Interactive comment on "Structure and functioning of epipelagic mesozooplankton and response to dust events during the spring PEACETIME cruise in the Mediterranean Sea" by Guillermo Feliú et al.
Referee 1: Tamar Guy-Haim (Referee) (tamar.guy-haim@ocean.org.il)
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Note: All the frames contain the comments of referee R1, and have been identified by a number. Our answers are below each frame, and the underlined paragraphs in yellow correspond to new or modified paragraphs in the manuscript. R2 refers to answers common to comments of referee 2.

Comment R1.1A
The manuscript by Feliú et al. presents a study of the zooplankton community structure at 12 stations along four Mediterranean Sea basins: Provencal, Algerian, Tyrrhenian, and Ionian basins during the PEACETIME cruise. This cruise was set to study the effect of Saharan dust depositions on processes at the air-sea interface. The authors sampled the epipelagic layer (0-300m) using two mesh-size nets during/after dust events.
Their major finding is that following Saharan dust deposition, the observed changes between basins are masked and that there is a larger contribution of small size fraction (<500 _m) to the mesozooplankton community. The authors also performed indirect (allometry-based) assessments of zooplankton metabolism and showed that this fraction also contributes significantly to the N and P fluxes in the epipelagic layer.
While this ms provides important information for the methodology of zooplankton sampling in the Mediterranean Sea, I had major concerns with incompatibility between its main aims as declared in the title, abstract and introduction, and the data presented.

Answer to comment R1.1A.
We have tried to answer all your comments and have substantially rewritten several paragraphs of the manuscript. We appreciate all your comments and we acknowledge that they have encouraged us to explore further our data. We hope that now the revised version of the manuscript shows more consistency between the main aims and the presented results.

Comment R1.1B
The most important environmental data for testing the research aim – the intensity and the spatiotemporal distribution of the Saharan dust deposition event – is missing. The ms lacks appropriate data on dust and nutrients. I understand that this information will be part of other/future publications, but I do think that it is mandatory to include it in this ms as well, and the final acceptance of this ms should be conditional on presenting these data.

Answer to comment R1.1B:
We have produced a full synthesis of the Saharan dust deposition events encountered during PEACETIME (See paragraph and table below). A lot of information is now presented in accepted and submitted papers of the special issue including the introduction paper by Cecil Guieu. We propose to include a synoptic table in our revised manuscript to summarize and comment on this information

Comment R1.1C
Other concerns relate to the lack of hypothesis setting, sampling justification, and statistical analyses.

Answer to comment R1.1C:
Hypotheses have been better formulated and new statistical analyses have been performed (See detailed answers below)

Comment R1.1D:
Also, this paper would benefit from some closer proofreading. It includes misuse of wording that can be easily corrected. It may be useful to engage a professional English language editor.

Answer to comment R1.1D:
The revised paper has been proofread by a native English speaker specialized in scientific publications.

Comment R1.1E: Nevertheless, the presented study is unique and scientifically worthwhile. I, therefore, suggest accepting this manuscript after major revision, pending correction of all major issue

Comment R1.2 :
Major issues that must be resolved
[1] The ms title "Structure and functioning of epipelagic mesozooplankton and response to dust events during the spring PEACETIME cruise in the Mediterranean Sea" as well as the abstract and introduction suggest/state that this study estimates the effect of dust deposition on zooplankton structure and function. Nevertheless, the ms does not include any data on dust deposition (e.g., in terms of Aluminum concentrations – see Measures Vink 2000 or Titanium in Dammshäuser et al. 2011) and nutrients in the sampling stations. Without this information, it is impossible to provide a reliable quantitative assessment of the effects of dust deposition on zooplankton communities.
The only dust-related information given within the ms (as personal communication with C. Guieu) is that on May 10 there was “a quite important dust event”. This, by all means, is not satisfactory for differentiating dust effects from natural variability.
Measures, C.I. and Vink, S., 2000. On the use of dissolved aluminum in surface waters to estimate dust deposition to the ocean. Global biogeochemical cycles, 14(1), pp.317-327. Dammshäuser, A., Wagener, T. and Croot, P.L., 2011. Surface water dissolved aluminum and titanium: Tracers for specific time scales of dust deposition to the Atlantic?. Geophysical Research Letters, 38(24).

Answer to comment R.1.2:
As the other referee raised a similar question (see comment R2.20), this answer is common to both.

As already mentioned in the general comment R1B, we have now produced a full synopsis of the Saharan dust deposition events encountered during PEACETIME. A lot of information is now presented in accepted and submitted papers of the special issue including the introduction paper by Cecile Guieu.
We present this new information in an additional paragraph in the Methods section.

Guieu et al., introductory paper) detailed how they used three regional dust transport models to identify major dust events during the PEACETIME cruise. Two major wet dust events occurred during the period (Table 2). The first concerned the whole southern Tyrrhenian basin, with predicted flux > 1g m-2 (Desboeufs et al. in prep.), and started on May 10, several days before the arrival of the vessel in this area. The dust event was confirmed by aluminium, iron and lithogenic Si measured in sediment traps at TYR with a lithogenic flux between 200 and 1000m of 150-200 mg m-2 (Bressac et al., in prep.). The second was located in the area between the Balearics and the Algerian coast and occurred from 3 to 5 June, with predicted flux of 0.5 g m-2 (Guieu et al., accepted) after the arrival of the vessel in this area (station FAST). The dust event was confirmed by on-board atmospheric dust deposition samples (Desboeufs, in preparation this special issue), water column observations (nutrients, trace metals) (Tovar-Sánchez et al. 2020) and tracers of dust deposition in sediment traps (pers. comm. C. Guieu). The highest aerosol mass concentrations (around 25 µg m-3) with the highest iron content (245 ng m-3) were measured at FAST between 1 and 5 June, and subsequently the highest trace metal concentrations in the surface micro-layer were measured on 4 June (Co: 773.6

pM; Cu: 20.1 nM; Fe: 1433.3 nM; and Pb: 1294.7 pM) (Tovar-Sánchez et al 2020). The chemical composition of rain samples at FAST confirmed wet deposition of dust reaching a total particulate flux of 0.012 g m-2 (Fu et al., in prep.). The Ionian basin was the only southern area not impacted by dust deposition during the PEACETIME cruise, and results obtained at the long-duration station ION will be used for comparison.

More details about the total trace metal concentrations in the dusty rain collected by Fu et al., (in preparation) were presented in Tovar-Sánchez et al (2020): trace metals values ranged from 180pM for Cd to 343nM for Fe (Cd: 180 pM; Co: 1380 pM; Cu:18.1 nM; Fe: 343 nM; Ni: 9.9 nM; Mo: 875 pM; V: 26.9 nM; Zn: 345 nM; and Pb: 788 pM)".

This information is summarized in the table below.

