	.003	1	.003	.121	.732
	Within Groups	.400	18	.022		
	Total	.403	19			

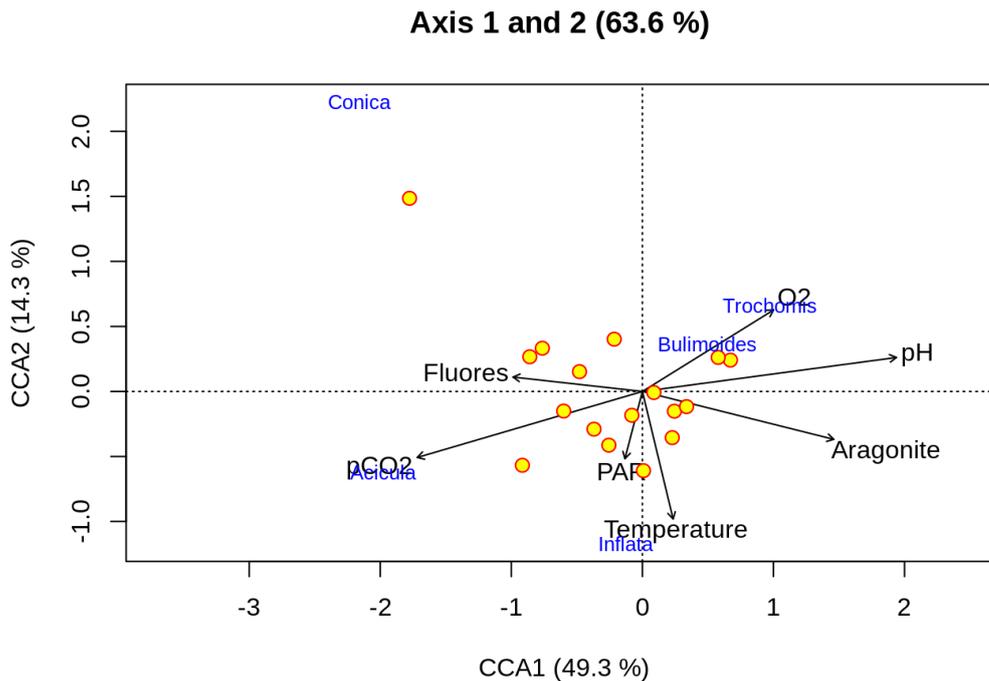


Figure 6. Triplot from the Canonical Correspondence Analysis (including the PAR variable), where the relation between species and environmental variables are obtained from the proximity (or remoteness) of species labels and environmental arrows (tips). The length of the arrow represents the importance (contribution) of the environmental variable to the data structure, thus we can see that the PAR variable, due to its short arrow, does not heavily contribute to pteropod community composition within the Mediterranean.

5. COMMENT: Given the correlation of majority of environmental parameters (temp, pH, omega, salinity; Fig 3D), it is really impossible to delineate a single driver. Contrary to Fig 5D, Fig 5C actually does not show the same co-linearity. Is this because of the exclusion of nitrate and fluorescence? The authors should attempt to present the correlation matrix of different parameters to the reader can understand the collinearity.

REPLY: In this study we use a CCA to better characterize the effects of environmental conditions on pteropod community composition. We conducted a parsimonious CCA in which environmental parameters that did not significantly affect the community were removed from the analysis (stepwise model selection). The significance of each variable was assessed using a permutation test.

A collinearity analysis was performed and those variables that showed a Pearson's correlation coefficient higher than 0.75 were removed from the model (Figure 7 here and to be included as a supplementary figure in the revised manuscript).

Fluorescence and NO_3 were used in the parsimonious CCA but removed as they did not significantly affect pteropod community composition. Based on the results of the parsimonious CCA, temperature and Ω_{ar} are significantly affecting 33.6% of the structure of the observed community during the sampling (Figure 8; $F = 4.05$, p -value < 0.01).

The methods have been updated:

A parsimonious Canonical Correspondence Analysis (CCA) was used to determine the significant environmental parameters affecting pteropod species composition. To avoid correlation among environmental variables (collinearity), those variables that showed a Pearson's correlation coefficient higher than 0.75 were removed from the analysis (Figure 6 - $p\text{CO}_2$, PO_4 , O_2 , salinity and pH).

