

Interactive comment on “Metazooplankton diversity, community structure and spatial distribution across the Mediterranean Sea in summer: evidence of ecoregions” by A. Nowaczyk et al.

Anonymous Referee #2

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Metazooplankton diversity, community structure and spatial distribution across the Mediterranean Sea in summer: evidence of ecoregions

by A.Nowaczyk et al.

General comments

This manuscript is focused on the results of zooplankton collection during the BOUM trans-Mediterranean survey conducted in June-July 2008. Data of abundance,

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biomass, and taxonomic composition of epipelagic metazooplankton are presented to depict the distribution of communities at 17 stations located along a 3000 km transect thought the western and eastern basins. This zooplankton dataset, acquired with integrated vertical tows in the upper 200 m layer and with two complementary analytical approaches (microscope and ZooScan-imaging) provides one of the very few synoptic overview of zooplankton distribution available for the open Mediterranean Sea. Moreover, the sampling was performed with a fine mesh net (Bongo, 120 μm), allowing the study of the small zooplankton compartment that is still rarely investigated. This is one of the most interesting aspects of this work, which contributes to widening the view of zooplankton in such an interesting oligotrophic environment. The paper does not present real novel ideas but, based on zooplankton communities, it supports the concept of Mediterranean regionalization that has been proposed by D'Ortenzio and Ribera d'Alcalà (2009) based on satellite images of chlorophyll-a, and also by Bianchi et al based mainly on benthos. Even if substantial conclusions are not reached yet on this crucial topic, this work contributes with new and good data. In my opinion, this work is worth to be published. However, the manuscript suffers of various problems and needs to be deeply revised before publication. The title needs to be changed. Methods need to be integrated and clarified. Introduction should present more clearly the goals of zooplankton sampling in the context of BOUM and the aims of the present work. Results need to be improved (see my specific comments below). Discussion is too fragmented and difficult to follow; it needs to be structured more fluently and at the same time to be further developed in depth. The English should be slightly improved and revised.

Specific comments

The title does not clearly reflect the contents of the paper and I suggest the following change: "Epipelagic metazooplankton across the Mediterranean Sea in summer: evidence of ecoregions". Indeed, only the upper 200 m layer has been investigated and this should be made clear in the title. Moreover, diversity should be removed because

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it has not been adequately analyzed (mainly identification at genus (not species) level, and no diversity indexes).

Introduction - This section is quite well organized, but I suggest to reduce the part regarding the ecoregions, which should be moved and expanded in Discussion. - At the end, the aims should be better defined in relation to clear ecological problems and not as simple descriptions of the content. - Moreover, this work on zooplankton distribution should be more clearly related to the aims of the BOUM project because the connection is not so clear now. Which were the precise goals for collecting and examining zooplankton during BOUM? I guess this manuscript presents only the structural part of those studies and it would be nice to have a quick but clear idea of the whole zooplankton investigation (structural and functional?). - - The novelty of this survey with respect to previous zooplankton studies should be also clarified. - I suggest to change the last sentence to better express this last important aim and substitute the verb “define” with “verify”.

Pg 3082, L22- complex marine environments: why complex ? explain

L22- change : ..and hot spots for marine biodiversity

L23- change: The Mediterranean marine biota. . .

L25,26- change: geological history of the basin (Furnestin, 1968. . . . What reported in brackets does not provide clear information unless it is explained more extensively, so I suggest to remove or extend it.

Pg. 3083, L1-13- Move to Discussion (see note above)

L5- change: Successive studies provided evidence. . .

L6-10- This part should be written in a better style, simplified and improved by adding information on which compartment of marine biodiversity was considered by Bianchi.

L8- change: More recently, the Strait of Messina was considered a separate biogeo-

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graphic region because of local endemism.... Say in which compartment the endemism was recorded.

L15- change: deficiency that reaches

L17- change: results in low phytoplankton biomass

L21- Delete: In addition

L21- change: emerged also from a survey from the Sicily Channel to the Levantine basin.

Pg 3084, L 1- change: ...such structures are represented by large river plumes.