New Table 2. Overview of the main characteristics of the wet dust events occurring during PEACETIME. Zooplankton sampling was carried out very close to a CTD cast except at FAST2 where the sampling was done between two casts respectively 9 hours after the first cast [a] and 16 hours before the second [b] .

Stations impacted by dust and cruise visit duration	Crusie strategy with regard to dust events	Dates, geographical characteristics and intensity of the dust events predicted by the model and by observations	Zooplankton sampling Date	Iron in aerosol ng m^{-3}	Nutrients below the nutricline NO3 (n mol/l) PO_4 (n mol/l)	Surface Primary production mg C m^{-3} d^{-1}	Water column (0-250) average Chl-a concentration mg m^{-3}	Depth range of the DCM strata (m)	Mean concentration of Chl-a on DCM strata mg m^{-3}	Ratio fluorecence phytoplankton F_{micro}:F_{nano}:F_{pico} within the DCM strata
Wet dust event FAST 02 to 07 June	Station FAST schedule and position detemined on board acording to metereologial event	**From 3 to 5 June; Impacted area: Betwen Baleares and Algerian coast; Predicted flux from models: 0.5 g m^{-2} (Guieu et al., accepted, Supp Info figure SI5); On-board atmospheric dust deposition observations confirmed a weak wet dust deposition of 0.012 g m^{-2} (Guieu et al., accepted). In sediment traps lithogenic flux was 40-60 mg m^{-2} between 200 and 1000m. Water column observations (nutrients, trace metals) (van Wambeke et al., in prep, Tovar-Sánchez et al. 2020, Bressac et al., in prep) show a clear imprint of the atmospheric deposition.**	FAST1: 04-06-2017	245.3	224 246	2.44	0,12	60-90	0,42	27:45:28
			FAST2: 06-06-2017	266.0	808 239	2.85	0,14[a] 0,18 [b]	60-100 [a] 70-90 [b]	0,38[a] 0,86 [b]	25:43:32 [a] 50:30:20 [b]
			FAST3: 08-06-2017	44.9	135 113	2.04	0,10	70- 90	0,42	20:49:31
Wet dust event Tyrrhenian 16 to 22May	TB stations schedule before the cruise. Model predicted a dust event 6 days before the arrival	From 10 to 12 may; Impacted area: whole southern Tyrrhenian sea; Predicted flux from models**:** >1 g m-^{2} (**Desboeufs et al. in prep)** **Dust event was confirmed by alumimium, iron and Lithogenic Si measured in sediment tramps at TYR with a lithogenic flux between 200 and 1000m was 150-200 mg m^{-2} (Bressac et al., in prep.)**	ST5: 16-05-2017	57.3	841 148	1.68	0,12	70-80	0,55	21:48:30
			TYR: 19-05-2017	162.3	n.d 127	1.77	0,11	70-80	0,61	33:40:27
			ST6: 22-05-2017	189.8	488 136	1.66	0,07	70-80	0,36	7:44:49
References of the data	Dulac (pers.com) Desboeufs et al. (in prep) Guieu et al. (accepted) .	**Desboeufs et al. (in prep) Guieu et al. (accepted) Bressac et al. (in prep) Tovar-Sánchez et al. (2020) van Wambeke et al. (in prep)**		Tovar-Sánchez et al. (2020)	van Wambeke et al. (in prep)	E. Maranon and M. Perez-Lorenzo	J.Uitz, C. Dimier	J.Uitz, C. Dimier	J.Uitz, C. Dimier	J.Uitz, C. Dimierl

Comment R1.3A:
Major issues that must be resolved ...
[2] The ms includes data on zooplankton communities, but is mainly descriptive and include hypothesis-free statistical comparisons. While descriptive articles are of values, it would be useful to set working hypotheses in the introduction, and, along with environmental and biological data, use appropriate statistical methods for testing these hypotheses. **H1** Such hypotheses could be bottom-up enhancement of zooplankton communities triggered by dust deposition, **H2** west to east gradient, etc.

Answer to comment R1.3A: As the other referee raised a similar question (see comment R2.1A), this answer is common to both.

We agree with the referee regarding this comment, which has been very helpful in stimulating a redefinition of certain aspects of the focus of the paper.

We have now stated these general hypotheses:

H1: Is zooplankton structure impacted by dust deposition?. *We hypothesize that Saharan dust deposition events had an impact on the zooplankton community in the Mediterranean Sea, modifying its abundance, biomass, metabolic rates (no observed changes except in in $C_{500\text{-}1000}$) and diversity (The changes we observed are mostly related to community structure and taxonomic diversity (RFD) and to size distribution) .*

H2: Hydrodynamical regions versus dust impact region

- It is difficult to relate zooplankton response to regional differences (lack of stations). Response is due to dust deposition and mainly affect the species diversity (RFD) and size structure (p1/p2 ratio)

H3: *How long should we observe the zooplankton structure in a given area to clearly observe the dust impact (p1/p2 ratio).*

The results providing a basis for validating these hypotheses have been analyzed following several statistical treatments as detailed in following answers. (See below, comments R1.2B, R1.2C, R1.2D)

The following paragraph was added at the end of the Introduction:

These objectives will serve to test the following hypotheses: whether the Saharan dust events impact the zooplankton community structure following deposition (H1), and if so, whether the effect would be immediately observable or after a lag time (H2). Finally, whether changes in zooplankton structure driven by dust deposition exceed regional differences under oligothropic conditions (H3).

Comment R1.3B
Major issues that must be resolved ...
It is unclear why multivariate data analysis was performed to explore spatial changes of environmental parameters – but not for the biological component. The relationship between environmental biological data could be examined, for example, using BIOENV/BVSTEP.

Answer to Comment R1.3B

We agree with the referee's suggestion regarding the lack of more in-depth investigation of the relationships between environmental and biological data. We have decided to add a new paragraph in the Results section

"3.3 Relationship between environmental variables and zooplankton community".

To complement our previous results according to the referee's suggestion, we have used the BIOENV algorithm to select the environmental variables best explaining the community pattern. The BIOENV results show that salinity and chlorophyll were the environmental variables best explaining the overall spatial distribution of zooplankton community (BIOENV; Rs = 0.657).

To take this analysis further and to better explore the relationships between the zooplankton community and the environmental conditions defining their habitats , we have also performed a Co-inertia analysis, which allows comparison of spatial trends in the two data sets.