The results have been updated:

The parsimonious CCA indicates that temperature and Ω_{ar} are significantly affecting 33.6% of the structure of the observed pteropod community at the time of sampling ($F = 4.05$, p -value < 0.01 ; Figure 8 here).

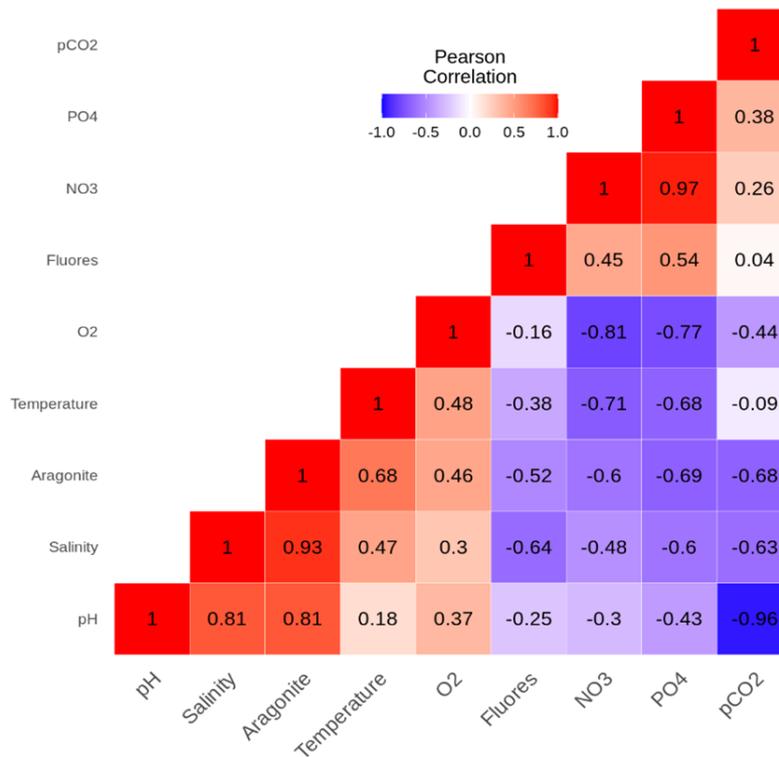


Figure 7. Pearson's Correlation matrix showing the high correlation between several environmental variables. For the CCA, the most biologically relevant of correlating variables were kept. Variables used in the analyses were: NO₃, temperature, Ω_{ar} and fluorescence. The variables removed from the analyses were pCO₂, PO₄, O₂, salinity and pH.

