

## ***Interactive comment on “Influence of chemosynthetic ecosystems on nematode community structure and biomass in the deep eastern Mediterranean Sea” by N. Lampadariou et al.***

**N. Lampadariou et al.**

nlamp@hcmr.gr

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### **Reply to Anonymous Referee #2**

We would like to thank Reviewer 2 for his/her very positive and constructive comments. We have addressed all the remarks but one (see General Comment No.2) and have amended the manuscript accordingly to include all the suggestions made. Our response to individual comments can be seen below.

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### **General comments**

The reviewer made four general comments which we addressed as follows:

1. The investigated mud volcanoes are the same described by Ritt et al. (2012). By checking the sampling cruise and the coordinate of samples, I guess that the samples investigated in this manuscript come from the same stations of the cited authors (Ritt et al., 2012). If this is the case, the Authors have access to all the chemical characteristics and all the trophic variables for each habitat mentioned in the manuscript. This impressive dataset, and in particular all the environmental parameters relevant for the ecology of the meiofauna, should be adequately taken into account and thoroughly discussed. I also would like to enquire if the Authors have changed the name of the stations from Ritt et al. (2012), and, if so, for what reason.

#### **Reply**

This comment is twofold: (a) take into account the physicochemical parameters published by Ritt et al. (2012) and discuss them in relation to the observed patterns and (b) explain why the station names between this study and the study by Ritt et al. (2012) are different. Our actions for these two comments will be as follows:

- (a) Our aim in the present study was to discuss distribution patterns of meiofauna and nematodes from mud volcanoes. The physicochemical parameters were published in great detail by Ritt et al. (2012) and we feel that a repetition here would be redundant. Nevertheless, in the new version of the manuscript we will use the available environmental data to assess which parameters explain better the observed patterns (e.g. by running correlation analyses). Depending on the output of the analyses we may upload these results as supplementary material and in any case, we will use them

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to discuss thoroughly the relationship between the available parameters and meiofauna.

(b) We used a slightly different naming scheme as we thought that ours was more descriptive and easy to understand. Nevertheless, we understand the concerns of the reviewer and in the new version of the manuscript we will use the exact same naming scheme used by Ritt et al. (2012).

2. The Authors sampled a control station in an area outside the influence of the mud volcano, but only one core was sampled. In my opinion only one core as a control is not acceptable. I understand the difficulties of sampling in the deep-sea, but I am also aware of the environmental variability, and I think that one core is not ecologically representative. I suggest removing this station from the article.

**Reply**

Not accepted: We understand the concerns of the reviewer and fully agree that only one control sample is not sufficient to make meaningful ecological comparisons. However, we prefer to keep this sample in our analysis because it had a high percentage of *Manganonema*, something which we considered quite important and therefore discussed thoroughly. Unfortunately, due to sampling restrictions, we were able to collect only one sample from one control station, whereas the original plan was to use more samples from more control stations. Ritt et al. (2012) studying the macrobenthic communities from the same stations had also only one control sample from the Amsterdam mud volcano, although they were able to collect a control sample from a reference station near the Napoli mud volcano. As we stressed above, for the purposes of our manuscript we prefer to keep the control sample in our analysis, yet we will state very clearly in the new version of the manuscript that this sample can not be regarded as a typical control sample, thus caution should be taken with regard to inferences.

3. The vertical distribution data of nematodes are not strong enough to support the  
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sentence of the Authors saying that nematodes displayed deeper penetration in the seep areas. The profiles on display are, in the majority of the cases, “normal” nematode deep-sea vertical profiles, with only a sub-superficial peak in the summit location: also in this case, however, the inversion is not marked). I suggest removing this sentence from the abstract and the discussion.

**Reply**

Accepted: This comment was also made by Reviewer 1 who had strong reservations about our strong statements on this matter. As we agree with both reviewers we have taken a number of actions (see reply to General Comments No.2 of our reply to Reviewer 1), amongst with also to remove the sentence from the Abstract and the Discussion as Reviewer 2 suggests.

4. The Authors use the words “habitats” and “microhabitats” as a synonymous. For example in paragraph 2.1 they wrote “In total, ten different microhabitats were sampled five from the Amsterdam and four from the Napoli mud volcano . . .” and few lines below: “The five habitats from the Amsterdam mud volcano were:..”. I invite the Authors to firstly define the term “habitat” and “microhabitat” (is there a spatial differentiation? ), and then to check carefully all the text in order not to confuse readers.

**Reply**

Accepted: The Reviewer is right since we used the two terms as synonyms. We will correct the problem by using exclusively the term microhabitats, which was also used by Ritt et al. (2012).

**Specific comments**

The reviewer made also a substantial number of more specific and technical comments which we addressed them all as follows:

1. Abstract The abstract provide a concise and complete summary. However, the vertical distribution data of nematodes are not strong enough to support the conclusion made by the Authors. I would suggest removing this part. Please see general comments

**Reply**

Accepted: This comment is also related to comment No. 2 of the General Comments section (see our answer above). In brief, amongst other actions we will remove this sentence from the Abstract.