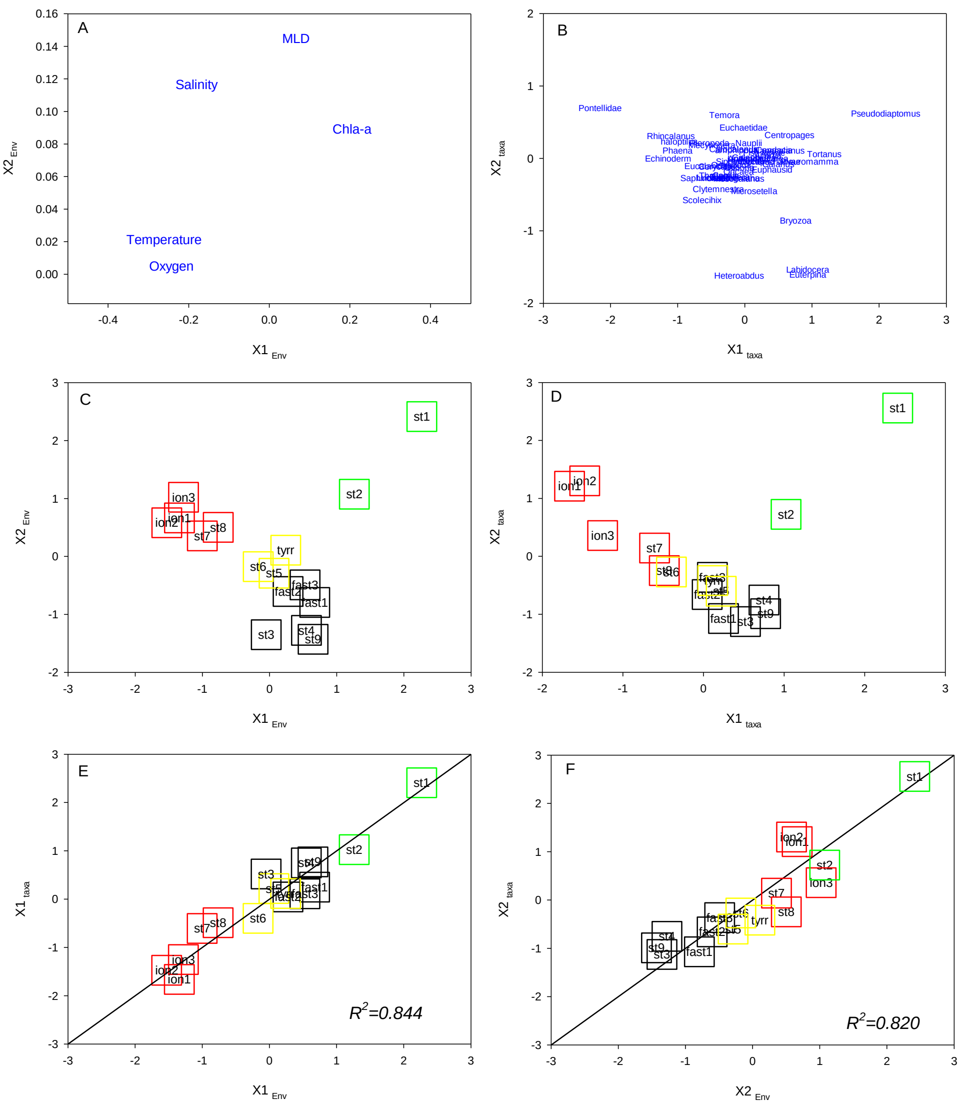
Accordingly, we propose these new paragraphs:

Methods

The relationships between the biological and the environmental variables were also studied by coupling multivariate analyses of two datasets. The first dataset featured the abundances of all the zooplankton taxa identified from the 200µm net samples, and the second recorded environmental variables (the same as for the PCA analysis). A factorial correspondence analysis (FCA) and a principal component analysis (PCA) were performed on these two data sets, respectively. Then the results of the two analyses were associated through a co-inertia analysis (Doledec and Chessel, 1994) performed using ADE-4software (Thioulouse et al., 1997). Prior to the analyses, the data were log-transformed to tend towards the normality of the distributions.

Results

The first factorial plane of the Co-inertia analysis (Figure 9) explained 96% of the total variance, with 79 % due to the first axis. On both spaces ('Environment' and 'Zooplankton'), the first axis opposes the IB stations associated with high temperature and salinity values and several zooplankton taxa (namely Echinoderm larvae and some copepod taxa, ie Pontellidae, *Rhincalanus* spp., *Haloptilus* spp. and *Phaena* spp.) to the PB and AB stations characterized by higher chlorophyll concentrations and by some copepod taxa (mainly *Pseudodiaptomus* spp., *Tortanus* spp. and *Pleuromama* spp.). On this axis, TB stations have an intermediate position, close to the coordinate zero. The second axis opposes northern (st1 and 2 of PB) and southern (AB) stations sampled in the Western Mediterranean basin. On this axis, PB stations are characterized by higher chlorophyll and salinity and deeper MLD, compared to AB and by the association with *Pseudodiaptomus* spp., whereas southern AB stations are associated with the copepods *Heterorhabdus* spp., *Labidocera* spp. and *Euterpina* spp. As in the preceding multivariate analyses, we note that St 8 and 9 from the IB tend to be closer to the TB stations than to the Ion station on the first factorial plane, particularly in the 'Zooplankton system'. The association between the environmental context and the zooplankton community is high with good correlation between the normalized scores of the stations ($R2=0.844$ and $R2=0.820$ for X1 and X2 axes, respectively), and by the positions of the plots of these stations close to the equality lines (i.e. X1 zooplankton = X1 Environment or X2 zooplankton = X2 Environment).



New figure 9: Co-inertia analysis. Ordination on the plans (1, 2) of the environmental variables (A) and the abundance of the zooplankton taxa (B) and of the stations in the 'Environment system' (C) and in the 'Zooplankton system' and plots of the stations on the first (C) and second (D) axes of the two systems. The line represents the equality between the coordinates on the two systems. Coloured squares identify the different regions: green = PB, black = AB, yellow = TB and red = IB.

Discussion
Interestingly, in the Co-inertia analysis the stations impacted by the dust (FAST and TB stations) are grouped on the left side of the relationship between X2 axis of environment and zooplankton. In addition, their succession in this graph is consistent with the sequence observed in the virtual time series of RFD (with FAST1 as the initial station before the dust deposition and TYR and St6 corresponding to day 9 and 12 after the dust event) showing the coupled impact of dust on both environment and zooplankton.

Comment R1.3C
The contributions of significant taxa to specific stations/basins could be tested using SIMPER procedure.

Answer to Comment R1.3C
We agree with the referee and we have done the test. These new paragraphs have been added to the manuscript

In section 3.2 of the manuscript, we added this new paragraph:
"The SIMPER analysis shows that the lower average similarity between the stations is in PB (64.79 %) mainly due to *Para/Clausocalanus* spp. The rest of the basins share a higher internal similarity 78.43 %, 79.79 % and 78.03 % for AB, TB and IB respectively. Another interesting point highlighted in the SIMPER analysis is the lower average dissimilarity between TB and ST7 and ST8 from (20.25 %), this dissimilarity increases when the comparison is made between TB and the rest of the stations included in IB (29.04 %); this is in agreement with the NMDS analysis (Figure 8) that related ST7 and ST8 with TB rather than with the stations in their basin."
In section 3.3 of the manuscript, we have added this new paragraph:
"Results of the PERMANOVA analysis on the environmental variables and on diversity on taxa are summarized on the following in Table 5. Interestingly, based on the zooplankton diversity of TB and IB, their difference is more significant when ST7 and ST8 are removed from IB and placed on TB (based on the NMDS cluster, Figure 8), whereas it is not the case when considering environmental variables (see Table below). This suggests that the similarity between st7 and st8 and the TB stations is not linked to the environmental context."