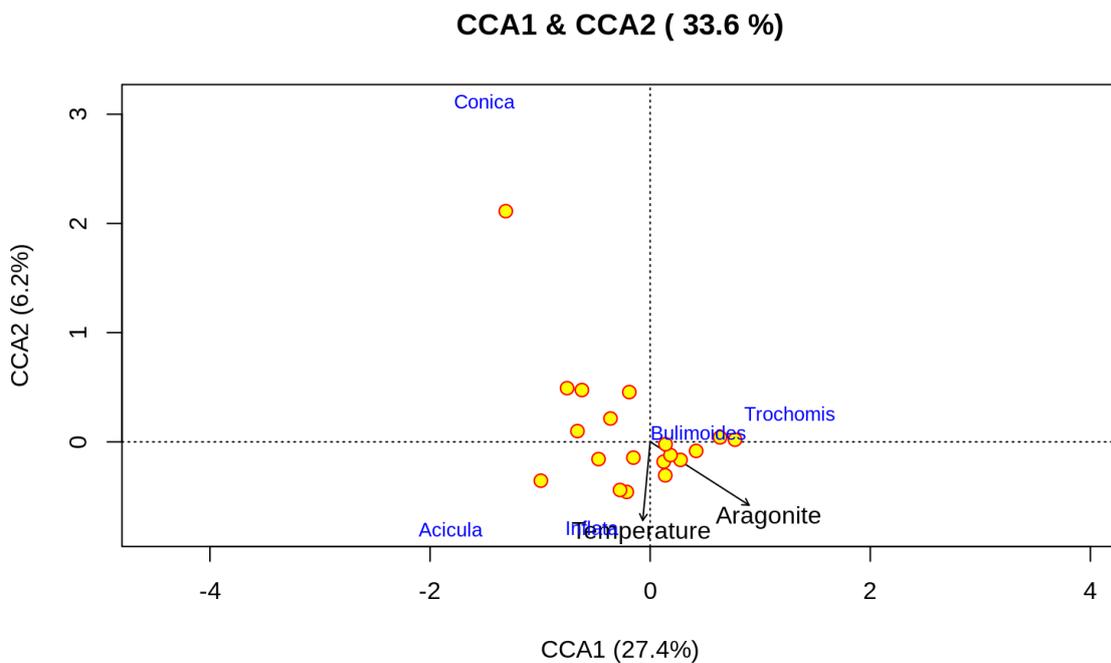


Figure 8. Parsimonious CCA indicating that temperature and Ω_{ar} are significantly affecting 33.6% of the structure of the observed community during the sampling ($F = 4.05$, p -value <0.01). 'Aragonite' indicates Ω_{ar} . 'Temperature' is obscuring the word Inflata (*H. inflatus*). The yellow circles indicate individual stations.

6. COMMENT: PCA analyses actually shows very complex relationship between environmental parameters and different pteropod species (Fig 3C), some with negative and the other species with no interaction with carbonate chemistry. However, what is baffling is the fact that PCA graphs for each species (Suppl Figure 1) actually shows that the environmental drivers are different depending on the basin. In such way, the distribution in the E part is driver by different set of parameters than the W part. In my opinion, this invalidates current analyses by subdividing the basin into E-W instead of dealing with the data on the station level. I would strongly suggest the reiteration of the analyses on the station level to (in)validate the current results.

REPLY: The parsimonious CCA and Pearson's correlations with total abundance and individual species abundances take a station based approach as the correlations are formed at the level of station. Our analyses and discussion has shifted to a more multifactorial approach based on the correlations between individual species or taxon and environmental parameters.

We maintain the division between the eastern and western basin as there are general but clear differences between the marine environment in the east versus the west as well as with species abundances (see response to comment #2 and 3).

7. COMMENT: In addition to #3, I disagree with the findings that omega is a major driver of pteropod distribution, or if it is, the co-authors need to do more throughout job to prove this.

REPLY: The results of the CCA and Pearson's correlations indicate that temperature and Ω_{ar} are important drivers of pteropod community composition. We appreciate the reviewer's comments and have clarified the discussion –

particularly the section on Ω_{ar} and temperature as factors influencing pteropod populations in the Mediterranean Sea. Please see our reply to comment #5 for the results of the CCA.

8. COMMENT: In the absence of one driver interpretation, I suggest that the authors stick to a multiple parameter interpretation. They delineated that various different parameters impact pteropod distribution but in the discussion they have abandoned this results and reduce it all to one, omega saturation state level.

REPLY: We agree about reshaping our discussion and the focus on Ω_{ar} saturation as the main driver of pteropod distribution, and have broadened our focus to a multiple driver analysis in the discussion. We have also shifted the focus of the discussion to a more species based approach, as per the results of the Pearson's correlations and the CCA which indicate differences in the relationship of species to environmental variables. The parsimonious CCA and the Pearson's correlations indicates that temperature and Ω_{ar} are significantly impacting pteropod community composition and abundances, and in the discussion we elaborate on the mechanisms for which these 2 parameters could drive the changes.

ADDITIONAL

9. COMMENT: There should be some background on the abundance and species distribution of pteropods in the sub-basin of the Mediterranean. That has been a well-studied topic, both spatially and temporally but authors do not include any of such data and such, fail to establish the baseline knowledge. In addition, there are no comparison of this study with the previous study on pteropods, which would give it a better comparison and evaluation.

REPLY: An additional section on pteropods in the Mediterranean has been added to the Discussion to give more context to our results. Table 2 compiles relevant published pteropod studies in the Mediterranean Sea and is included here and in the supplementary material of the revised manuscript.

