bg-2020-126 General comments

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 larger contribution of small size fraction (<500 µm) 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 an 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. 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.

Other concerns relate to the lack of hypothesis setting, sampling justification, and statistical analyses. Also, this paper would benefit from some closer proof reading. It includes misuse of wording that can be easily corrected. It may be useful to engage a professional English language editor.

Nevertheless, the presented study is unique and scientifically worthwhile. I therefore suggest accepting this manuscript after major revision, pending correction of all major issues.

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. Guiro) 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).

[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. Such hypotheses could be bottom-up enhancement of zooplankton communities triggered by dust deposition, west to east gradient, etc. 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. The contributions of significant taxa to specific stations/basins could be tested using SIMPER procedure. In addition, two-way ANOVA should be performed to test difference of univariate parameters (abundance, biomass) between size class X ecoregions/basins.

Minor comments

Title

Add “deposition” to the title (“dust deposition events”).

Abstract

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

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.

Materials and Methods

L85: state whether the Bongo net was towed vertically or in oblique mode.

L83-85: was flow meter used to quantify the filtered volume? Or was it calculated based on net dimensions?

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.

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.

L128: detail ZooProcess version, add citation.

L128-129: which software was used for automatic classification of vignettes?

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

L155-156: shortly describe the model for assessment of oxygen consumption, and excretion of NH3 and PO4.

L164: add citation for PRIMER software.

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. In addition, PERMANOVA can be used to statistically test the differences between basins. Also, the contribution of certain species for the dis/similarities between stations and basins should be statistically explored using SIMPER.

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.

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).

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.

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).

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}.

L188-198: no statistics! See above comment re ANOVA.

L199: “$C_{300-500}$ biomass is positively correlated with Chl-a (r=-0.54, p=0.024)”. r is negative so it is negatively and not positively correlated.

L211-223: missing statistics – see comment above re SIMPER.

L229: change sub-header to “zooplankton community changes at long-duration sampling stations”.

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.

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.

L293-310: additional comparison should be performed to Mediterranean studies that used 100 µm 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.

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 assessment 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.

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, seems that the combined data (fig 2c) include these fractions from the flowCAM only. Shouldn't these fractions be accumulative?

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.

Fig. 4: correct Chla-a to Chl-a

Table 3: is biomass presented in all mentioned studies as dry weight?