New Table 5. PERMANOVA analysis on the environmental variables and on zooplankton taxa abundances: Pair-wise tests with unrestricted permutation of raw data (number of permutations: 999) for the comparison between the zones. Resemblance worksheets are based on Euclidean distance.

Groups	Environmental variables			Zooplankton taxa abundances		
	t	P(perm)	Unique Perms	t	P(perm)	Unique Perms
PB, AB	3,78	0,044	28	2,08	0,049	28
PB, TB	3,24	0,101	10	2,01	0,094	10
PB, IB	5,65	0,043	21	2,47	0,056	21
AB, TB	1,79	0,014	84	1,65	0,008	84
AB, IB	5,91	0,001	400	1,67	0,004	404
TB, IB	4,59	0,016	56	1,57	0,045	56
TB+st7 and 8, ION ST	1.65	0.159	56	1,90	0,019	56

Comment R1.3D
In addition, two-way ANOVA should be performed to test difference of univariate parameters (abundance, biomass) between size class X ecoregions/basins.

Answer to comment R1.3D
Following the referee's suggestion, differences in the abundance and biomass of the zooplankton between size classes and basins were tested using two-way ANOVA. One-way ANOVA with Scheffé post-hoc analysis was also applied for comparison of the each size class between basins. Prior to analyses, data were log-transformed and tested for homogeneity. Dunnett's test was used in case of non-homogeneity.

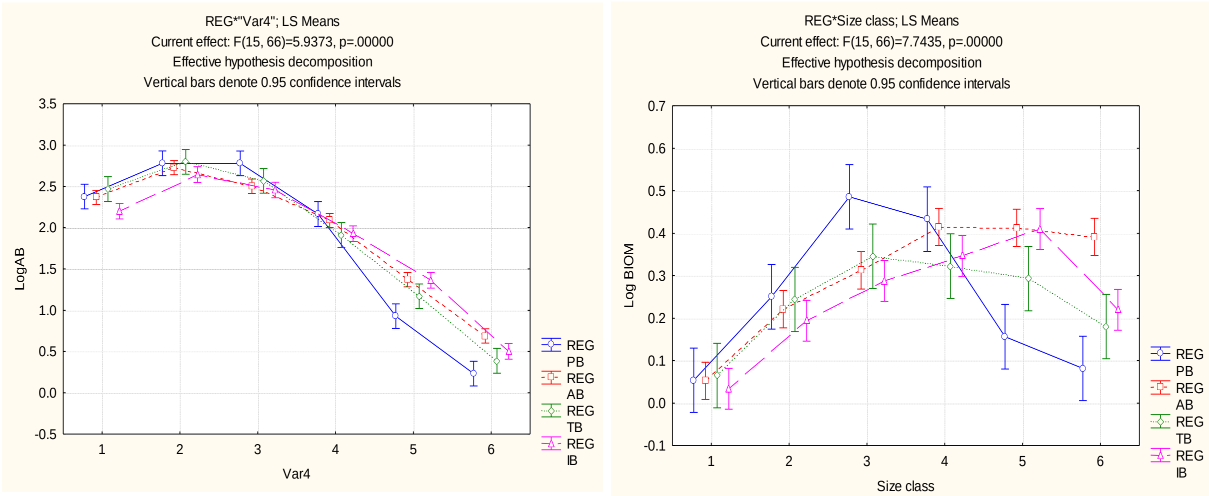
This new paragraph has been added to the manuscript

The two-way ANOVA shows that the PB basin is characterized by significantly lower abundance and biomass in the upper size classes (1000-2000µm and >2000µm) compared to the other areas (p<0.05). One-way ANOVA results show that both total zooplankton and mesozooplankton present significantly higher abundance in PB than in IB, whereas their total biomass was not significantly different between the areas (p>0.05). Significant differences in abundance and biomass between areas were found in the size classes C300-500, C1000-2000 and C>2000 and the biomass of C<200 (P<0.05) (Table 4).

New Table 4: Results of the one-way ANOVAs performed to test differences between areas (PB, AB, IB and TB) in abundance and biomass data for the different zooplankton size classes, for total zooplankton (cumulative of all size classes) and for mesozooplankton (ESD between 200 and 2000 µm) between the areas. Significant p-value <0.05 are marked in bold. ns= non-significant difference. Values of F and p in italic mark where Dunnett's test was used. In the post-hoc analysis homogeneous group with the lowest and highest values are noted with "a" and "b" respectively. PB= Provencal basin, AB= Algerian basin, TB= Tyrrhenian basin, IB= Ionian basin.

	Abundance						Biomass					
			Sheffé post-hoc						Sheffé post-hoc			
Size class	f	P	PB	AB	TB	IB	F	p	PB	AB	TB	IB
C200	3.19	0.067	*ns*	*ns*	*ns*	*ns*	3.64	**0.048**	a	a	a	a
C200-300	3.46	0.055	*ns*	*ns*	*ns*	*ns*	2.55	0.109	*ns*	*ns*	*ns*	*ns*
C300-500	4.4	**0.029**	b	ab	ab	a	5.03	**0.020**	b	a	ab	a
C500-1000	*3.01*	*0.076*	*ns*	*ns*	*ns*	*ns*	1.75	0.214	*ns*	*ns*	*ns*	*ns*
C1000-2000	14.77	**0.000**	a	b	ab	b	17.87	**0.000**	a	b	ab	b
C>2000	9.25	**0.002**	a	b	ab	ab	11.63	**0.001**	a	b	a	a
Total	5.51	**0.015**	b	ab	ab	a	*3.2*	*0.066*	*ns*	*ns*	*ns*	*ns*
Total mesozooplankton (200-2000 µm)	5.03	**0.020**	b	ab	ab	a	*1.06*	*0.405*	*ns*	*ns*	*ns*	*ns*

Figure: two-way ANOVA to test difference of abundance (left) and biomass (right) between size class X ecoregions. (This figure is only displayed in this answer but not shown in the revised version of the manuscript)



Minor comments

Comment R1.4
Title Add “deposition” to the title (“dust deposition events”). ☑

Answer to comment R1.4
We agree to change the title following the referee's suggestion and according to the new results in the manuscript .
We propose this new title: "Structure and functioning of epipelagic mesozooplankton and response to dust deposition events during the spring PEACETIME cruise in the Mediterranean Sea."

