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

1. **COMMENT:** The sample depth of 200 m is relatively shallow for sampling many of the species. The early work of Rampal (1975) very clearly showed that many species exhibit seasonal depth preferences, with some populations spending many months of the year at depth in excess of 700 m. I do appreciate the difficulties in accessing this work as it is an unpublished thesis (in French) but it is available by request from a number of international libraries and is absolutely essential reading for this type of work in the Mediterranean. I suspect this is why Cavolinia inflexa were underrepresented and did not appear to be tied to any environmental parameter as, at this time of year, most of the population is found below the sampling depths and the samples will have just picked up the odd individual. Rampal J. Les thécosomes (molluques pélagiques). Systématique et évolution - Écologies et biogéographie Mediterranéennes [These doctoral]. Université Aix-Marseille I1975.

REPLY: We appreciate the reviewer's comment that gave us the possibility to clarify the sampling strategy of this work. 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 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, although some pteropod species could be found at depths >500 m (Figure 1 from Bednaršek et al. 2012).

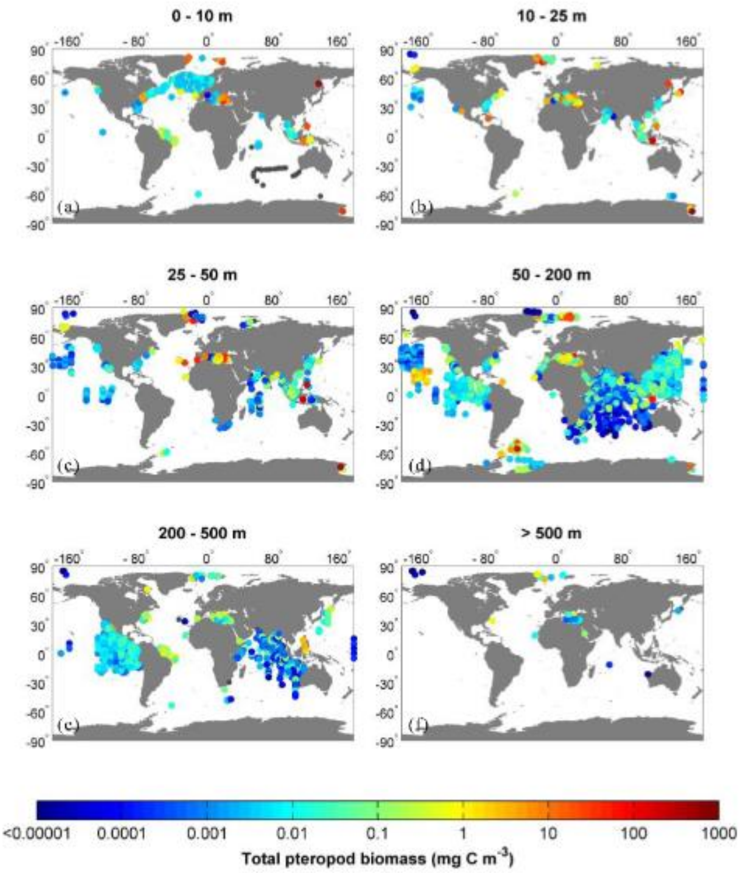


Figure 7. Pteropod carbon biomass (mg C m^{-3}) for six depth intervals: **(a)** surface (0–10 m), **(b)** 10–25 m, **(c)** 25–50 m, **(d)** 50–200 m, **(e)** 200–500 m, **(f)** ≥ 500 m.

Figure 1. 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 2A 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 2B 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 and to be added as a supplementary table).

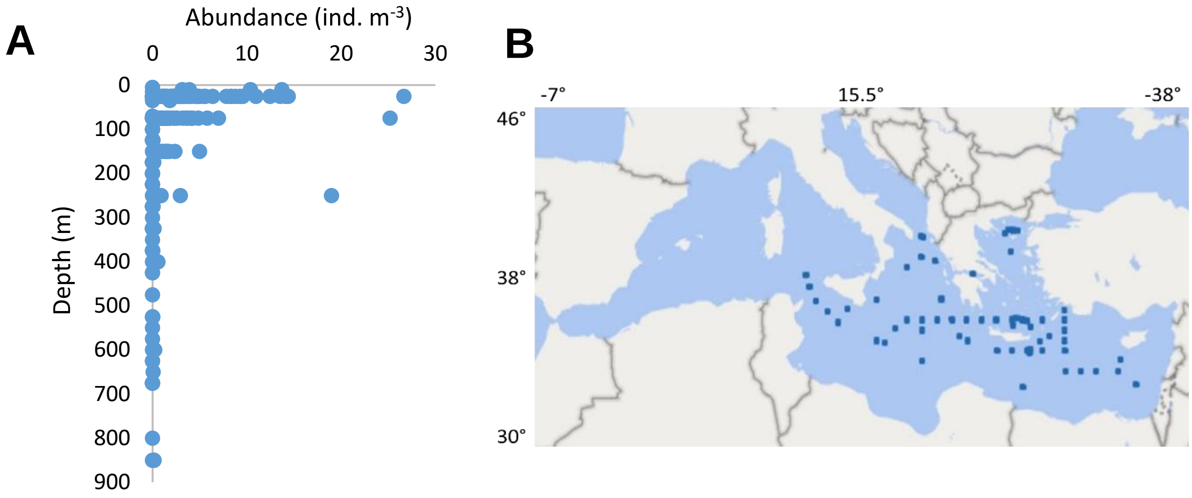


Figure 2. A Abundance of pteropods (expressed as ind. m^{-3}) in the Mediterranean Sea from the dataset in Bednaršek et al. (2012) only including sites where pteropods have been collected below and above 200 m; **B** Location of stations within the Mediterranean Sea where data were collected above and 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).

We obtained a copy of Rampal's thesis (1975) that was mentioned by the Reviewer. This was not readily accessible and also (as the reviewer said) in French but we have been able to partially translate it. The data from this thesis are also not published and the author states that "The heterogeneity of the samples, in terms of collection period, sampling methodology and type of net, sampling depth, etc. does not allow one to perform a quantitative Mediterranean-wide study". We do, however, understand the value of this seminal work and we refer to it in the revised version of the manuscript accordingly.