2. Introduction P18133 line 1 The Authors define cold seeps as “extreme environments occurring in a wide variety of geological settings along both active and passive margins”. This definition is too generic, cold seeps differ from other extreme environments because the emission of gases from the seafloor is typically not associated with a significant temperature rise.

**Reply**

Accepted: We will refine our definition in the new version of the manuscript according to the suggestion.

3. P18133 lines7-8 “ More recently, seep communities have been also reported in the Mediterranean Sea (Corselli and Basso, 1996)”. There are several recent publications that are missing (e.g. Holland et al 2003, - 2006, Camerlenghi and Pini, 2009, Savini et al., 2009, etc..)

**Reply**

Accepted: We will update our list of citations including the suggested references as well as any other recent reference we missed.

4. P18133 lines19- 21 Please provide a reference for this sentence.

**Reply**

Accepted: We will support the sentence with a few references.

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5. Material and methods P18135 lines 5-13 The question 2 and the question 4 are similar and it is better combine both in a unique question.

**Reply**

Accepted: This was also requested by Reviewer 1. We have reformulated our questions and reduced them in two as follows:

6. P18135 lines 18 and 21 The definition of habitat and microhabitat needs attention. See general comments.

**Reply**

Accepted: As answered above we will use the term microhabitats exclusively throughout the manuscript.

7. P18137 lines 5-7 Only one core cannot be considered a control. See general comments.

**Reply**

We have provided a detailed argumentation why we would like to keep this station in the manuscript and we hope that the reviewer and the editor will understand our reasoning.

8. P18138 lines 14-17 “As soon as the cores were retrieved, the overlying water was filtered through a 32  $\mu$ m mesh size sieve and the material retained on the sieve was backwashed into the plastic containers where samples were stored for later laboratory analysis.” Where are these data?

**Reply**

Accepted: Our phrasing was probably confusing. What we meant was that the overlying water was filtered and backwashed into the container where the 0-1 cm slice was stored. This is usually done in meiofaunal studies because the epibenthic meiofauna might be suspended into the water column during the core transportation from the sampler to the laboratory, where slicing takes place. These

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suspended specimens belong to the 0-1 cm slice. We will rephrase the sentence in order to make this clear in the new version.

9. P18138 lines 24-25 “.nematodes from each of the remaining sections were randomly picked out, mounted on glycerine slides and identified to species level” Have the Authors mounted nematodes directly on slides without follow a formalin–ethanol–glycerol treatment? Mounting directly nematodes on slides could be problematic for species identification.

**Reply**

Accepted: The Reviewer is right. Of course we have evaporated them first to pure glycerol by following a formalin–ethanol–glycerol treatment. We will correct the sentence in the new version.

10. Resultats P18140 Line 7 “. . .and the lowest at the Lamellibrachia field of Napoli (8 taxa) and the control station (9 taxa).” In table 2, 9 taxa are also reported in the Reduced Sediment of the Amsterdam.

**Reply**

Accepted: The Reviewer is right. We will correct the sentence in the new version.

11. P18140 Lines 8-12 I suggest the Authors to add a table reporting taxa presence/absence in the different habitats.

**Reply**

Accepted: We will include a presence/absence table. However, as we already have a table providing details on the major meiofaunal taxa (Table 2), we feel that it would be better to provide this new table as supplementary material.

12. P18140 Lines 13-17 Please add the samples referred to the data showed.

**Reply**

Accepted: We will refer to the samples in the new version.

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13. P18140 Line 17 “All other taxa, including both permanent and temporal meiofauna, contributed less than 1% (Table 2).” The list of other taxa is missing.

**Reply**

Accepted: This problem will be corrected with the inclusion of a new table as supplementary material, which will include a list (presence/absence or densities) of all the meiofaunal taxa (see also reply to Specific comment No. 11)

14. P18140 Line 27 I recommend the authors to add a table with the statistical results. For each ANOVA, F ratio and df should be shown.

**Reply**

Accepted: We will add a table either in the manuscript or as supplementary material.

15. P18141 Lines 15 and 19 The authors wrote: “. . .the genus Sabatieria was represented by a complex of three species”.. and then authors describe Sabatieria sp. 4. Please check and correct it.

**Reply**

This comment was also made by Reviewer 1 and we repeat our answer below.

When identifying nematodes to species level, people tend to use what we call working species, i.e. Sabatieria sp.1 Sabatieria sp.2 etc. It is also common practice, a group of scientists working within the same laboratory to use a consistent naming scheme for all projects. For example, if we find 4 Sabatieria species in one project, they will be named with Sabatieria sp.1 through sp.4. If we carry out another project and find only one Sabatieria species, which however is the one we named Sabatieria sp.4 in the previous project, we keep naming it sp.4 in order to avoid confusion as well as to be able to analyze the data together. Renaming the working species constantly, so as to have always an ascending species numbering, would make it after a while impossible to recall which species is which.