Comment R1.5
Abstract L27: it is hard to understand the meaning of “long station”. I suggest changing to “long-duration sampling station”.☑

Answer to comment R1.5
We agree to change the term 'long station' to “long-duration sampling station" following the referee's suggestion.
We have rewritten these sentences as follows:

L27 "Whereas in the Algerian basin (long-duration station FAST)"
L70 "the short-duration stations ST1 to ST9, and the long-duration station TYR"
L71 "whereas two long-duration stations ION and FAST, lasting 3 and 5 days respectively"
L86 "night tows were also performed for the long-duration stations FAST and ION"
L225 At the long-duration stations FAST and ION, strong variations in slope

Comment R1.6
Introduction L41: For P limitation the following paper may be cited:
Thingstad, T.F. and Rassoulzadegan, F., 1995. Nutrient limitations, microbial food webs and 'biological C-pumps': suggested interactions in a P-limited Mediterranean. Marine Ecology Progress Series, pp.299-306.

Answer to comment R1.6
We agree to add the citation following the referee's suggestion, considering that this is a specific paper rather than a review.

Comment R1.7
Materials and Methods L85: state whether the Bongo net was towed vertically or in oblique mode

Answer to comment R1.7
We agree to specify how the net was towed following the referee's suggestion.
We have rewritten this sentence as follows: "The Bongo frame was vertically towed from 300 m depth to the surface at a constant speed of $1 ms^{-1}$".

Comment R1.8
Materials and Methods L83-85: was flow meter used to quantify the filtered volume? Or was it calculated based on net dimensions?

Answer to comment R1.8

We agree to specify how we quantify the filtered volume, following the referee's suggestion. The volume was calculated based on the net ring diameter and the length of the towed cable.
We have rewritten this sentence as follows:
"Sample volume was estimated based on the ring diameter and the towed cable length".

Comment R1.9
Materials and Methods L83-87: clarify why was the entire epipelagic depth strata (0-300m) chosen for sampling. It can be hypothesized that dust deposition will mostly affect the uppermost strata that is more affected by atmospheric deposition, thus sampling all along 0-300m may mask such possible effect. Nevertheless, diel migration and fecal pellet deposition may affect the whole epipelagic community.

Answer to comment R1.9: As the other referee raised a similar question (see comment R2.6), this answer is common to both.

During the PEACETIME cruise, there were no zooplankton specialists on board due to the high pressure of other tasks (Atmospheric and oceanic sampling, on-board mesocosm studies).
The zooplankton net sampling strategy had to be defined before the cruise and the time devoted to zooplankton sampling was short, only for one tow between CTD casts.
Under these conditions, the best compromise based on previous studies (See table 3 in the manuscript) in these regions was to sample in the epipelagic water column. This depth was chosen to be sure that the zooplankton community in the epipelagic layer was collected.
Considering that bad meteorological conditions could affect the verticality of the net, using a 200 m cable length could not sample the whole epipelagic layer.
Also note that the observed impact on zooplankton is more significant because it integrates the whole water column.

Comment R1.10
Materials and Methods L89: which model of FlowCAM was used? Were the flow cells of type FOV (field of view)? Non-FOV flow cells are not efficient for quantitative measurements, and are best used for qualitative-only assessment of plankton as the transport of particles via the flow cell is not constrained (see Detmer et al 2019).
Detmer, T.M., Broadway, K.J., Potter, C.G., Collins, S.F., Parkos, J.J. and Wahl, D.H., 2019. Comparison of microscopy to a semi-automated method (FlowCAM®) for characterization of individual-, population-, and community-level measurements of zooplankton. Hydrobiologia, 838(1), pp.99-110.

Answer to comment R1.10
We agree to add the FlowCAM model to the manuscript and to specify that we use a FOV flow cell. We consider that it is a necessary addition to the manuscript.

We propose these new sentences:
-"The samples were processed using FlowCAM® (Fluid Imaging Technologies Inc. Series VS-IV, Benchtop model)"
-"For the fraction $N_{100F<200}$, a 4X magnification and 300 µm FOV flow cell were used and the analysis was carried out up to 3000 counted particles. For the fraction $N_{100F200-1000}$ a 2X magnification and 800 µm FOV flow cell were used and the analysis was carried out up to 1500 counted particles

Comment R1.11
Materials and Methods L128: detail ZooProcess version, add citation.

Answer to comment R1.11
We agree to change paragraph following the referee's suggestion. We propose this new sentence:
"After scanning, the images were processed with ZooProcess (version 7.32) using the image analysis software Image J (Grosjean et al.,2004; Gorsky et al., 2010)"

Comment R1.12
Materials and Methods L128-129: which software was used for automatic classification of vignettes?

Answer to comment R1.12
We agree to mention the software used for the vignettes classification, and we propose this new sentence: "the Plankton Identifier software (http://www.obs-vlfr.fr/~gaspari/Plankton_Identifier /index.php, last access: November 2019) was used for automatic classification of zooplankton".

Comment R1.13
Materials and Methods L135: ESD size categories 0.2 to 2.0 µm does not make sense (likely mm). Also, results show also 0-100 _m and 100-200 µm categories (Fig. 2).

Answer to comment R1.13
We have noted the typing mistake in the sentence: µm will be changed to mm. Thesentence is rewritten as: "The data were classified in size categories of 0.1 mm of ESD from 0.2 to 2.0 mm".

Comment R1.14
Materials and Methods L155-156: shortly describe the model for assessment of oxygen consumption, and excretion of NH3 and PO4

Answer to comment R1.14: As the other referee raised a similar question (see comment R.2.28), this answer is common to both.

We agree to better describe the model used following the referee's suggestion. To do that, we now show the explanatory equation:
We propose this new paragraph:

"Ammonium and phosphorus excretion and oxygen consumption rates were estimated using the multiple regression model by Ikeda et al. (1985) with carbon body weight and temperature as independent variables.

$$\ln Y = a_0 + a_1 \ln X_1 + a_2 X_2$$

Where ln$Y$ represent the ammonium excretion, phosphorus excretion or oxygen consumption. α_0, α_1 and α_2 are constant (see Ikeda et al. 1985), X_1 is the body mass (dry weight, carbon, nitrogen or phosphorus weight) and X_2 is the habitat temperature (°C)".

Comment R1.15
Materials and Methods L164: add citation for PRIMER software.

Answer to comment R1.15
We agree that this citation is necessary.
The added reference will be: "Anderson M.J., Gorley R.N. & Clarke K.R. 2008. PERMANOVA+ for PRIMER: Guide to Software and Statistical Methods. PRIMER-E: Plymouth, UK."

Comment R1.16A
Materials and Methods L161-177: multivariate analysis was done separately for environmental data (PCA) and biological data (nMDS). These datasets should be correlated using BIOENV/BVSTEP or RELATE procedures.
Comment R1.16B
In addition, PERMANOVA can be used to statistically test the differences between basins.
Comment R1.16C
Also, the contribution of certain species for the dis/similarities between stations and basins should be statistically explored using SIMPER.