According to Rampal (1975), *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 Rampal's study, *C. inflexa* is mainly present in the western Mediterranean (mainly in the north sector) and considered rare in the eastern basin (see Figure 3 [Figure 96 from Rampal, 1975]). Rampal's thesis indicates that *C. inflexa* is a temperate species and, in the Mediterranean Sea, is mainly concentrated above the 40th parallel. Based on the pteropod survey of Rampal, (1975) *C. inflexa* is abundant only in the Catalan Sea, Gulf of Lion and Ligurian coast and in our study, only 3 of the 20 stations are included within these regions.

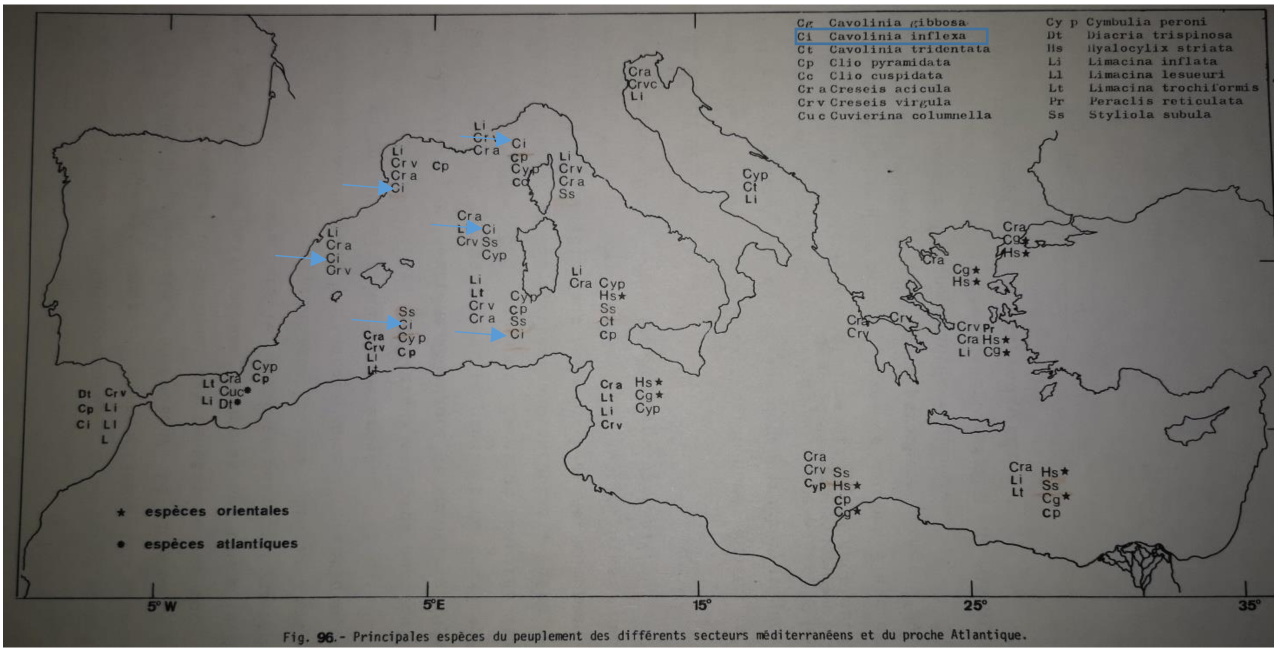


Figure 3. Figure 96 from Rampal's (1975) thesis showing the distribution of *Cavolina inflexa* in the north western Mediterranean.

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 the upper 200 m integrated 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) against the new CCA (where 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 S. 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 decided not to incorporate* S. subula *and* C. inflexa *into our statistical analyses of distribution in relation to the environmental parameters.*

2. **COMMENT:** Mediterranean pteropods have also been shown to undertake diurnal vertical migrations. At least two of the main species found in the nets exhibit a strong diurnal vertical migration and the sampling of each station was undertaken at different times in the day/night cycle, thus potentially skewing the data. See papers: Andersen V, Francois F, Sardou J, Picheral M, Scotto M, Nival P. Vertical distributions of macroplankton and micronekton in the Ligurian and Tyrrhenian Seas (northwestern Mediterranean). Oceanol Acta. 1998;21(5):655–76. Tarling GA, Matthews JBL, David P, Guerin O, Buchholz F. The swarm dynamics of northern krill (Meganyctiphanes norvegica) and pteropods (Cavolinia inflexa) during vertical migration in the Ligurian Sea observed by an acoustic Doppler current profiler. Deep-Sea Res Pt I. 2001;48(7):1671–86.

REPLY: We kindly acknowledge the reviewer's suggestion regarding the articles by Andersen et al., (1998) and Tarling et al., (2001) which show the day/night distribution variability in *C. inflexa* and *S. subula* to be below 200 m. As per our reply to the previous comment, we have decided not to include these two species in our statistical analyses dealing with species distribution.

The collection of day and night samples at each location was not anticipated in our research. However, we considered diurnal vertical migrations 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 4).

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 diurnal migration 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			
H. inflatus	Between Groups	.000	1	.000	.000	.987
	Within Groups	4.317	18	.240		
	Total	4.317	19			
L. trochiformis	Between Groups	.354	1	.354	1.029	.324
	Within Groups	6.198	18	.344		
	Total	6.552	19			
L. bulimoides	Between Groups	.251	1	.251	.789	.386
	Within Groups	5.726	18	.318		

	Total	5.977	19			
C. acicula	Between Groups	.002	1	.002	.056	.816
	Within Groups	.590	18	.033		
	Total	.592	19			
C. conica	Between Groups	.003	1	.003	.121	.732
	Within Groups	.400	18	.022		
	Total	.403	19			