This study aims to give the most comprehensive pteropod distribution within the upper 200 m across the Mediterranean Sea during the spring period. A previous investigation of the whole basin was made by Rampal (1975) who performed a comparative analyses of pteropod abundance within the different Mediterranean sectors. Unfortunately, the heterogeneity of the collected materials limited the quantitative approach of this study and the results are not presented in terms of pteropod concentration. The only published Mediterranean long-term study focusing solely on pteropods (Howes et al., 2015) is in the Ligurian Sea (water depth 0-70m depth). In this study, the dominant species in each family were C. acicula and H. inflatus, corroborating well with our overall findings. Contrary to our results, in this study, Limacinadae was found to be the least abundant family, which the authors contributed to a sampling bias which led to under-sampling. Abundance average of pteropods in Howes et al. (2015) over the period from 1957-2003 was 15.7 ind. m⁻³ for family Creseidae, and 5.5 ind. m⁻³ for family Limacinadae (this includes H. inflatus). Other studies investigating pteropod community abundance in different Mediterranean Sea regions (e.g. Ligurian Sea, Balearic Sea, Adriatic Sea, Tyrrhenian Sea) show average abundances of the entire pteropod community ranging from 0.34 – 5.9 ind. m⁻³ (Batistić et al., 2004; Fernández de Puelles et al., 2007; Granata et al., 2020; Manno et al, 2019; Table 3 here and added as a supplementary Table to the revised manuscript). Pteropod abundance in our study is as high as 5.14 ind. m⁻³ and within the same magnitude as most of these other Mediterranean studies, with an average of 1.22 ind. m⁻³ across the whole Mediterranean. In agreement with our results, Granata et al. 2020 and Batistić et al., 2004 also found H. inflatus to be the most abundant pteropod species. However, all the previous studies mentioned above differed in sampling methodology, were sampled over different seasons and time periods, and are coastal versus open sea stations, making a direct comparison between the studies and regions difficult.

Table 2. An overview of published pteropod studies in the Mediterranean

Region of Collection	Min-max conc. of pteropods community (ind. m ⁻³)	\bar{x} conc. (ind. m ⁻³)	Period of sampling	Collection depth	Water column depth (m)	Most abundant species/taxa	Net/mesh size	Reference

Ligurian and Tyrrhenian Seas (NW Mediterranean)	Study focuses on <i>C. inflexa</i> , <i>C. pyramidata</i> and <i>S. subula</i> . Min-max not provided	Day: <i>Cavolina inflexa</i> : 4.0 <i>Clio pyramidata</i> : 2.1 <i>Styliola subula</i> : 0.3 Night: <i>Cavolina inflexa</i> : 1.7 <i>Clio pyramidata</i> : 1.6 <i>Styliola subula</i> : 0.4	April, 1994	0-25 25-50 50-75 0-75 75-150 100-150 150-200 150-250 250-350 350-400 400-450 450-500 500-550 550-700	Various 700-2700	<i>Cavolina inflexa</i>	BIONESS 1 m ² mouth 500 µm mesh	Andersen et al., 1998
Southern Adriatic	Min: 0.38 Max: 57.68	2.87	April September November February June	0-50 50-100 100-200 200-300 300-400 400-600 600-1000	1242	<i>Heliconoides inflatus</i>	113 cm diameter 380 cm length 250 µm mesh	Batistić et al., 2004
Balearic Sea	Only monthly \bar{x} given. Min: 4 Max: 11	5.9	1994-2003 (all year round)	0-75 0-100	Various 78-200	<i>Creseis acicula</i>	Bongo-20 Plankton net 100 µm and 120 µm meshes	Fernández de Puellas et al., 2007
Ligurian Sea	<i>C. inflexa</i> : 1.59 (20-40 m) <i>C. pyramidata</i> : 0.06 (100-200 m) <i>H. inflatus</i> : 6.87(0-20 m)	0.3	April-May, 2013	0-20 20-40 40-60 60-80 80-100 100-200 200-400 400-600 600-800 800-1000 1000-1300	Various 1400-1639	<i>Heliconoides inflatus</i>	BIONESS multinet 1 m ² -mouth 230 µm mesh	Granata et al., 2020
NW Ligurian Sea	Creseidae: ~630 Cavoliniidae: ~790 Limacinadae (incl. <i>H. inflatus</i>): max 60.8	Creseidae: 15.7 Cavoliniidae: 13.8 Limacinadae: 5.5	1967-2003 (all year round)	0-75	~80	Creseidae	Juday Bogorov net 330 µm mesh 50 cm diameter	Howes et al. (2015)
Tyrrhenian Sea	Min: 0.00 Max: 4.02	<i>C. acicula</i> : 1.48 <i>C. conica</i> : 1.11 <i>H. inflatus</i> : 1.03	August, 2015	One depth for each station according to the sea	Various (73-185)	<i>Creseis acicula</i>	Bongo-40 200 µm mesh	(Manno et al., 2019)