Answer to comment R1.16A: See answer to major comment R1.3B
Answer to comment R1.16B: See answer to major comment R1.3C
Answer to comment R1.16C: See answer to major comment R1.3C

Comment R1.17
Materials and Methods L168-169: RFD analysis here is only descriptive.
To test differences between RFDs, RAD analysis (max rank normalization method) can be used: Saeedghalati, M., Farahpour, F., Budeus, B., Lange, A., Westendorf, A.M., Seifert, M., Küppers, R. and Hoffmann, D., 2017. Quantitative comparison of abundance structures of generalized communities: from B-cell receptor repertoires to microbiomes. PLoS computational biology, 13(1), p.e1005362.

Answer to comment R1.17
We agree that our previous description was mainly descriptive and following the referee's suggestion, we propose this new text and figures on the RFD comparison to be added in the manuscript.

In section 2.6 of the manuscript, we have added this new paragraph:
"In order to improve the interpretation of the RFDs, first we used a method derived from Saeedghalati et al. (2017) based on the ordination of normalized rank abundance distribution. Rank-abundance matrix was created with the data standardized by the total abundance. Resemblance was

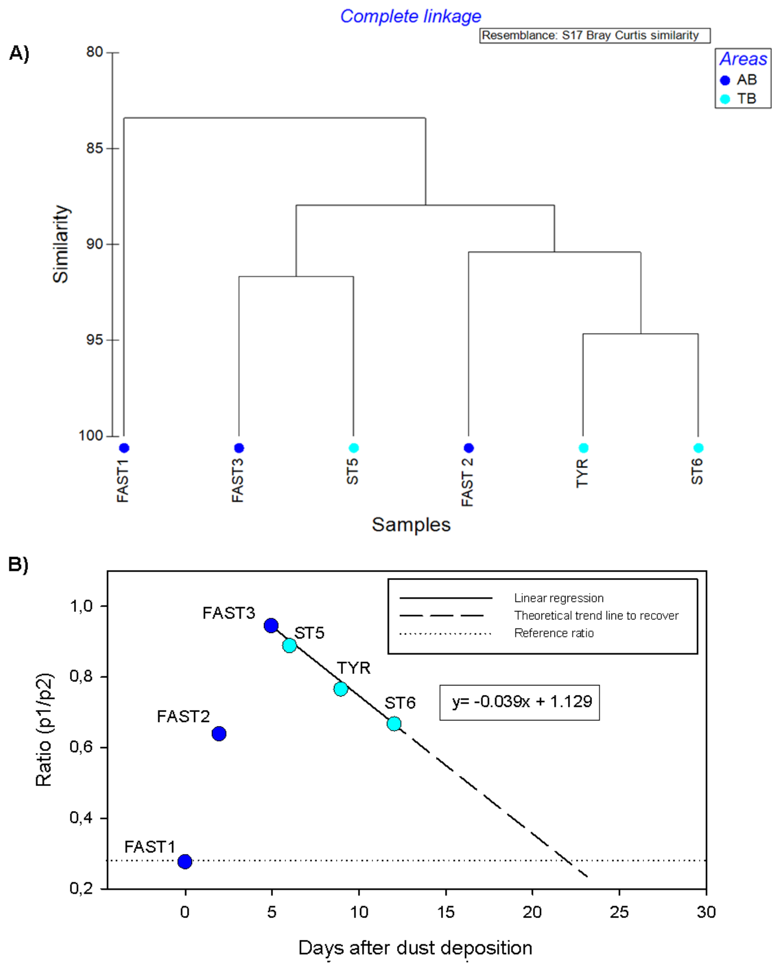
measured with Bray-Curtis similarity and a cluster was created using the complete linkage criterion. Secondly, a rank abundance distribution index was estimated following Mouillot and Lepretre (2000). The RFD for each station was separated into three portions: first the ranks with relative abundance <0.5 % were discarded (rare taxa, between 0 and 30% of the taxa according to all stations; by taking <1% we would discard between 18 and 49% of the taxa) and then the two parts were fitted with a linear regressions. One part with 4 highest ranks (see Mouillot and Lepretre for the justification) and the remaining portion with the following ranks (between 15 and 23 taxa, depending on the station). The slope for both upper and lower RFD portion was calculated (p1 and p2 respectively), then the p1/p2 ratios were estimated to quantify the differences between the RFDs of all the stations."

In section 4.4 of the manuscript, we have added this new paragraph:

The cluster analysis on the RFDs (Figure 11A) is in agreement with this succession of the time series (Figure 10F) by grouping the stations according to impact level of the wet dust deposition. It separates the initial condition (FAST1) from the most disturbed state (stations FAST3 and ST6) and identifies a transition phase before (FAST2) and after (TYR and ST6) the peak disturbance.

The changing trends in p1/p2 ratios (Figure 11B) show an interesting development, with a sharp increase until day 5 after the dust deposition and a progressive decrease towards the end of the virtual time series. The linear regression suggests that the community structure will deliver a p1/p2 ratio value similar to the initial value of the time series after 22 days. Is interesting to note that this delay corresponds to an average generation time of zooplankton organisms for this region.

New figure 11: Cluster analysis on rank frequency diagrams (A) and changing trends in the p1/p2 ratio (B) on the stations impacted by wet dust deposition.



In the revised version of the manuscript, we have focused only on the information concerning the virtual time series (see paragraph 3.4 Zooplankton community changes linked to dust deposition events during the PEACETIME survey and figures 10 and 11). But below we present the whole analysis available for the referee and any reader of the comments.

Figure: Rank frequency diagram for all stations of the PEACETIME cruise. Ac: Acartia spp.; Cal: Calanoid copepods; Cala: Calanus spp.; Cent: Centropages spp.; Cor: Corycaeus spp.; Euc: Eucalanus spp.; Halop: Haloptilusspp; Luci: Lucicutia spp.; Mecy: Mecynocera spp.; On: Oncaea spp.; Ot: Oithona spp.; P/Cla: Para/Clausocalanus spp.; Pleu: Pleuromamma spp.; Pont:Pontellidae; Tem: Temora spp.; App: Appendicularia; Cha: Chaetognatha; Dec: Decapods; Hydro: Hydrozoans; Ich: Ichtyoplankton; Ost: Ostracods; Poly: Polychaeta; Pte: Pteropods; Siph: Siphonophores; Thal: Thaliaceans. (This figure is only displayed in this answer but not shown in the revised version of the manuscript)