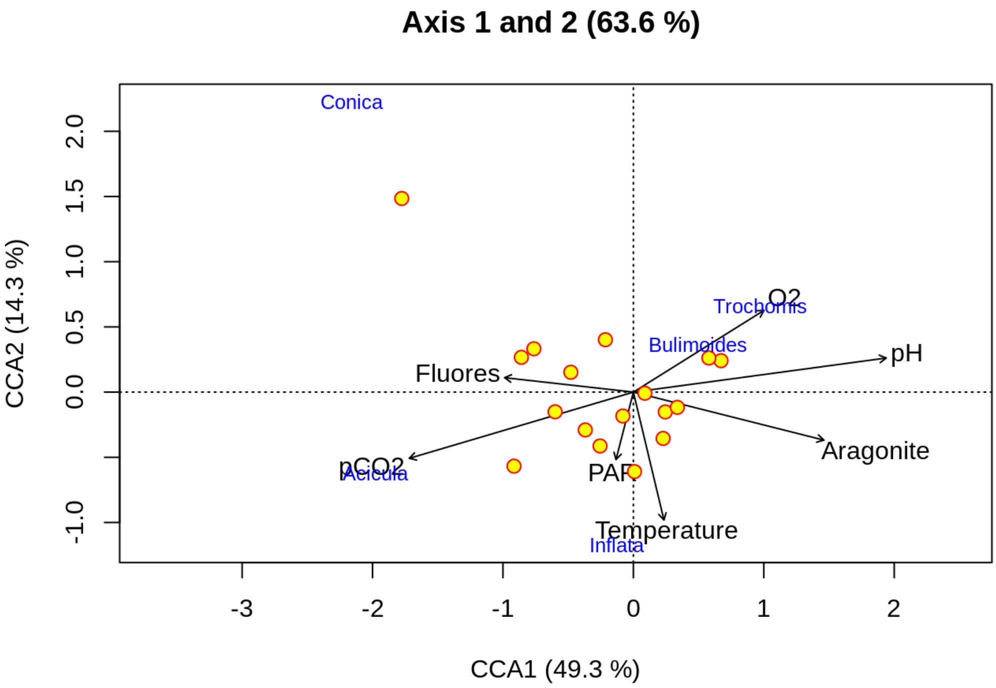


Figure 4. Triplot from the Canonical Correspondence Analysis (including the PAR variable), where the relation between species and environmental variables are obtained from the proximity (or remoteness) of species labels and environmental arrows (tips). The length of the arrow represents the importance (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.

Introduction

3. **COMMENT:** The introduction seems to lack a depth of research, especially of older material which is still useful background on pteropod ecology. Would be good to see the original references, e.g. Lalli and Gilmer, Rampal, as well as the more modern ones, to support statements, as many of the modern studies referred to merely added to the field of knowledge already in place.

REPLY: Thank you for this input. We have revised the introduction according to this comment and have added original references together with the more recent as follows:

There are few studies within the Mediterranean Sea that focus solely on pteropod community distribution (eg. Howes et al., 2015; Manno et al., 2019; Rampal, 1967) and those are limited to very restricted geographical and/or coastal regions. The thesis manuscript of Rampal (1975) was the first study investigating the pteropods distribution and ecology across the Mediterranean Sea, combining samples collected with different methods and within different periods and regions. Most recently, Bednaršek et al. (2012) estimated the global distribution of pteropods by merging a large number of datasets collected globally as well as in several Mediterranean regions. To our knowledge, there is no published peer reviewed study on pteropod abundance and distribution across the Mediterranean Sea, covering the whole basin and relatively large biogeochemical gradients, using a consistent sampling and processing methodology.

Pteropods are passive feeders, utilising large mucous webs to collect food particles (Lalli and Gilmer, 1989). These organisms play an important role in both the trophic system and biogeochemical cycling, linking phytoplankton and larger pelagic predators, such as carnivorous zooplankton, fish, cephalopods, and birds (Fabry, 1989; Lalli and Gilmer, 1989). Pteropods are known to perform diel vertical migration, however peaks in abundance are thought to be related to seasonal vertical migrations linked to reproduction (Rampal, 1975).

4. **COMMENT:** It would be good to include some indication of why the Mediterranean is a ‘climate change hotspot’.

REPLY: The Mediterranean Sea was referred to as a climate change hot-spot in Giorgi (2006) and Lionello and Scarascia (2018). The region is experiencing an increase in temperature that is exceeding global trends (Cramer et al., 2018), and sea temperatures are expected to rise by 1.5-2°C by the end of this century, with atmospheric warming likely to be 20% faster than the global average (Lazzari et al., 2014; Lionello and Scarascia, 2018). This is outlined in the third paragraph of the introduction section.

5. **COMMENT:** An organism’s suitability for use as a sentinel species depends on a number of factors, not only a perceived sensitivity to a climate driver, but also a sound understanding of its ecology which I am not convinced we have yet for pteropods.

REPLY: We agree with the reviewer’s comment. Our study does not definitively suggest that pteropods should be used as a sentinel group. Their possible sensitivity to seawater Ω_{ar} indicated by our results support that it could be an important environmental parameter modulating their distribution, an idea that has been touted by other studies (e.g. Bednaršek et al., 2019, Bednaršek et al., 2016; Manno et al., 2017). We will rephrase this part in the manuscript to indicate this.

Updated text in the introduction:

Pteropods are considered susceptible to changes in the carbonate saturation state (Ω) due to their aragonite shell, which is a more soluble form of calcium carbonate compared to calcite (Mucci et al., 1989). According to a recent systematic review and meta-analysis of thresholds of ocean acidification impacts on thecosome pteropods (Bednaršek et al., 2019), they were touted as suitable indicators for assessing the impacts of changes in water chemistry associated with climate change.

6. **COMMENT:** Line 30: pray should be prey

REPLY: Thank you. Updated.

7. **COMMENT:** throughout: ar should be subscript when following Omega to denote saturation state of aragonite.

REPLY: Thank you. Updated throughout the paper.

8. **COMMENT:** There should be a space between the distance and the unit, e.g. 200 m

REPLY: Thank you. Updated throughout the paper.

9. **COMMENT:** Line 33-34: This is a strong statement, there are many other factors that contribute to an organism's suitability for use as a sentinel organism, make this statement more precautionary.

REPLY: We have already addressed this comment about the use of pteropods as sentinel organism where we show the new sentence changed to be more precautionary form (please see comment # 5).

10. **COMMENT:** Line 37: there is extensive work on Mediterranean pteropod distribution by Rampal which is not covered in this introduction.

11. **COMMENT:** Lines 43-44: again, the work of Rampal should be considered here.

REPLY: We have already addressed these comments. We agree with the reviewer and we refer to the work of Rampal (1975) in the introduction, highlighting that Rampal's study was the first investigation of pteropod distribution and ecology across the Mediterranean Sea (see comment # 1, 2 and 3).

Methods:

> **12. COMMENT:** Line 123-125: what direction was the tow? Vertical, oblique? It would be useful to know the depths of the stations.

REPLY: The Methods section has been updated with:

The plankton towing was performed with the vessel moving at approximately 1 nautical knot, with an oblique sampling direction.