		<i>L. trochiformis</i> : 0.64 <i>L. bulimoides</i> : 0.33		bottom (min depth 0-65, Max depth 0- 170				
Eastern Mediterranean	Sicilian Channel: Max. 120 ind. m ⁻³	Sicily Channel: 2.07 Ionian Sea: 0.56 Cretan Sea: 1.00 Cretan Passage: 2.32 Rhodes area: 2.94 Levantine Sea: 1.37	October- November	300	Various: 449-4359	N/A	WP-3 net 113 cm diameter 200 µm mesh	Mazzocchi et al., 1996
Ligurian Sea	<i>Study focuses solely on Cavolina inflexa Max: \bar{x} 1.64 (0-200 m)</i>	Not provided	September, 1997	0-500 with intervals 0-25 25-50 50-75 100-125 125-150 150-200	Not provided	<i>Cavolina inflexa</i>	MOCNESS 1 m ² mouth 300 µm and 2000 µm meshes	Tarling et al., 2001

COMMENT: The introduction fails to identify what exactly will be investigated in this paper – and that is not the population response to climate change. More structure hypothesis testing needs to be presented in the Intro.

REPLY: Thank you for your suggestion and giving us the opportunity to clarify the aims of our study. The sentence referring the population response to climate change has also been removed.

Updated text:

This study aims to investigate pteropod abundance distribution across the Mediterranean Sea at a large spatial scale, which has been identified as a gap within pteropod research. Further, the presence of a west-east natural environmental gradient enables us to investigate the interaction between pteropod distribution and environmental parameters during the spring season. We also present the relationship between pteropods distribution and another major group of planktic marine calcifier, foraminifera (single-celled, calcareous zooplankton). Investigating this relationship between pteropods and foraminifera is important as ocean acidification has been shown to cause ecosystem shifts

due to altered competition between calcareous species, likely resulting from the physiological responses of individual species (Kroeker et al., 2013). Foraminifera were collected during the same research cruise campaign (data published in Mallo et al., 2017) and are therefore directly comparable with this study on thecosome pteropods, giving us the opportunity to investigate the ecological relationship between groups of prime calcifying zooplankton in the Mediterranean Sea.

10. COMMENT: How were satellite data obtained and averaged; daily/monthly? How was this done? Do you have fluorescence of chl-a data? Different figures and tables have different parameter enlisted.

REPLY: The satellite data is only relevant to Fig 1. Methods text: Surface chlorophyll a concentration was obtained from MODIS Aqua L2 satellite data (NASA Goddard Space Flight Centre, 2013; Fig. 1). The data are showing the surface chl-a satellite-derived of the section 1 (stations 1-13; please see Figure 1 of the manuscript for the relevant dates) and here is used to illustrate differences in chlorophyll concentration across the Mediterranean during the time of sampling. Those data are not used in the analysis as fluorescence data were collected during the sampling. Fluorescence was measured at each station and was included in the analysis. This data can be found in a data base (<https://doi.pangaea.de/10.1594/PANGAEA.822153>) and previously published at D'Amario et al., (2017).

11. COMMENT: Usually studies report the abundances in ind/m², not m⁻³. Can this be, for comparison reasons, amended with 200 m vertical depth.

REPLY: Many studies report m⁻³ for abundance, including many articles that we reference in this paper, making the values useful for comparing and contrasting results (Fernández de Puellas et al., 2007; Howes et al., 2015; Mazzocchi et al., 1996). As most of our statistical analyses have been conducted with these values, we have maintained the values as is, using m⁻³. Where we are making comparisons with papers that have used ind. m², we have converted these values to ind. m³, as done for Batistić et al. (2004).

12. COMMENT: How were abundances enumerated?

REPLY: This information can be found in the methods section: 'Pteropod abundance was determined for each station and species were identified and counted using a Leica z16 APO binocular light microscope. Pteropod abundance within the water column was calculated as individuals per cubic meter (ind. m⁻³). Pteropods were grouped into four target families: Limacinadae, Heliconoididae, Cavoliniidae and Creseidai; and further into seven target species: *Heliconoides inflatus*, *Limacina trochiformis*, *Limacina bulimoides*, *Creseis acicula*, *Creseis conica*, *Styliola subula* and *Cavolina inflexa*. The online plankton portal (www.planktonportal.org) was used to aid in the identification of pteropods to species level.'