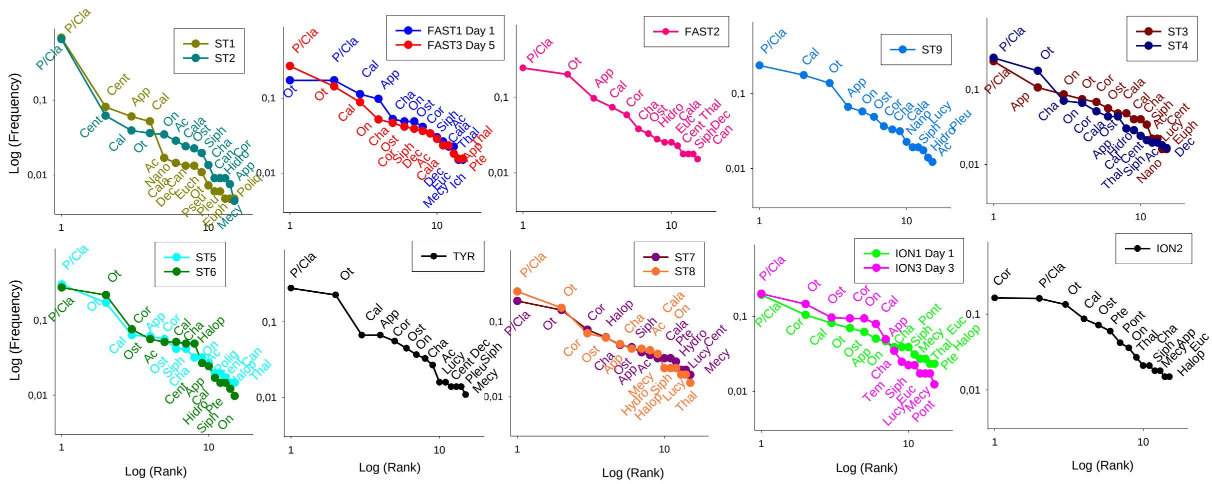


Figure: Cluster analysis on rank frequency diagrams of all the stations in the PEACETIME survey. (This figure is only displayed in this answer but not shown in the revised version of the manuscript)

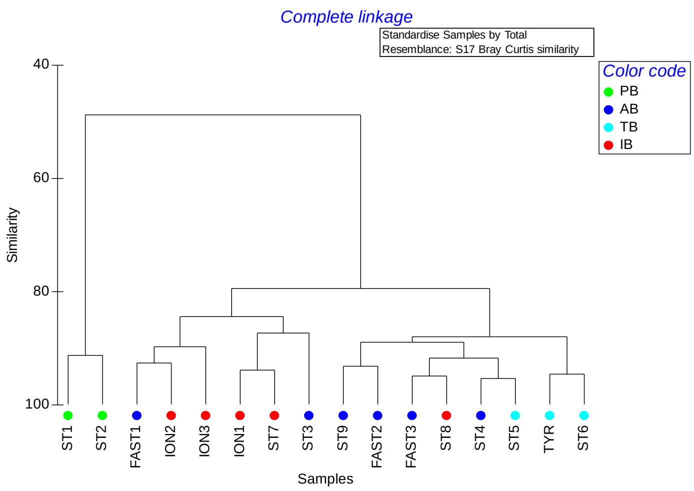
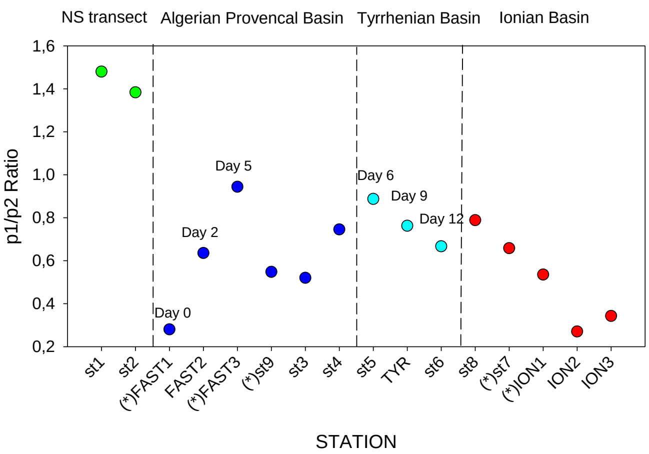


Figure: Changing trends in the p1/p2 ratio of all the stations in the PEACETIME survey. (This figure is only displayed in this answer but not shown in the revised version of the manuscript)



Comment R1.18
L168-171: it would be more convenient to summarize all Spearman rank correlations and t-tests in two tables. I could not find t-test results except one testing the difference between N100 and N200 mesh (L107).

Answer to comment R1.18
Following the referee suggestion we propose this new table to be added on the manuscript.

New Table 3. Summary table of Spearman rank correlations. T°= temperature; Sal= salinity; Chl-a= Chlorophyll; MLD= Mix layer depth; pp= primary production. Bold characters indicate significant Rs value (p<0.05)

	Correlation coeff.					
Abundance	*T°*	*Sal*	*Chl-a*	*DO*	*MLD*	*PP*
$C_{<200}$	-0,49	-0,43	0,32	**-0,61**	-0,16	-0,37
$C_{200-300}$	**-0,58**	-0,37	0,48	**-0,58**	0,08	-0,24
$C_{300-500}$	-0,51	-0,19	**0,52**	-0,45	0,21	-2,28
$C_{500-1000}$	**-0,56**	-0,50	0,23	-0,49	-0,06	0,05
$C_{1000-2000}$	0,29	0,01	-0,28	0,33	-0,34	0,35
$C_{>2000}$	**-0,12**	**-0,53**	-0,15	0,08	-0,50	-0,16
Total abundance	**-0,67**	-0,44	**0,56**	**-0,68**	0,08	-0,28
Biomass						
$C_{<200}$	**-0,61**	-0,48	0,42	**-0,71**	-0,08	-0,36
$C_{200-300}$	**-0,52**	-0,29	0,52	-0,51	0,12	-0,14
$C_{300-500}$	-0,49	-0,18	**-0,53**	-0,46	0,19	-0,27
$C_{500-1000}$	-0,45	-0,43	0,17	-0,41	-0,11	0,14
$C_{1000-2000}$	0,24	-0,05	-0,37	0,32	-0,39	0,30
$C_{>2000}$	-0,18	**-0,61**	-0,10	-0,02	**-0,53**	-0,10
total biomass	**-0,58**	**-0,62**	0,24	-0,43	-0,27	0,08

T-test was changed for ANOVA and the table is now included. See answer to comment R1.3D

Comment R1.19
Results L176-178: "mostly influenced by: how was this determined? Visually? This can be tested statistically using correlation between the environmental factors and the 1st axis (or also the 2nd axis) of the PCA.

Answer to comment R1.19
We agree with the referee's suggestion, and have performed a correlation analysis between the environmental variables and the scores of the stations on the two axes of the PCA. We propose the following sentence.
"The first axis (62 % of the variance) is mostly influenced by temperature and dissolved oxygen, as shown by their high correlations with the scores of the sampling points on this axis (r= 0.95 with p=0.000 and r=0.92 with p=0.000, respectively), whereas the second axis (28.3 %) is mostly influenced by MLD (r=-0.75, p=0.01), salinity (r=-0.75, p=0.001) and Chl-a (r=-0.57, p=0.022)."