This is the same technique used for the foraminifera study performed on the same samples Mallo et al., 2017.

The bottom depth of each station has been added to Supplementary Table 1.

> **13. COMMENT:** Please indicate what pH scale was used.

REPLY: The pH was a calculated value on the total scale. This has been updated in the Methods section of the manuscript.

> **14. COMMENT:** Depth of tow is pretty shallow for many of the pteropods listed here, see Rampal 1975 for average depth distributions throughout the year. Could be that many of the species have been under-sampled.

> **15. COMMENT:** Looking at the sampling depths of used here study, I am not confident that this is the case and several common species may have been under sampled.

REPLY: We have addressed the concern of the reviewer about the potential underestimation of pteropods due to the sampling methodology in previous replies (see comments # 1 and 2) where we refer to Rampal (1975) as well as to more recent literature in order to a) support the validity of our strategy and b) provide a more conservative approach in the interpretation of species having a depth distribution possibly spanning depths below 200 m. Consequently, we removed *C. inflexa* and *S. subula* from the statistical analyses of pteropod distribution among the environmental parameters.

16. **COMMENT:** Please explain why a CCA was chosen as opposed to another correspondence analysis, e.g. RDA?

17. **COMMENT:** It is my understanding that CCAs are better in more controlled environments when there is a high confidence that the community has been exhaustively sampled as CCA inflates the importance of rare species.

REPLY: In this study we use a CCA to better characterize the effects of environmental conditions on pteropod community composition. The CCA can be seen as a weighted form of RDA applied to a species matrix of contributions using a Chi-square statistic (Borcard, et al 2018), giving a better approach for count data (as it is based on the Chi-square distribution). Moreover, because the effects of the environmental variables on community data isn't always linear (e.g. temperature thresholds), in the CCA the species are ordered following their ecological optima (unimodal relationship), allowing us to make better ecological interpretations of the species assemblages and the effects of the environmental variables (Borcard et al., 2018). 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. The functions *cca* and *ordistep* from the "vegan" package were used for the CCA and the permutation test, respectively (Oksanen et al., 2019).

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 5 here and to be included as a supplementary figure in the revised manuscript).

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 6; $F = 4.05$, 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 (pCO_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 6 here).

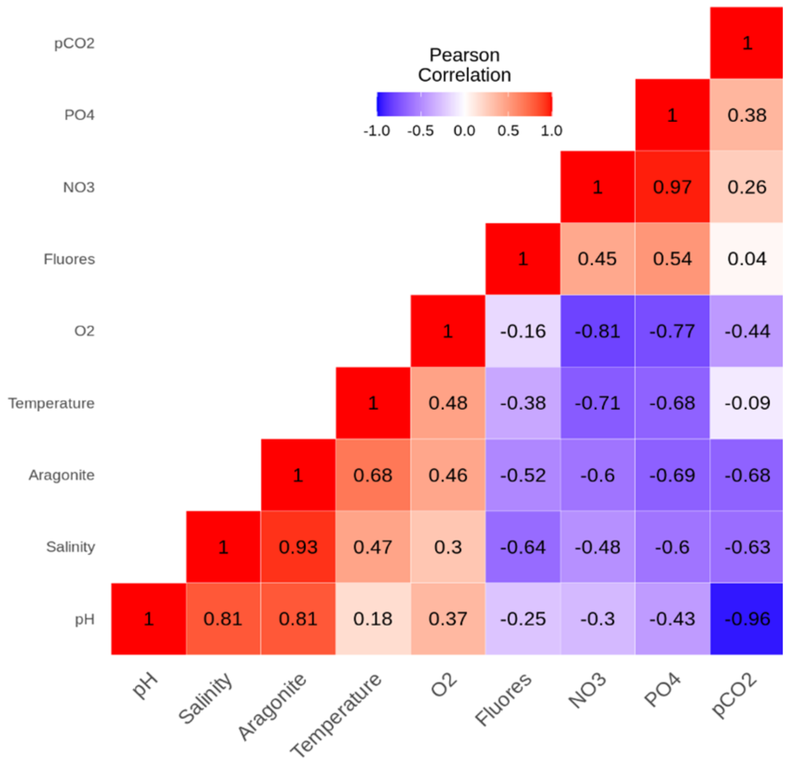


Figure 5. Pearson's Correlation matrix showing the high correlation between several environmental variables. For the CCA, the most biologically relevant of correlating variables were kept. Variables used in the analyses were: NO_3, temperature, Ω_{ar} and fluorescence. The variables removed from the analyses were pCO_2, PO_4, O_2, salinity and pH.

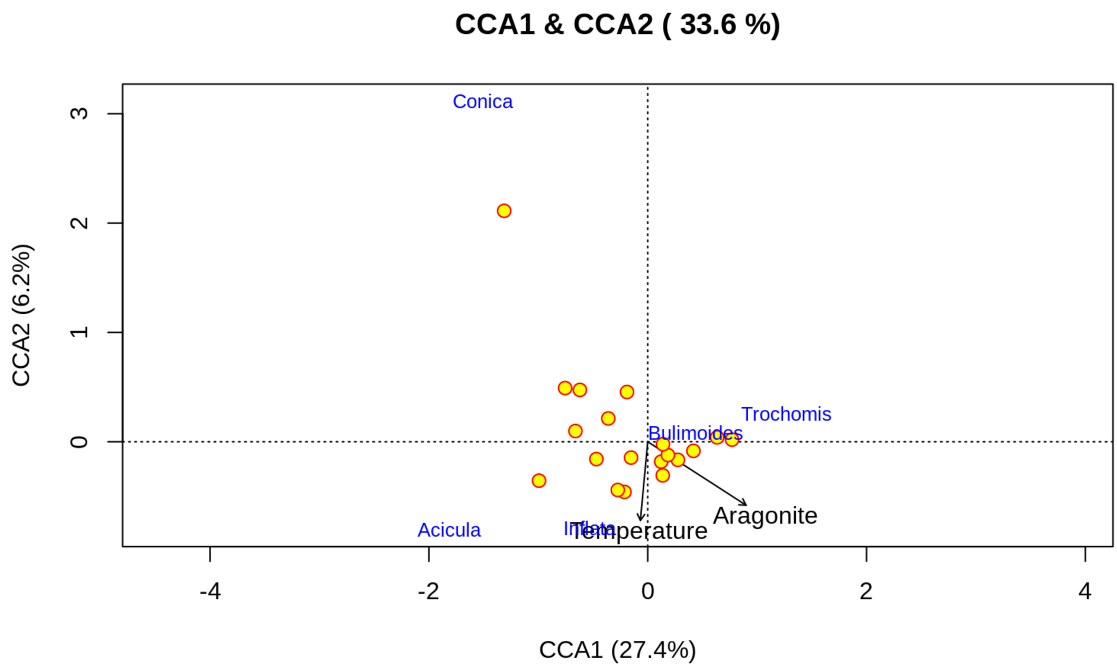


Figure 6. Parsimonious CCA indicating that temperature and Ω_{ar} are significantly affecting 33.6% of the structure of the observed community during the sampling (F = 4.05, p-value <0.01). 'Aragonite' indicates Ω_{ar}. 'Temperature' is obscuring the word Inflata (*H. inflatus*). The yellow circles indicate individual stations.