13. COMMENT: How were the samples preserved and what was pH of the solution?

REPLY: We will update the text and the following clarification in the methods:

Plankton samples were preserved on board in a 4% formaldehyde solution that was buffered with hexamethylenetetramine to reach pH 8.2 and were stored in 500 ml polycarbonate bottles at 4°C in the dark. pH was measured in all the samples, at the beginning, during and the end of the storing period to ensure that the state of the pteropod shells were not affected by the preservation technique.

14. COMMENT: How do authors explain the salinity as a dominant driver, is it possible that the current distribution is water-mass related, and thus, the species have affinity to specific water mass (rather than to an environmental parameter). Please, comment on this and explain, why could you exclude the advection as a driver behind observed distribution.

REPLY:

We cannot definitively exclude the potential role of lateral advection as a factor affecting pteropod distribution, however, the large-scale circulation of the surface of the Mediterranean Sea has been described as sub-basin-scale and mesoscale gyres interconnected and bounded by currents and jets (Figure 9; Millot and Taupier-Letage, 2012). The general circulation flow can impact coastal regions and heavily influences local current dynamics. Mediterranean shelf areas are relatively small and are separated from deeper regions by steep continental shelf breaks. As such, it is more likely that the coastal pteropod work in the Mediterranean Sea is heavily influenced by currents than the deeper, ocean-like stations of this study.

Note that salinity was removed from the CCA to avoid collinearity with Ω_{ar} . The Pearson's correlations and CCA triplot indicated that Ω_{ar} was a greater driver of pteropod community composition and abundance in the Mediterranean.

We have clarified the potential role of advection in the revised manuscript:

Updated text:

We cannot definitively exclude the potential role of lateral advection as a factor affecting pteropod distribution, however, the large-scale circulation of the surface of the Mediterranean Sea has been described as sub-basin-scale and mesoscale gyres interconnected and bounded by currents and jets (Millot and Taupier-Letage, 2012). The general circulation flow can impact coastal regions and heavily influences local current dynamics. Mediterranean shelf areas are relatively small and are separated from deeper regions by steep continental shelf breaks. As such, it is more likely that the coastal pteropod populations in the Mediterranean Sea are heavily influenced by currents than the deeper, ocean-like stations of this study.

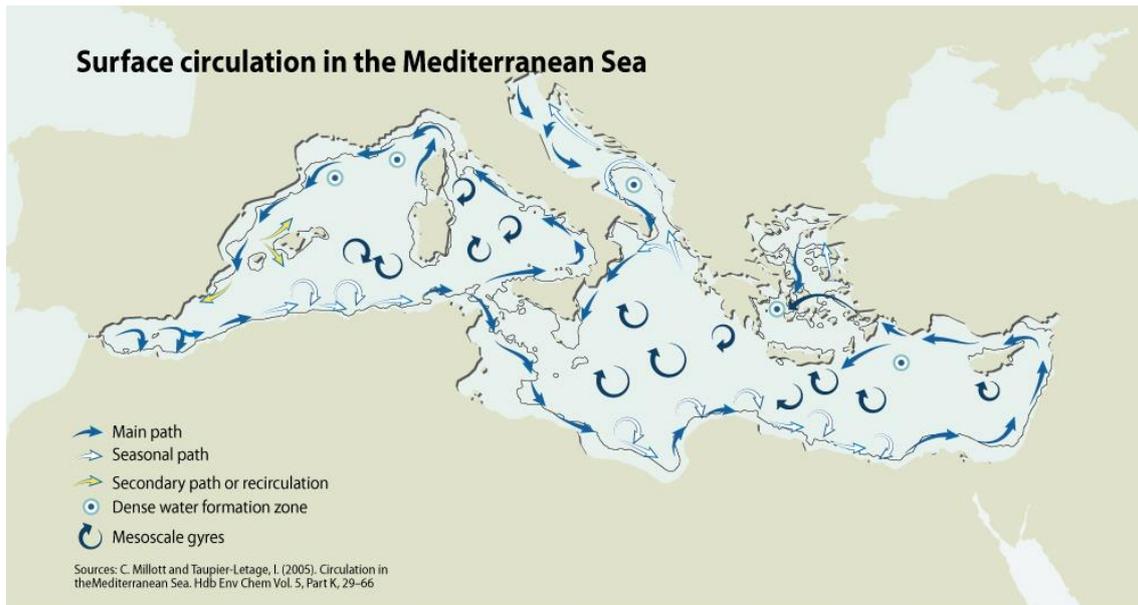


Figure 9. Surface circulation in the Mediterranean Sea. There is a general circulation flow around the coastal regions of the Mediterranean Sea, whereas the deeper regions generally form mesoscale gyres.

