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Interactive comment on "Biological and environmental rhythms in (dark) deep-sea hydrothermal ecosystems" *by* Daphne Cuvelier et al.

Anonymous Referee #1

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General comments:

The paper by Cuvelier et al. is an interesting study that uses time series analyses, conducted concurrently at two different hydrothermal vent settings in two different oceans. It is a unique study that deserves attention and it is good to see such work being done. However, there are some important scientific issues that need to be addressed.

A major finding of the paper is that patterns in temperature and tubeworm behavior were seen at both the Pacific (NEP) and Atlantic (MAR) sites that correspond to 6 hour time intervals, which the authors conclude is linked to tidal patterns. Additionally, they note that the same effect is seen 6 hours apart between the two sites which is a product of the time difference between the two sites. The 6 hour periodicity might be present,

however, the link to tidal patterns is not sufficiently developed. There is no data on the tidal rhythms or whether the increases or decreases in tubeworm appearances or temperature values correspond to specific events of the local tidal patterns. In order to come to the conclusion that the periodicity seen in this study is indeed linked to the tides, tidal data needs to be examined and presented within the context of the results of this study.

The other major issue I have with the manuscript in its current form is the use of statistical tests. Some of them are not quite appropriate and others can be tweaked. Details on this are listed below, under specific comments.

It appears from the results, that by and large, not a lot of changes overall were seen. The mussel and shrimp densities at MAR and the pycnogonid densities are the only ones that show an increase over time. This brings up a number of issues and considerations that ought to be treated in the discussion of the paper. For example, one major issue is the spatial extent: the areas analyzed are very small and the authors should include a discussion of the spatial scales at which appreciable changes in the megafaunal community can be observed. In the cases of the increases in densities of taxa, it is surprising that the discussion includes no references to successional patterns. The authors do mention that the mussels represent a climax community at shallow Atlantic vent sites, but there is no discussion of recruitment or colonization as being possible explanations for the observed increases in densities. And, the overall stability is not discussed very well either. Though there is a brief reference to differences in the level of dynamism in vent communities being possibly linked to spreading rates, this is not discussed very much despite stability being one of the major findings.

The writing itself needs considerable improvement. First, it should be read by a native English speaker since there are a number of grammatical errors and sentences that appear to be lost in translation. Secondly, the discussion, particularly the part with reference to the different taxa is written as a list of short, highly abbreviated paragraphs. This needs to be improved upon, restructured and rewritten so that a cohesive story

is presented as opposed to a list of short comments. For example, paragraphs should not end with a new thought or idea such as line 432, on page 12 which states 'Both species were considered predators or scavengers.' This is an important aspect to the biology of the snails discussed within this paragraph, without a doubt, but it is something that should be expanded upon, and should not be the final, concluding sentence of a paragraph that up to that point has not made any mention of trophic relationships or feeding biology. As it stands now, this part of the discussion reads basically like bullet points instead of a cohesive discussion.

Specific comments:

Introduction: In the key questions in the last paragraph: the first question is 'are tidal rhythms discernible in both vent settings?' It would be better to perhaps say 'are rhythms discernible in both vent settings that correspond to tidal patterns?' Since making the actual connection between the patterns seen in this study and tides is beyond the scope of the study.

The introduction should include some background about the major faunal groups and community structure at the two study sites. This is presented currently in the Methods section and certainly more details can be presented there, but the Introduction should also contain this information because understanding the settings is important contextual information.

Methods and Results: A number of key methodological information is missing. Though it is mentioned that the MAR observatory was positioned to face the Eiffel Tower edifice, no such information is given about the NEP observatory, such as whether it is also facing a chimney structure or not. If it is also placed facing a chimney structure, then this should be clearly stated early on in the manuscript, because chimney communities differ from areas of diffuse flow (and even host different morphotypes of Ridgeia tubeworms) which would mean that this study is examining chimneys on vents from two different oceans, which is very specific.

It is not mentioned, but clear from the photos, that the camera is positioned facing forward. In this case, there has to be clear details on how the spatial extent of the field of view was calculated. This is very important information and I am surprised that it has been left out. Other details about the imagery is also missing, for example, since video cameras were used, I assume that video stills were taken at the appropriate time points and those video stills were analyzed and used for marking the animals (in which software?), but these details are not present in the manuscript.

I think that it is inappropriate to use tubeworm abundances or tubeworm densities since in reality, what was counted where the extended plumes. Throughout the text, this should be changed to visible plumes or extended plumes, etc. and not tubeworm density.