18. COMMENT: Please explain why a BLRM was chosen for the analysis as opposed to, for example, a GLM?

19. COMMENT: Please report on the results of the BLRM in the main body of text, a p value should be reported, at least.

RESPONSE: The Binary Logistic Regression Model (BLRM) is not a different (opposite) analysis but a specific case of a Generalised Linear Model (GLM) for a binary distributed variable. The BLRM was used to investigate the environmental drivers of higher abundance in pteropods using an aggregation of pteropod abundance into two major groups (binary model) relative to their distribution. However, we decided to remove the BLRM (the binary GLM) from the pteropod analysis as a GLM based on the aggregated pteropod data is not the most suitable approach when using a small number of observations showing high variability, which is common in planktic organisms.

We have retained the Pearson's correlations, which investigate the relationship between the total and individual species abundances and the environmental parameters.

Removing the BLRM from our statistical analysis does not majorly affect the results or change our interpretation as the results of the CCA and Pearson's correlations still show that temperature and Ω_{ar} are the major drivers of spring pteropod community composition and abundance in the Mediterranean Sea. We are aware that correlation is not necessarily causation, but the interpretation of our results was motivated by the likely mechanistic dependency of pteropod distribution on these main environmental drivers (temperature and Ω_{ar}). The Pearson's correlations also indicate that salinity and O_2 are positively correlated with total pteropod abundance and that NO_3, PO_4 and pCO_2 are negatively correlated with total pteropod abundance, and therefore we have shifted our Discussion section to incorporate these results.

The methods have been updated:

Pearson's correlation coefficients were calculated to determine if there were any significant relationships between the environmental parameters and total and individual species abundance.

The results have been updated:

The results of the Pearson's correlation show that total spring pteropod abundance across the Mediterranean Sea is positively correlated with Ω_{ar}, O_2, pH, salinity and temperature and negatively correlated with NO_4, PO_3 and pCO_2 *(Supplementary Table 3).* Heliconoides inflatus *was positively correlated with temperature and Ω_{ar} and negatively correlated with the nutrients NO_3 and PO_4.* Limacina trochiformis *is positively correlated with pH and Ω_{ar} and negatively correlated with* pCO_2. Limacina bulimoides *is positively correlated with pH and O_2 and weakly negatively correlated with* pCO_2. Creseis acicula *and* C. conica *are not correlated with any environmental variable.*

> **20.** **COMMENT:** I am not familiar with varimax rotation, please clarify for the reader.

REPLY: We conducted a varimax rotation for the PCA which is used when there is high collinearity among some of the environmental variables (Figure 5). The varimax rotation is used to keep the explanatory variables uncorrelated.

The methods have been updated:

As there was high collinearity among some of the environmental variables (Figure 5 here and added as a supplementary figure to the revised manuscript), a varimax rotation was applied to delineate the factor structure.

21. **COMMENT:** It might help to reassure the reader that the depth or sampling time did not skew the results by including these as variables and assessing any correlation with pteropod abundance.

REPLY: The issue of diel vertical migration and collection depth is an important one and we have addressed this comment earlier in the text (Comment # 2 pages 7-9).

Results:

22. **COMMENT:** I am not clear where the foraminifera appeared from, there is no mention of foraminifera collection in the methods or any rationale for their inclusion in the analysis prior to this point, please include in methods and some rationale for their inclusion in the introduction.

REPLY: This paper is providing the first comparison of pteropod and foraminifera distribution in the Mediterranean Sea. Foraminifera are single-celled marine eukaryotes producing calcite and little is known about how the distribution of these important groups of calcifying zooplankton differs. The foraminifera were collected in the same plankton towing samples as the pteropods of this study and the results were published in BG (https://www.biogeosciences.net/14/2245/2017/), adding value to this work.

As mentioned, the published foraminifera study used data collected from the same set of samples and has been already published. This was mentioned in the introduction and we refer to Mallo et al. (2017) for details on the methodology. We realise that for clarity this information could be expanded on in order to provide a better rationale for their inclusion. We added the following text in the Introduction, Methods and Results sections.

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:

Planktic foraminifera and pteropods were collected together in the same plankton tow 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 ($\overline{x}$=2.13 ind. m^{-3}) Mediterranean than in the Western Mediterranean ($\overline{x}$=0.47 ind. m^{-3}), while foraminiferal populations showed a contrasting abundance distribution (Figure 7 here and to be added to the revised manuscript) with higher abundance in the Western Mediterranean ($\overline{x}$=1.87 ind. 10 m^{-3}) than in the Eastern Mediterranean ($\overline{x}$=0.96 ind. 10 m^{-3}).