L13- change: ..characteristics of plankton communities and the whole.

L18: the statement “considering scales from the single process to the whole basin” is unclear and it should be changed. What process? In any case, a process is not a scale.

L19-24: this last part should be rewritten (see note above).

Materials and methods -This section lacks some pieces of information that must be added to make all procedures clear and eliminate ambiguities. -I recommend to indicate clearly in Figure 1 where the Authors place the limit between the western and eastern basins, which are mentioned quite often in the text but are not clearly defined. - “Microscope” must replace “microscopic” throughout the manuscript (Tables and Figures included). -It is stated that, for the counts at the microscope, taxonomic identification was done at species level, sex and developmental stages when possible. From the author’s expertise, I would have expected to read more about species. In contrast, most (if not only) genera are presented in Results. -The procedure of subsampling and counting at the dissecting microscope and at the Zooscan are not clear at all. Were the respective subsamples taken in parallel or in succession? Was the Zooscan subsample taken after the one of the microscope was put back in the original

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sample? Was the count made on the same subsample for microscope first and then Zooscan? All these details are not trivial because the information they hold indicates how the counts were representative of the original sample and how the two methods could be compared. I recommend explaining these procedures very clearly and exhaustively. -Regarding the cluster analysis (2.3), I understand that it was performed separately on total zooplankton community (74 taxa) and on copepods (54). If I have correctly understood (the 54 copepod taxa were included in the 74 zoo taxa), I question this choice. Copepods are the dominant part of zooplankton and obviously influence the results of total zooplankton clustering. So, the two dendrograms in Fig. 4 appear redundant. -Zooplankton abundance and biomass were related to numerous physical, chemical and biological parameters (phytoplankton, ciliates), but for these non-zoo parameters, none information is given about how they were collected/analysed. This part must be added with synthetic but clear information, referring to other contributions from the same cruise, if necessary.

2.1- change title: Cruise track and zooplankton sampling

L4- add the nationality of the RV

L4- ...Atalante (Fig. 1)

L8- delete (Fig.1). Not clear if sampling strategy is only for zooplankton or also CTD, or everything.

L14- change:...and equipped with filtering. ...

L19- change:...collection one sample was preserved. ... Say how it was calculated the volume of filtered sea water for each sample.

L22- change (no new paragr.): the other sample was kept fresh and split in two parts with a Motoda splitter for later taxonomic identification and abundance.

L23- change: immediately concentrated onto GF/F. How was it concentrated? Was it stored in liquid nitrogen? This sample disappears from this manuscript. I guess the

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data of gut content will be presented in another manuscript, but this should be at least mentioned here.

L27- change: ...and small copepods from water samples collected with the CTD/rosette. At each selected depth, the content of a 12 L Niskin bottle was gently collected on a 20 μm mesh net and fixed in Why to use Lugol as fixative for small copepods and nauplii instead of formol? Was the same sample examined also for protozooplankton? This should be mentioned here.

Pg 3086, L8- add: ..dried in the oven

L10,11- change: ..of the filter and referred to the unit volume (mg DW m⁻³). In addition... Was the mass spectrometer used onboard? Say clearly.

L15- change: Microscope counts.

L16- change: In the laboratory on land, taxonomic identification and counts of zooplankters in the Bongo samples were made. . .

L17- change: . . .species were counted in sub-samples (. . .) and the whole sample was examined for rare species .. What does it mean “larger organisms”? Which size? Were they counted only as rare species? I guess this was not the case, so this part must be rephrased.

L21- This section seems mainly focused on copepods. What about the other groups? Were they all also identified at species level?

L24-delete: Special care was taken to separate. Change: Organisms were carefully separated. . . .

Pg 3087, L11: define “NB-SS” the first time you mention it.

L12,13- the biovolume was converted into wet weight. How was it compared with the dry weight measured as in 2.2.1?

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L18- change: ..variation of zooplankton abundance. State clearly if the statistical analysis was done on data from the counts at the microscope or/and Zooscan.