In general, density should not be used at all. In both cases, the surface filmed and analyzed is considerably less than 1 m2 which means that all the density numbers are extrapolations and I don't think that is appropriate. Since within a site, the same area is filmed and examined for all 23 days and time points, the use of numbers of individuals instead of extrapolated densities would be more appropriate. Similarly, for microbial mats, use area coverage instead of percentage of area (and was percentage and density calculated based on filmed area or analyzed area?)

There is no explanation as to why areas of microbial mats were examined at 12 hour intervals and not at 6 hour intervals like the fauna.

Due to the difference in depths and ambient temperatures between the two study sites, raw temperatures should not be used at all. Instead, rescaled temperatures (raw temperature – ambient temperature) should be used and presented. The authors even say that there is a 2 degree difference in ambient temperatures between the sites and they say that even when this is taken into account, the NEP temperature recordings have a higher mean and maximum temperature. However, that does not mean that the distributions are necessarily different. A simple t test should be done to test if they

are significantly different or not. The temperature data shown, for example, in Figure 5 seems to indicate that they are not significantly different since they appear to basically differ by about 2 degrees, which is the difference in ambient temperature between the two sites.

I am not convinced it is appropriate to use a linear regression model to state if changes in densities over the 23 day period were significant or not. The independent variable is time, which is actually specific time points. It is important to have Figure 3 to show the trends, but fitting a line to these data and using that to say the changes are significant or not is, I believe, incorrect. The buccinid density graph really illustrates this, where the densities increased, then decreased and then increased again. That clearly does not mean that overall, in the study time period, buccinid densities showed a decrease, or should be represented by a downward sloping best fit line (as it is in the paper).

The differences in analyzed areas between the two study sites needs to be considered very carefully. I understand that the setup could not accomplish getting the same spatial extent for the fields of view, certainly, that would have been near impossible to achieve. However, when comparisons are made, for example, in the discussion about pycnogonid densities differing greatly between the two study sites, this difference in FOV extents needs to be kept in mind. In fact, it would be very difficult to constrain whether differences in densities or numbers of a specific taxon between the two study sites is a real difference or due to sampling artifacts. Therefore, such discussions need to be treated very cautiously.

There are some inconsistencies in terms of what was analyzed. For example, anemones are mentioned in the text, but are not in Table 2 which lists all the animals analyzed. Similarly, in the results (lines 229), mention is made of ophiuroids, which are not mentioned anywhere else before. And line 232 talks about a fish, which is also mentioned in Table 2, but was actually not seen in the stills, but in other video footage, which means, it was seen at other time points. Discussion of trends seen outside the time points relevant to this study should be discussed separately because it is has the

potential to introduce bias (large, flashy fauna are easily seen and focused on).

Limpets are mentioned and it is also said that they were not quantified (understandably so, because they are very small and numerous), but they are not shown in Figure 2.

In general, the results and the discussion appear to have three major themes that should be dealt with in separate sections. The first is spatial trends and associations between taxa within each study site, the second is comparisons between the two sites and the third is temporal trends. These are often intermixed and the paper would benefit by having them discussed separately. There will be some overlap between them, but currently, the results and discussion comes off as being very patchy and leaping from one point to another, without complete development of each point. Splitting into different sections might help to make the paper more cohesive.

I suggest adding two figures or analyses: first, in addition to figure 3, which shows densities plotted for the different time points, the authors could benefit by having a similar figure, but with difference in numbers from the previous time point (6 hours) on the x axis instead of numbers.

Secondly, I strongly suggest having a figure with tubeworm appearances (and anything else that shows the 6 hour pattern) vs. temperature. And in fact, regression models could be applied to these and it would strengthen your case that temperature can be used to predict tubeworm behavior.

The discussion about the same taxon inhabiting bare substrate at one site but not at the other is very problematic, because the FOV for NEP does not include bare substrate at all. In fact, the caption for Figure 2 even lists bare substrate as being an MAR only feature. If bare substrate is not present in the images of NEP, then it is not possible to say that NEP taxa that are seen on bare substrate at MAR are not seen on bare substrate at NEP.

In the discussion, certain taxa names are introduced for the first time, e.g., Bythograei-

dae, Bythitidae, and Majidae. These names do not appear in the Introduction or Methods, even when the animals are being introduced and they do not appear in Table 2 which lists the animals studied. The manuscript would benefit by keeping reference names for taxa consistent throughout the manuscript.

The first part of section 4.2, ie, the discussion about mussel valve openings is problematic. By opening valves, do the authors mean that one of the siphons are visible and extended or simply open? Mussels filter water through their inherent and exhalant siphons and fully opened valves are generally only seen in sick or dead individuals. Therefore simply talking about mussels valve openings does not seem appropriate, or should be explained further.