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There are also many practical difficulties involved in such a renaming. For example, during the identification process, one would make a drawing of the species so as to use these drawings as an identification guide. Renaming would mean to go back and correct all the drawings, as well as all the data sheets and then the computer files etc. For all the above reasons we would prefer to avoid renaming our species. This way, by just reading the publication we can go back and find out quickly which species we are referring to. Nevertheless, we understand the confusion this might create to the reader, and therefore, in the new version of the manuscript we will provide the names of the three *Sabatieria* species encountered in our study in parentheses the first time we refer to them.

16. Discussion P18144 p 4.1 Please see general comments about environmental data.

**Reply**

Accepted: As explained in our reply to General Comment No. 1 (1a), we will do our best to use all the available environmental parameters in order to better explain the observed patterns.

17. P18144 p 4.2 Please see general comments about vertical distribution

**Reply**

Accepted: The comment about the vertical distribution has been raised several times by both reviewers and we will take a number of actions to address this issue (see our detailed answer to General comment No. 2 of our reply to Reviewer 1).

18. P18144 line 2 Have you data below the 5 cm? Where are they?

**Reply**

We did sample down to 10 cm sediment depth but the 5-10 cm layer was taken as a bulk sample. We usually do this to check if there is a deeper penetration of animals in the sediment. Most of these samples had only a few nematodes

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(no other meiofaunal group) thus we did not include them in our analysis. Moreover, in most meiofaunal studies from the Mediterranean, only the top 5 cm are considered since below this sediment depth meiofauna is very sparse. Thus, the inclusion of these data would only cause confusion. For example, in the vertical distribution graphs, the last layer would be as much as the first 5 layers. Also we did not identify nematodes from below 5 cm. For the above reasons we prefer to leave this as personal communication by V. Kalogeropouou, who was the person counting the groups in the 5-10 cm layer.

19. P18146 lines 6-8 "Another striking feature of the control station was the fact that the genus *Manganonema*, a genus that is found only rarely and in very low numbers (Zeppilli et al., 2011b), was ranked second." This is not true, looking in table 3 we can observe that *Aponema* is first with 12.9%, *Thalassomonhystera* is second with 11.9% and *Manganonema* is third with 6.2%.

**Reply**

Accepted: The reviewer is right, we will make the correction.

20. P18147 lines 22-29 and P18148 lines 1-10 Please see general comments about vertical distribution

**Reply**

Accepted: The comment about the vertical distribution has been raised several times by both reviewers and we will take a number of actions to address this issue (see our detailed answer to General comment No. 2 of our reply to Reviewer 1).

21. P18148 lines 28-29 Syntax error: remove ambient

**Reply**

Accepted: The word will be removed.

22. P18150 lines 1-2 ".. beta diversity has been widely neglected in the marine environment, particularly in meiobenthic studies (Sevastou et al., 2011)." This is not

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true, there are several studies on beta diversity of meiofaunal community (e.g. Danovaro et al 2010, VanGaeveer et al 2010, Leduc et al 2012, etc. . .)

**Reply**

Accepted: The reviewer is right as we used a wrong phrasing here. We will rephrase this sentence to make our statement clear (i.e. that beta diversity has been used only recently in meiofaunal studies). We will also update the citations including other existing beta diversity studies (also the ones suggested by the reviewer).

23. P18152 lines 2-4 "In addition, many of the other putative species identified from the reduced samples appear to be new, suggesting possible endemism to seep environments at the species level." How many species are new to science? Could you give a percentage?

**Reply**

Accepted: A precise answer is not possible as it would require a lot of taxonomic work. Moreover, nematode taxonomy is largely dependent on good male specimens, which are not always available, particularly in the case of not so abundant species. Nevertheless, we will make an estimate for the more abundant species. We will look into this and, if possible, we will add this information in the new version.

24. P18153 line 3 Syntax error: substrata

**Reply**

Accepted: Will be corrected.

25. Table 1 Here the coordinates are expressed in decimals of minutes, while in the figure 1 they are expressed in minutes and seconds. Please use the same conversion.

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**Reply**

Accepted: Will be corrected.

26. Table 2 Please add a list of the group "other taxa" The column with the number of taxa could be removed from the percentage table, it is confusing.

**Reply**

Accepted: The column with the number of taxa will be removed and the required list with the group of "other taxa" will be available in a new table (see Specific comment No. 11). Explanation on what "other taxa" includes will be also provided in the caption of Table 2.

27. Figure 1 Please add N and E to the coordinate. Please see comments on table 1 about minutes/seconds conversion

**Reply**

Accepted: We will add N and E to the coordinates and we will also use the same notation in Figure 1 and Table 1.

28. Figure 4 Please see general comments

**Reply**

Accepted: The comment about the vertical distribution has been raised several times by both reviewers and we will take a number of actions to address this issue (see our detailed answer to General comment No. 2 of our reply to Reviewer 1).

29. Figure 5 Why have you not transformed the nematode species matrix?

**Reply**

We have tried all sorts of transformations that gave similar results and illustrations. Moreover, the one with the non-transformed data resulted in a 2D configuration with a better stress value. Therefore, there was no reason to prefer presenting the results of transformed data instead of the ones based on raw data.

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