L22- For such a small number of samples (17), it seems to me that 10% of occurrence as a limit for considering rare specie is quite high. Is there any particular statistical reason for this choice? Please explain in the text.

L23- It must be clearly defined how the “indicator species” were identified, which criterion or computation was applied? Knowing that SIMPER routine was used can be informative only to PRIMER users.

Pg 3088, L2: PON, POP, N/P are better defined as biogeochemical parameters than biological parameters.

Results -The variability within each area (and not only among areas) should be addressed -Copepod nauplii are not efficiently collected by 180 μm mesh net and they should be removed from data of the Bongo samples and from Table 2. Nauplii should be eliminated also for the comparison between the microscope and Zooscan counts (3.6) of the Bongo samples and this exclusion should be clearly mentioned in M&M and in Results. -The record of *Cosmocalanus darwini* if the first one for this species in the Mediteranean and should be presented in Results, not only in Discussion. -As in my note at M&M, I suggest to present only clustering for zooplankton and not copepods (obviously included in the former). -In the Cluster Analysis (3.4) I do not see the results of the indicator species (pg. 3087, L23), which I expect to read in a table. -The day-night variability has been correctly analyzed only at 3 stations located in areas of particular hydrological features (anticyclonic eddies). The results acquired cannot be considered sufficient to represent a generalized pattern for the whole Mediterranean. I am aware of the work done and I can understand the necessity to include these data in the present manuscript, but I would be very cautious in presenting and discussing these results. -*Oncaea* is misspelled (*Oncea* in the ms, tables, figures)

Pg 3088, L11- change: temperature above... and up to 27...

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L17- “trend in oligotrophy”: data of surface chl concentration indicative of this trend should be presented.

L20- say the areas where the min and max values of chl at DCM were measured.

L21- change: Zooplankton abundance and biomass distribution.

L22- repeat here the zoo sampling layer: Zooplankton abundance in the upper 200 m layer estimated from the microscope counts varied. . . .

Pg 3089, L21- change:..of total metazooplankton abundance and were. . .

Pg 3090, L1- change: larvae were

L4- change: The genus *Corycaeus* was

L9- change: the western Mediterranean.

L10- from fig 3, I see that *C.darwinii* was absent in the eastern basins.

L13- change: metazooplankton abundance while.

L15- change: of the eastern basin.

L14- change: at the coastal station (st. 27) There was indeed only 1 coastal station

L17- in the rest of the transect.

L21- change: Stations B and C located in the eddies clustered in a distinct subgroup.

L23- *Ctenocalanus* spp. Why spp? To the best of my knowledge, only *C. vanus* is reported in the Mediterranean. Please, check and report correctly.

Pg. 3091, L3- change: . . .stations accounted for 10%...

L6- “small-sized copepods”: which size range?

L12- change: . . .most stations with the exception of stations 7 and 24.

L13,14- change: of the highest abundance was observed from 25 to 90 m in the west-

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ited number of stations. However, it is not sufficiently developed and convincing, and I recommend to re-work and comment more in depth this issue. - The part on the west-east gradient does not carry any different info with respect to what already mentioned in Introduction (pg. 3083) with similar or same statements. I would expect some effort to explain the observed patterns with thoughts and considerations in relation to the environmental characteristics as emerged from such an integrated cruise. - Comments on *C. darwini* distribution are quite confusing and this issue should be clarified (see note below). - The comparison between the data of nauplii and small-copepod abundance obtained with bottles and nets should be removed from Discussion (pg. 3095, L17-28). It is now quite clear and obvious that nets of 120 or 180 μm mesh are not appropriate for sampling such tiny animals. - I am skeptical about the part on “diel vertical migration”, see above my comments to Results - Some explanations or hypotheses should be presented about the abundant zooplankton at st. 13 e 17 and high biomass at st 7.

Pg. 3093, L10: change: ...transect was. . .

L12,13- among the quoted papers, the recent review by Siokou et al (BGS 2011) on plankton in the open Mediterranean should be mentioned

L14- the paper by Galienne and Robins (2001) on *Oithona* is not specifically on the Mediterranean as the others cited here.