I do not know what software was used to mark and count the animals, but if the animals were physically marked, then it might be a good idea to examine the extended tubeworms more closely to see if there is periodicity in appearances among individuals. For examples, are half the worms extending out of their plumes at a certain time while the other half remain in their tubes and at the next interval, do you see the retracted ones extended and the extended ones retracted, or is it random in who is retracted or extended at any time point?

When talking about periodicity of the more mobile animals like pyconoginids and snails, etc., it is important to keep in mind the time and spatial scales: Currently, I don't think it has been shown conclusively that the observed periodicity is real periodicity and not the result of mobile animals moving in and out of a small area of focus at their own individual paces.

Though there is information on and a discussion of the CHEMINI system for measuring iron, no discussion or mention is made of sulfide. This is a very big gap in the discussion since sulfide is the fuel for the chemosynthesis based animals, and also a determinant of other animal distributions due to its toxicity. I understand that there was so sulfide sensor and therefore real sulfide measurements were not possible. However,

temperature, oxygen and iron are correlated with sulfide and can be used as a proxy to a certain extent for sulfide. Even if real concentrations of sulfide are not included, sulfide itself should be discussed because it is the source of energy in this system and one of the main reasons why tubeworms extend out of their tubes.

Technical corrections:

Please proofread for corrections to English grammar and sentence constructions.

Figure 1: The inset pictures are very small, and I think, the ones showing the FOVs are not necessary here, since they are presented in Figure 2. A better figure would be the map and the instrumentation. If the authors do decide to include the pictures of the FOVs, please make sure that the caption states clearly what all the images are. Currently, the caption does not explain what the smaller pictures are.

Figure 2: In addition to the sketches with the animals and substrates interpreted, one sample image in its original form, without interpretations drawn in, needs to be included as well for each site. Ideally, instead of a composite sketch, just one sample image should be presented, with and without the interpretations drawn in (and a reference can be made to Table 2 for a comprehensive list of animals seen at the two sites). This provides the opportunity to see what is being analyzed. These images also need scale bars. And, the white arrow that is mentioned in the caption, which is supposed to be pointing to the fluid exit, is not in the figure. Additionally, there is no mention whatsoever, of 'mussel background' anywhere in the text but it is drawn in in this figure.

Figure 4: The x axis is labeled incorrectly on the figure: it states 'hours', but the scale bar reads 0 to 40, but it should read 0 to 552 if it is hours. The caption reads that the x axis contains periods of 12 hours and this makes more sense, since 552 hours would equal to 46 12 hour periods. Secondly, as mentioned before, real areas should be used instead of percent areas. In fact, this is a reason why using percent cover is inappropriate: since the MAR FOV is much larger than the NEP FOV, the use of percent cover gives a very different view, namely that much more of the NEP is covered

in microbial mats than at MAR. This is not necessarily true, it just so happens that the area in question at the NEP site is much smaller and a similarly sized microbial mat there gives the impression of being much larger because the overall study area is much smaller.

Figure 7: what are the dashed lines?

Figure 8: The caption should mention why there is a box drawn in the graph for NEP Fe.

Table 1: remove coordinates and write out the full form of latitude and longitude. The last line, for turbidity has a '/' for NEP, this should be changed to N/A.

Table 2: In number of images, please spell out that 93 is the total, and 9 or 5 are the number of images that are missing, or could not be recorded. However, given that in both cases, video stills were taken, is it not possible to take an image just before or just after the specific time in question?

For surfaces, perhaps cms might be more appropriate since they are both much smaller than 1 m2.

Surface analyzed: it says to refer to Fig X, please change to refer to the correct figure in question.

The listing of taxa in this table needs to be more consistent. For example, if you put a descriptive category in the left column ('engineering species') then similar descriptive terms should be used for the others (mobile predators, scavengers, etc.). Basically, the same general type of information should be in the same column, instead of having a descriptor in one row and class or phylum names in the others. In the second and third columns, the order should be consistent. For example, you start with phylum (Mollusca), then family (Mytilidae), followed by common name in parentheses and the next line has the species name, which is a good format to follow. Similarly, for NEP, it should then read Annelida, Siboglinidae (tubeworms) and the species name on the next

line. So, next, should be Annelida, Polynoidae (scaleworms) and then multiple species on the next line. With M. fortunata, these higher categories and common names are left out (and / should not be used to indicate not available). Finally, since anemones are also present and discussed, they should be included in this table as well.

Table 4: As mentioned before, conduct a statistical test on the distributions of the rescaled temperature values to see if they are significantly different or not and include the results in this table.

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