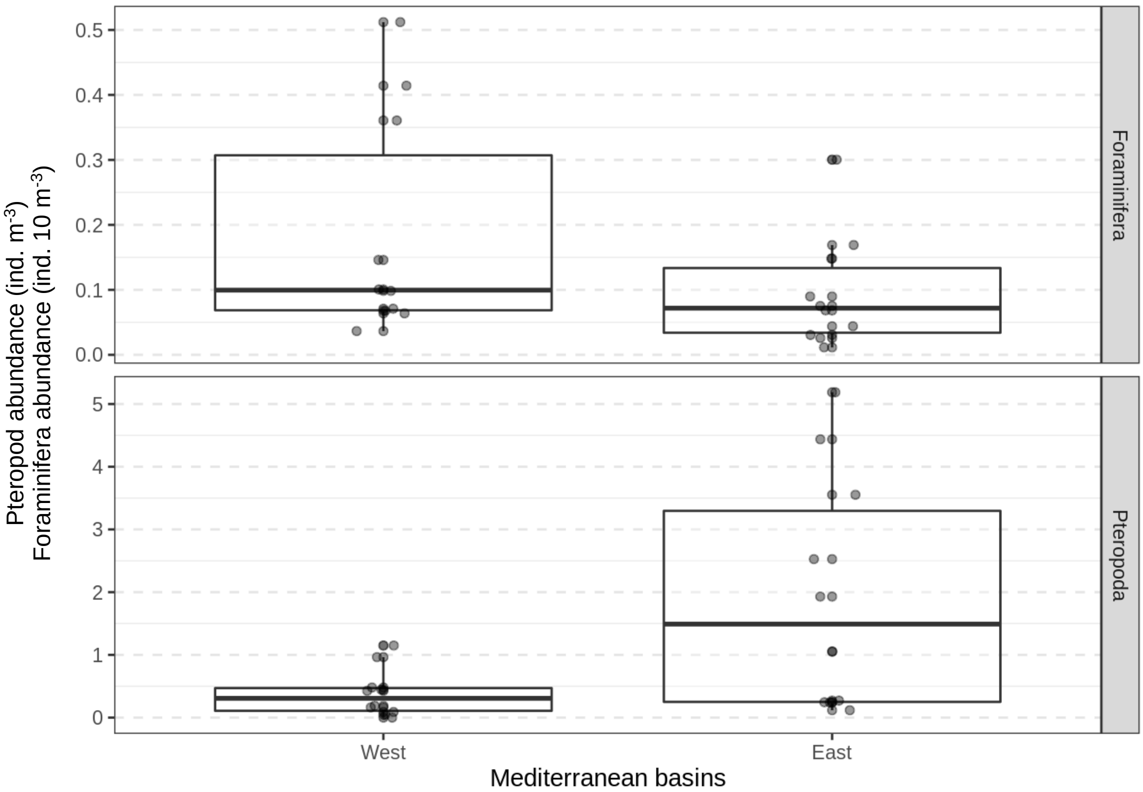


Figure 7. Box plot showing the contrasting abundance distribution between pteropods and foraminifera between the East and West of the Mediterranean.

23. COMMENT: Some way of distinguishing whether the station numbers are located in the east or the west would be helpful.

REPLY: This is indicated in the methods sections: '..between western stations (1-7a and 19-22) and eastern stations (9-'16-18').' We have also added a figure from Mallo et al. (2017) to Figure 1 in the revised manuscript which indicates each station and transect of the cruise (Figure 7 here).

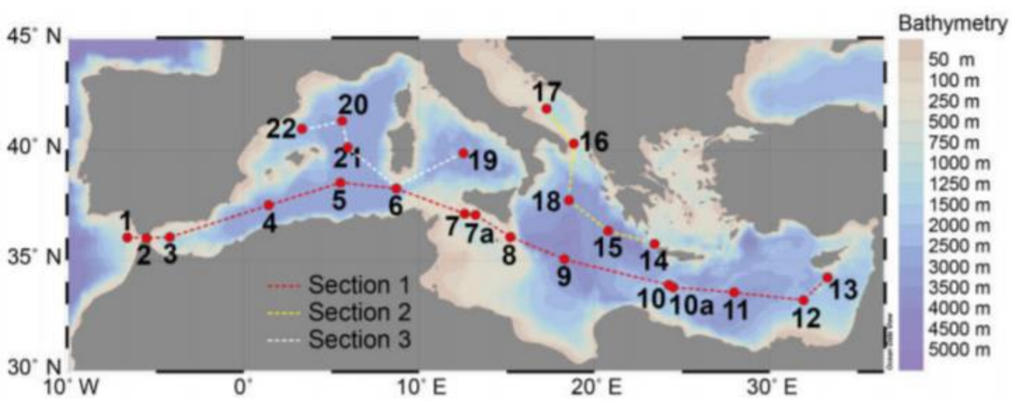


Figure 7. Sampling stations of each individual transect (Ocean Data View).

24.COMMENT: Line 197 (and throughout): please use correct terminology to avoid confusion, this should be principle components 1 and 2.

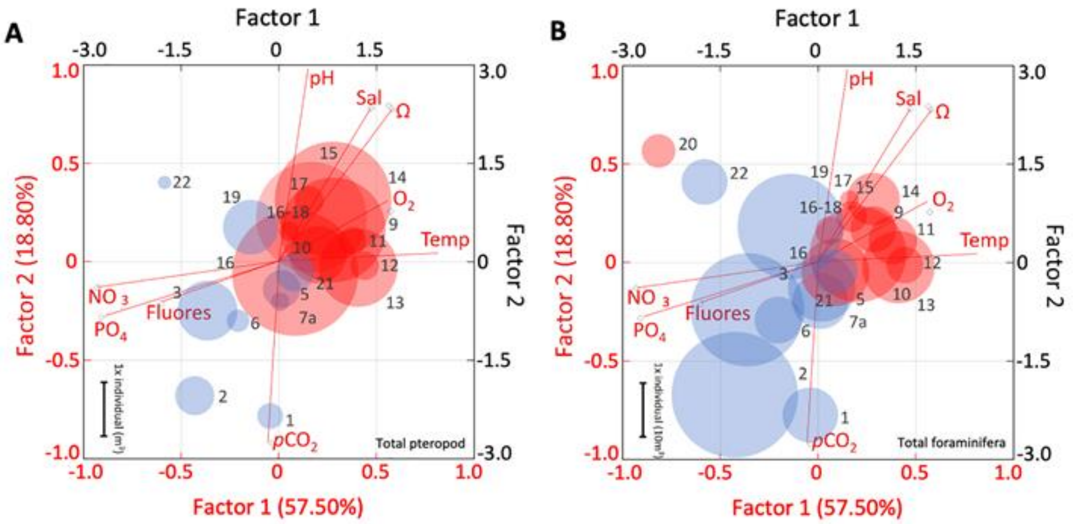
REPLY: Thank you. This has been updated.

25.COMMENT: Fig 3: this figure is very unclear and I struggle to relate the description of the figure in the text to the figure, itself. I assume that this is essentially two plots overlaid which is why there are two different axes? If not, I don't understand how the red axis relate to the PCA coordinate and the black to station coordinates as both colours cover factors 1 and 2.