15. COMMENT: Forams do not have a proper introduction, very confusion for a reader.

REPLY: We appreciate your comment which has given us the ability to clarify the inclusion of foraminifera in our study. The foraminifera do not have a large introduction (as for pteropods) as we are using foraminiferal data previously analysed in the study of Mallo et al., 2017. Foraminifera were collected during the same cruise and from the same tow sampling of our pteropod collection. We have updated the Introduction, Methods and Results section to better clarify this point.

Introduction:

We also present the relationship between pteropods distribution and another major group of planktic marine calcifier, foraminifera (single-celled, calcareous zooplankton). Investigating this relationship between pteropods and foraminifera is important as ocean acidification has been shown to cause ecosystem shifts due to altered competition between calcareous species, likely resulting from the physiological responses of individual species (Kroeker et al., 2013). Foraminifera

were collected during the same research cruise campaign (data published in Mallo et al., 2017) and are therefore directly comparable with this study on thecosome pteropods, giving us the opportunity to investigate the ecological relationship between groups of prime calcifying zooplankton in the Mediterranean Sea.

Methods:

Samples for foraminifera were collected in the same net as the pteropod samples at all of the same stations and preserved using the same methodology. Foraminifera were identified to species level using a light microscope and following the guidelines and taxonomic nomenclature of André et al. (2013), Aurahs et al. (2011), Hemleben et al. (1989) and Spezzaferri et al. (2015), depending upon each species. For a more detailed description of collection, preservation and taxonomic identification methods, please refer to Mallo et al. (2017).

Statistical Methods:

Planktic foraminifera total abundance and distribution presented in Mallo et al. (2017) were compared to the pteropod data from this study. The tow samples from Mallo et al. (2017) were collected during the same cruise and within the same nets as the pteropods of the present study, allowing a direct comparison of these two groups of key planktic calcifiers. To compare the abundance of pteropods and foraminifera within specific regions of the Mediterranean Basin, we used a Generalised Linear Mixed Model (GLMM) with a gamma distribution. As the magnitude of the abundance data is very different between pteropods and foraminifera (almost one order of magnitude), the abundance data was transformed to its logarithmic scale to make abundances from both groups comparable. For this analysis, the Mediterranean was split into two basins: “western” stations (1, 2, 3, 5, 6, 7, 19, 20, 21, 22) and “eastern” stations (11, 12, 13, 14, 15, 16, 17, 16-18).

To run the GLMM, the functions “glm” in the glmmTMB package was used (Brooks et al., 2017).

Results:

The results from the GLMM comparing the aggregated abundance of pteropods and foraminifera between the two basins (Eastern and Western basins), indicates that there are significant differences between the abundance of both taxa (chisq = 29.27, $p < 0.05$), between the Eastern and Western Mediterranean basins (chisq = 5.57, $p < 0.05$), and also in their interaction (chisq = 4.97, $p < 0.05$). These results indicates that an inverse relationship between taxa abundance and Mediterranean basin exist.

Pteropod abundance is distinctly greater in the Eastern ($\bar{x}=2.13$ ind. m^{-3}) Mediterranean than in the Western Mediterranean ($\bar{x}=0.47$ ind. m^{-3}), while foraminiferal populations showed a contrasting abundance distribution (Figure 10 here and to be added to the revised manuscript) with higher abundance in the Western Mediterranean ($\bar{x}=1.87$ ind. $10 m^{-3}$) than in the Eastern Mediterranean ($\bar{x}=0.96$ ind. $10 m^{-3}$).