Table. Correlations between environmental variables and the axes of the PCA. (This table is only displayed in this answer but not shown in the revised version of the manuscript)

	X1	X2
Temperature	.9564	-.2174
	p=.000	p=.419
Salinity	.6294	-.7476
	p=.009	p=.001
Dissolved Oxigen	.9213	-.1154
	p=.000	p=.670
Chlorophyll-a	-.7572	-.5680
	p=.001	p=.022
MLD	-.3880	-.7460
	p=.137	p=.001

Comment R1.20
Results L179-184: also here seems that non-statistical assessment was done to relate biological data to environmental parameters. See above comment on the use of statistical procedures (BIOENV, RELATE, PERMANOVA).

Answer to comment R1.20: See answer to Comment R1.3C

Results Comment R1.21
L186-L261 and throughout the text: abundance of zooplankton is presented in ind.m-2 instead of ind.m-3. Similarly biomass, mgDW.m-2 instead of mgDW.m-3.

Answer to comment R1.21
Throughout the text, the data was presented in m^{-2}, because we estimate metabolic rates in the integrated water column. In the comparative table (New Table 7), the data was presented in m^{-3} to be able to compare them with published data.

Comment R1.22
Results L188-198: no statistics! See above comment re ANOVA.

Answer to comment R1.22: See answer to comment R1.3D

Comment R1.23
Results L199: "C300-500 biomass is positively correlated with Chl-a (r=-0.52, p=0.042)". r is negative so it is negatively and not positively correlated.

Answer to comment R1.23
Following the referee's suggestion, we noted the typing error. We propose this new sentence:
"$C_{300-500}$ biomass is negatively correlated with Chl-a (r=-0.52, p= 0.042)".

Comment R1.24
Results L211-223: missing statistics – see comment above re SIMPER.

Answer to comment R1.24: See Answer comment R1.3C

Comment R1.25
Results L229: change sub-header to "zooplankton community changes at long-duration sampling stations".

Answer to comment R1.25 : In the new version of the manuscript, we have changed the name of this section to:
3.4 Zooplankton community changes linked to dust deposition events during the PEACETIME survey

Comment R1.26
Discussion L265-278: the methodological concerns are presented as a major outcome of this study although they are mostly a by-product. Nevertheless, they provide important conclusions. I would reorder the sections, and put this as the second or third section.

Answer to comment R1.26
We agree that this methodological aspect is a by-product of the paper, but we think that it is better for the reader to be aware early in the Discussion of the argumentation about the adequacy (and limitations) of our sampling and analytical strategy. Consequently we prefer to keep this paragraph at the beginning of the Discussion section.

Comment R1.27
Discussion L293-310: the authors compare the zooplankton abundances and biomass measured in this study (372000 ind m-3 and 1707 mgDW m-3 respectively reported in L186-187 and in figure 4) to other studies (table 3). Unlike their statement that the results of this study are in the same order of magnitude as previous studies – it seems that the changes are enormous! For example, Donoso et al 2017 measured 608 ind m-3 and 64 mgDW m-3. This is a change of 3 orders of magnitude in abundance and 2 in biomass! Note that the reported values are not the same in figure 4 and table 3. Please explain these differences.

Answer to comment R1.27
Throughout the text, all estimations (abundance, biomass and metabolic rates) are in m^{-2} to express those values as integrated in the water column. In table 3 the abundance and biomass from other

papers were expressed in m^{-3} in order to use the same values as they appear in the respective publications.
Tables 3 and Figure 4 had the same values, the differences are in the unit and also because in table 3 the abundances and biomass were expressed as mean values ± Standard Deviation of the respective basin

Comment R1.28
Discussion L293-310: additional comparison should be performed to Mediterranean studies that used 100 _m mesh in addition to a larger mesh size and measured abundance and biomass. For example: Koppelmann, R., Böttger-Schnack, R., Möbius, J. and Weikert, H., 2009. Trophic relationships of zooplankton in the eastern Mediterranean based on stable isotope measurements. Journal of Plankton Research, 31(6), pp.669-686.

Answer comment R1.28
All the papers cited for comparison use samples from areas close to the PEACETIME track, there are more studies in Mediterranean Sea that use 100 µm nets but since they were sampled in areas that are far away, those data are not comparable with our study

Comment R1.29
Discussion L389-410: phytoplankton biomass and production assessment were calculated from Chl-a rather than being measured directly. Similarly, zooplankton carbon demand, oxygen consumption and excretion was based on multiple assumptions, including the use of constant biomass to carbon conversion factor, carbon to Chl-a ratio, Redfield ratio and respiratory quotient.
While these are all legitimate methods, it must be discussed that over large geographical scales that include environmental gradient – these factors may vary and thus these assessments may be inaccurate.
For example, Minutoli Guglielmo 2009 showed an increasing trend in ETS activity from west to east: Minutoli, R. and Guglielmo, L., 2009. Zooplankton respiratory Electron Transport System (ETS) activity in the Mediterranean Sea: spatial and diel variability. Marine Ecology Progress Series, 381, pp.199-211.

Answer to comment R1.29

We agree with the referee's comment and we propose to add this new sentence in section 4.3:

"By using allometric relationships relating zooplankton grazing and metabolic rates to size structure, zooplankton impacts (top-down vs. bottom-up) on primary production have been investigated. We are aware that using constant conversion factors may limit the analysis of the spatial variation, since these factors may display temporal and geographical variations (Minutoli and Guglielmo, 2009). However, our sampling strategy based on a limited number of stations sampled did not enable us to consider temporal and spatial variations accurately and our main goal was to have rough estimations of the epipelagic zooplankton mediated fluxes at the scale of the PEACETIME cruise."

comment R1.30
Figures and Tables. Fig. 2: in the Zooscan and FlowCAM analyses, there is a small overlap in the fractions 300-400 and 400-500. Nevertheless, it seems that the combined data (fig 2c) include these fractions from the flowCAM only. Shouldn't these fractions be accumulative?

Answer to comment R1.30:

Classes 300-400 and 400-500 µm should not be cumulative because it is the same part of the sample analyzed twice. If we accumulate them, we will count the same size class twice.

Comment R1.31
Figures and Tables. Fig. 3: show the % of total variance in the labels of each of the axis (PCs). If possible overly the environmental vectors (fig 3B) on the PCA (fig 3A) instead of showing them in two different images.

Answer comment R1.31:
We agree and the correction will be done in the figure.

Comment R1.32
Figures and Tables. Fig. 4: correct Chla-a to Chl-a

Answer comment R1.32:
We agree and the correction will be done in the figure.

Comment R1.33
Figures and Tables. Table 3: is biomass presented in all mentioned studies as dry weight?

Answer to comment R1.33: Throughout the manuscript, we use the term dry weight, but in table 3 one of the cited papers publishes the data in wet weight.
*In table 3, all values are expressed in dry weight except one marked with ***. We will mention this in the legend of the table in the revised version.*

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