26.COMMENT: Fig3 A and B: Please add to the figure description to make it clear what the coloured circles relate to, I assume that they are abundance at station and that red is east and blue is west? The overlapping of the circles renders them meaningless as it is impossible to assign the circle to the corresponding station number.

REPLY: For this figure, we have used the same approach as Mallo et al. (2017). The red axes are associated with PCA coordinates and the black axes are associated with the station coordinates. This approach was used to better visualise the relationship of the sampling stations with the environmental parameters.

Figure 3 in the manuscript is two plots overlaid on top of each other. This has been made clearer in the Figure text box. Rather than giving the option to pinpoint each particular station, this figure aims to illustrate the differences between the east and west of the Mediterranean in terms of abundance and the relationship to environmental variables.



Updated Figure 3 text: PCA graphs of environmental factors overlaid with absolute abundance values on station scores of **A** pteropods at all stations (ind. per m^{-3}) and **B** foraminifera at all stations (ind. per $10m^{-3}$). Stations in blue indicate western Mediterranean stations (1-7a and 19-22) and stations in red indicate eastern stations (9-'16-18'). The size of the circles represents the total pteropod abundance at each station. The red axes are associated with PCA coordinates and the black axes are associated with the station coordinates.

> **27.COMMENT:** Fig 3 C: If the analysis was performed on the community as a whole, I am unclear how am unclear how C. inflexa was removed. Was it removed from just the plot or the full analysis?

REPLY: *Cavolina inflexa* was not included in the plot as it was not significantly correlated with any variables. As mentioned above, we decided to remove *C. inflexa (*along with *S. subula)* from the discussion of the species distribution and the analyses, as based on the reviewer's suggestion, these species may have been underestimated due to sampling depth.

Discussion:

> **28.COMMENT:** The authors rightly point out that this study represents a "snapshot" in time and, as such, I would be wary at relating the results

so strongly to ocean acidification factors and the inferring pteropod suitability as an indicator species. It is my opinion that we simply do not understand enough about pteropods to use them as an indicator species.

REPLY: We agree about toning down the possible interpretation related to the link between our results with ocean acidification (OA) and seawater carbonate chemistry. This work is contributing to previous field and laboratory studies addressing the sensitivity of pteropods to OA. According to a recent systematic review and meta-analysis of thresholds of OA impacts on thecosome pteropods (Bednaršek et al., 2019), pteropods were touted as appropriate indicators for assessing the impacts of changes in water quality associated with climate change. Please refer to comment # 5 regarding pteropods as an indicator group – while we are not directly advocating here for pteropods to be used as an indicator species, these results do add support to the idea that has been more strongly suggested in other studies (Bednaršek et al., 2016; Manno et al., 2017).

29.COMMENT: How do the mean pteropod abundances found in this study compare to other abundance estimates from the Mediterranean? There are several time series that might provide a more temporally averaged estimate of abundance that could be used to validate this ‘snapshot’. Pteropod abundance is patchy and highly seasonal, we are not entirely sure what controls their lateral distribution on the water column but we do know that there are strong seasonal variations in their depth profiles (See Rampal, 1975) which may be skewing the results due to relatively shallow depth of the tow.

REPLY: An additional section on pteropods in the Mediterranean has been added to the Discussion to give more context to our results. A table compiling relevant published pteropod studies in the Mediterranean Sea 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, Limacinidae 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 Limacinidae (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 to 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 3. 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 *C. inflexa, C. pyramidata* and *S. subula*). Min-max not provided	Day: *Cavolina inflexa*: 4.0 *Clio pyramidata*: 2.1 *Styliola subula*: 0.3 Night: *Cavolina inflexa*: 1.7 *Clio pyrimidata*: 1.6 *Styliola subula*: 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	*Cavolina inflexa*	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	*Heliconoides inflatus*	113 cm diameter 380 cm length 250 µm mesh	Batistić et al., 2004
Balearic Sea	Only monthly x̄ given. Min: 4 Max: 11	5.9	1994-2003 (all year round)	0-75 0-100	Various 78-200	*Creseis acicula*	Bongo-20 Plankton net 100 µm and 120 µm meshes	Fernández de Puelles et al., 2007
Ligurian Sea	*C. inflexa: 1.59 (20-40 m)* *C. pyramidata: 0.06 (100- 200 m)* *H. inflatus: 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	*Heliconoides inflatus*	BIONESS multinet 1 m^2 mouth 230 µm mesh	Granata et al., 2020
NW Ligurian Sea	Creseidae: ~630 Cavoliniidae: ~790 Limacinidae (incl. *H. inflatus*): max 60.8	Creseidae: 15.7 Cavoliniidae: 13.8 Limacinidae: 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	*C. acicula*: 1.48 *C. conica*: 1.11 *H. inflatus:* 1.03 *L. trochiformis:* 0.64 *L. bulimoides:* 0.33	August, 2015	One depth for each station according to the sea bottom (min depth 0-65, Max depth 0-170	Various (73-185)	*Creseis acicula*	Bongo-40 200 µm mesh	(Manno et al., 2019)
Eastern Mediterranean	Sicilian Channel: Max. 120 ind. m^{-3}	Sicily Channel: 2.07 Ionion 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	*Study focuses solely on Cavolina inflexa* Max: x̄ 1.64 (0-200 m)	Not provided	September, 1997	0-500 with intervals 0-25 25-50 50-75 100-125 125-150 150-200	Not provided	*Cavolina inflexa*	MOCNESS 1 m^2 mouth 300 µm and 2000 µm meshes	Tarling et al., 2001

30. COMMENT: Comparison with foraminifera: It would be more useful to compare the pteropod abundance and distribution with a non-calcifying species to really provide some insight as to whether the differences observed are due to calcification energetics.

REPLY: The motivation for looking at the relationship between pteropods and foraminifera is important as OA 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). What is interesting in this study is that when nutrients become limited (oligotrophic conditions), we see a change in calcifying plankton communities, and here we attempt to provide some insight as to potential biological or environmental factors driving this change. The comparison with a target, non-calcifying group in order to provide some insight into whether the differences observed are due to calcification energetics is an interesting approach that should be investigated in future studies, but is outside the scope of this work.

31.COMMENT: The depths sampled are also relatively shallow for some planktonic foraminifera, e.g. O. universa, which prefer deeper waters.