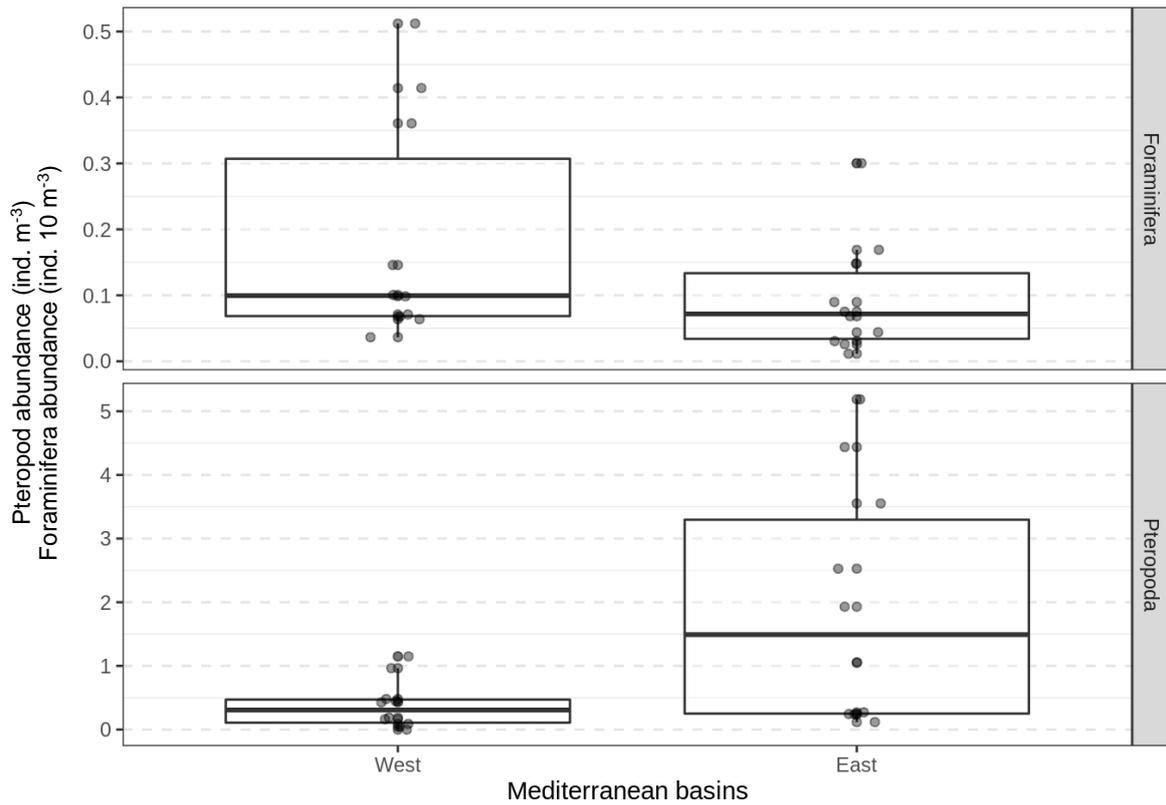


Figure 10. Box plot showing the contrasting abundance distribution between pteropods and foraminifera between the East and West of the Mediterranean.

16. COMMENT: Figure 4 does not show Adriatic and NE part, the latitudes do not align.

REPLY: The depth profiles shown in figure 4 represent the 3 main longitudinal sections sampled during the 2013 research cruise. The station numbers are also indicated in the figure. For clarity, we will add a map to Figure 1 of the manuscript (Figure 11 here) indicating the 3 sections. The Adriatic station is #17.

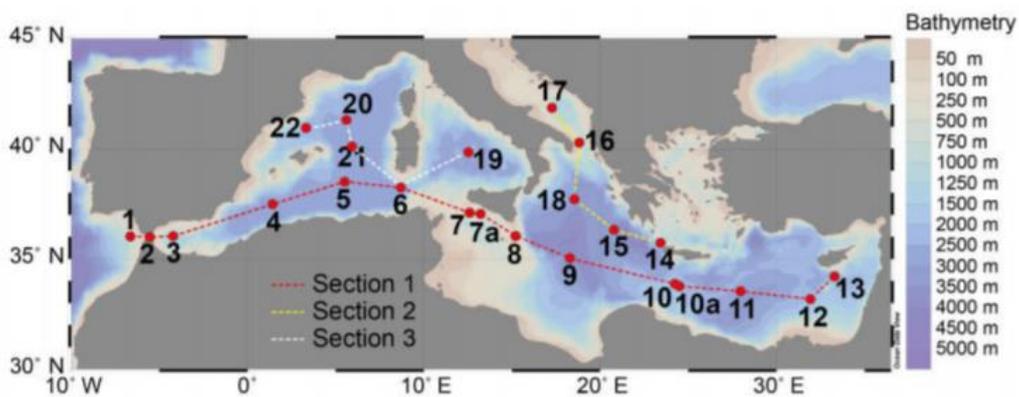


Figure 11. Sampling stations of each individual transect (Ocean Data View).

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