L17-19- this part on *C.darwini* should be moved to Results

L20-25- It would be very interesting to get a more clear picture of *C.darwini* distribution. What is presented here is quite confusing: common in the Red Sea, but abundant in the S-W Med suggesting an Atlantic origin, but never recorded in the Atlantic. These pieces of information and suggestions are quite puzzling.

Pg.3094, L2- change: West-East gradient

L3- change: eastward decrease of zooplankton (not westward!)

L4-7- change: Mediterranean that was also recorded during other trans-Mediterranean

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surveys conducted in June-September (...) and during an autumnal cruise from the Sicily Channel to the Levantine Sea (..).

L7-10- change: In contrast, the biomass distribution...transect. This parameter showed generally...as previously reported (...)

L11- change: Several metazooplankton species showed...Sea, characterizing the western or the eastern basins.

L14-16- change: The relative abundance of these two large-sized copepods might explain the lower NB-SS slope in this region.

L23,24- “Both metazooplankton and copepods” suggests that they are two different entities!

Pg. 3095, L1- change: the eastern Mediterranean Sea... .

L5- change: were responsible of differences in mesozooplankton community structure

L6,7- where are the data of chl decrease and DCM deepening at the eddy stations? They should be shown here in Results or other papers should be quoted.

L12- change: Riandey (not Riandez)

L13- “to promote calanoid copepods”: please explain how they should be promoted: with an increase in numbers, in biomass, in reproductive rates, etc.?

L16- “Comparison of mean values”: this is an odd and useless title

L17- “Mean integrated abundance”: you probably mean “Depth averaged abundance”, please change.

L22-24- this part is quite misleading and I suggest to remove it (see my note above). This study was not designed to make a technical comparison among nets and methods. It does not make much sense to state that zooplankton abundance was higher in the present than in previous cruises, when also seasons and layers were different.

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L28 and pg 3096 L1- “large” and “small” organisms should be defined more precisely in terms of size (mm), also mentioning species for example.

Pg 3096, L15- “> 500 μm ECD” but at 3.7 (pg 3092, L11) it was written > 300 μm : what is the right value?

L15- change explains the day-night differences observed in zooplankton numbers and biomass. . .

L16-19- this part with species should be moved to Results

Pg. 3097, L4- change: in the eastern basin (not western)

L6,7- the statement on nauplii distribution should be moved after the one on “the patchy vertical distribution..”

L11- say where, in which Med basin, Alcaraz had observed this distribution

L12- The role of environmental parameters is very superficially addressed

L15, 16- what should it explain the potential role of temperature? Be more explicit and clear

L23-26- These two statements are repetitions

Pg 3098, L9 pg3099,L3- change: D’Ortenzio (misspelled)

L13, L17,18- again, “metazooplankton” and “copepods” are presented as two separately different communities.

L18- change: eastern (not western!)

L20- say where st 13 is located and try to explain the reasons of this rich zooplankton situation

L22- change: concentration; cells

L23- what is “a marginal effect (boarder between SC and IB”)? Sure there is not a clear

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border between the two regions. Please explain

L24- change: Ribera d'Alcalà (incomplete name)

L26- change: for zooplankton (no comma) abundance, biomass and community structure

Table 2 - Report in the legend the depth layer of reference (0-200 m). Remove nauplii and add the stdev values. Check carefully the spelling, because some names (Oncaea, Euphausiids, Polychaetes) are misspelled.

Figure 1 - Levantine in the map (not Levantin)

Figure 2 – Zooplankton abundance and biomass as dry weightat the stations in five Mediterranean areas. Mean and.

Figure 3 – Macrosetella/Microsetella spp. The panel with A.clausi and A.negligens is not clear to me

Figure 5 – The “station” label on top panel must be indicated. In the legend:within the upper 200 m layer across the.

Figure 6 – Microscope (not microscopic)

Figure 7 – In the legend: indicate the sampling layer; d) is missing and j) does not appear in the figure.

Figure 8- Delete “issued”

Figure 9 – Microscope (not microscopic)

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