REPLY: We refer to the BGD and BG paper Mallo et al. 2017 (https://www.biogeosciences.net/14/2245/2017/bg-14-2245-2017.html) for detailed discussion and interpretation of foraminifera results.
During the sampling the deep chlorophyll maximum was situated close to 200 m depth and we did not expect to find high numbers of foraminifera between 200 and 350 m. Pujol and Grazzini (1995) performed a study on the distribution

pattern of foraminifera across the Mediterranean and indicated that *O. universa* has a preference for surface waters.

32. COMMENT: Linking pteropod abundance to environmental parameters The study does not make mention of the fact that several other studies of pteropod abundance (time series) have found that pH does not have a significant effect on the abundance of pteropods through time, therefore it seem unlikely that it would have a significant effect through space. Notably, Howes et al (2015) did not find any negative effect of decreasing pH on Eastern Mediterranean pteropods. Logically, one would assume that if the effects of a gradient on pH was impacting the distributions such that pteropods were favouring the Western Mediterranean, that a decrease in the already less favourable Eastern basin would lead to a decrease in their numbers there, however this is not the case.

REPLY: We refer to Howes et al. (2015) in our manuscript. We are aware that Howes et al. (2015) did not find any negative effect of decreasing Ω_{ar} in the pteropod time series from the North Western Mediterranean coastal site. However, the results of Howes et al. (2015) is based on Ω_{ar} variability over 36 years (1967-2003) in one location (Point B, Bay of Villefranche in the Ligurian Sea), which does not reflect the potential influence of Ω_{ar} variability over the large geographical scale on pteropod distribution. Further, Howes et al. (2015) focuses on a coastal population (70 m water depth), and like many coastal inhabitants, these organisms are more likely to be able to cope with wide changes in environmental parameters, as coastal environments experience large natural variability. Aragonite saturation in Howes et al. (2015) ranged from 4.3 to a minimum of 3.1 and was therefore highly saturated. In this study, aragonite saturation ranged from 3.6 to 2.7, and while these are conditions that are still highly saturated, studies indicate a relationship between negative impacts on calcification under reduced Ω_{ar} (Comeau et al., 2010a, 2010b).

Finally, our study showed that even if decreases in Ω_{ar} over the time (1967-2003) does not impact pteropod abundance in the same location (Howes et al. 2015), the variability of Ω_{ar} values between two distinct geographical regions can be a driver of the geographical distribution of pteropods. In brief, the results from Howes et al. (2015) do not contradict the results of the present study. We have added some of the points raised above to the revised Discussion section to clarify the different approach related to large scale distribution study versus a single coastal station time series. Both approaches are extremely important for ecological studies and provide different types of insights.

33. COMMENT: The authors mention that the significance of the results are being driven by the Limacinidae, but how do they explain this? I find it strange that the effects of Omega ar would be affecting one family of pteropods but not another when (as far as we know) they both have the same method of calcification and they both calcify the same polymorph of CaCO3.

REPLY: The discussion has shifted to a more species based response as, based on the Pearson's correlations and the CCA, different species show individual relationships with different environmental parameters.

Although thecosome pteropods produce the same polymorph of calcium carbonate, so far we are not aware of any studies showing whether they calcify in the same way or not, including in relation to the energy budget devoted to the calcification process. The energetic costs associated with the biomineralisation of calcium carbonate in pteropods are poorly understood. Different pteropods family/species may present a different sensitivity to Ω_{ar}.

> **34. COMMENT:** Looking at Fig 3C, only L. trochiformis appears to be strongly positively correlated with Omega ar, while S. subula seems to be telling the opposite story, please discuss these results.

REPLY:

Please refer to our response to comment #1. *Styliola subula* has been removed from the statistical analysis as it can have deeper distribution than 200 m and their abundance here would likely be underestimated. The CCA has been conducted again and Ω_{ar}, along with temperature, is one of the significant factors influencing community composition. While not one species shows a distinctly strong relationship with Ω_{ar}, there is a positive relationship with the most abundant limacinid species: *H. inflatus, L. trochiformis* and *L. bulimoides.* This means that the relationship with Ω_{ar} is mainly associated with the limacinid pteropods, who make up the majority of our recorded pteropod abundances across the Mediterranean.

> **35. COMMENT:** Please also include a discussion on the reasons why C. inflexa are not correlated to any variable.

REPLY: Please refer to our response to comment #1. *Cavolina inflexa* has been removed from the statistical analysis as it has deeper distribution than 200 m and their abundance here would likely be underestimated. It is also likely that the habitat (environmental parameters) within the upper 200 m does not reflect their main habitat conditions.

Conclusions:

> **36. COMMENT:** I do not think that the results of this study can be taken to indicate that pteropods display suitability as an indicator species and I think that the assertion that they are correlated with Omega ar is an oversimplification of the results, especially when this is driven by one pteropod family and not others included in the analysis. It seems likely that the results have been skewed by sampling technique and station depth and this should be investigated before assigning the results to Omega ar.

REPLY: The quality of the data collected for this study have been obtained using a robust and consistent sampling technique as well as a well-established lab processing approach. We have already commented on the sampling depth above. We agree about reshaping the conclusion of this work and we have substantially modified this part of the Discussion in the revised manuscript as well as some of the statistical approach. Please refer to our response to comment #

1 regarding sampling depth (species removal, acknowledging limitations). Based on the results of the parsimonious CCA and the Pearson's correlations, as well as the biological importance of Ω_{ar} to pteropod shell development, we maintain that Ω_{ar} is an important factor influencing spring pteropod community distribution in the Mediterranean Sea. We have taken a more conservative approach in the manuscript and have removed the suggestion that our results directly add support to the suitability of using pteropods as an indicator species. Based on the changes mentioned above we modified the text as follows:

This work shows the presence of a positive correlation between the distribution of the pteropod community in the upper 200 m and the natural west-east Ω_{ar} and temperature gradient in the Mediterranean Sea. Overall, we suggest that the higher abundance of pteropods in the oligotrophic sector of the Mediterranean Sea is a combination of higher Ω_{ar} that is energetically favourable for calcification, and their ability to withstand starvation (Busch et al., 2014). Further, this study provides new insight into the ecology of shelled pteropods and foraminifera and highlights that in specific oligotrophic regions, diet strategy could also play an important role in regulating the pelagic calcifier zooplankton communities.

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