Reply to Anonymous Referee C4052

General comments

The present study by Siano et al. describes the importance of parasitism of Amoebophryia species on dinoflagellates as part of the microbial food webs. For this purpose authors did an exhaustive work counting by FISH these dinospores that belong to the Marine Alveolates group across the Mediterranean Sea. Authors try to find patterns, relating dinospores abundances with total eukaryotes, and with abiotic parameters (temperature, nutrients...), as well as their capacity to infect dinoflagellates, looking at stained prepartions under the epifluorescence microscope. Although the study is interesting, I found several uncertainties that need to be clarified before the ms were suitable for publication.

In all stations sampled authors presented the abundance of total eukaryotes and total parasites, then, if dinospores are infecting agents of dinoflagellates why authors do not show the abundance of nanoflagellates and dinoflagellates separately?

Our paper is part of a special issue of the BOUM cruise. Data on dinoflagellates and nanoflagellates are supposed to be published in two separated papers and this is the main reason for which these data were not included in this paper.

Furthermore, since some dinoflagellates can ingest the parasite, how authors could be sure that the dinospores observed inside of all dinoflagellates shown in Fig. 3 are infecting it?

It is true that dinoflagellates could ingest dinospore and that therefore a positive probe signal could be detected in non-infected cells. However the fact that different phases of infection (development of the parasite, beehive stage, etc.) are observed for a given species, even if in different specimens, is a proof that the species can be infected by Amoebophyridae. For species showed in the figure 3 (in the new version of the manuscript Fig. 4) and listed in table 1 different infection phases have been observed in the analyzed samples. This shows that those species are potential host of Amoebophyridae.

Specific comments

Material and Methods

1-Page 7397. In my opinion would be better that the second paragraph of the oceanographic context should be moved to the Results section, under this title, and the first paragraph, included in M&M under a subsection entitled Sampling Site.

We agree with the referee.

The first paragraph of the 'oceanographic context' has been included in a paragraph entitled 'sampling strategy' and the remaining paragraph of the oceanographic context has been moved into the result section.

2- Page 7397. Since, in the results section authors are relating abundances of dinospores with nutrients, description of the method used in the determination of these variables should be included in M&M

Done, a sentence has been introduced in the paragraph 'sampling strategy'.

3- Page 7398. Authors filtered from 50 to 200 ml of fixed seawater through 0.22 μ m. Although authors are working in an oligotrophic- ultraoligotrophic system 50 ml- 200 ml on 0.22 μ m from my experience, could produce an accumulation of bacteria, particles, nanoflagellates, and many other things, that sometimes make difficult to see clear the wanted microorganisms. Perhaps I am wrong but I think it would have been better to use 0.6 μ m filters. I also believe that authors could concentrate one or two liters samples to count dinoflagellates, in order to have a better quantitative dinoflagellate abundances

Here probably the referee confused the filter used for dinospore eukaryotic cells counts (anodisc 0.22 μ m, filtration of 50 to 200 ml of water) with those used for identification and prevalence done on infected dinoflagellates (a sub-sample collected using plankton net, which represented several liters of water). This was already stated in the 'sampling strategy' (ex: 'sample collection and storage') but a further clarification has been made afterwards.

In both case we never observed over saturation of the filters and we were able to clearly see of the target organisms.

4- page 7398. Samples of dinoflagellates were collected using vertical net. Until which depth were they taken?

From 150m to surface. Added in the text.

Results

Page 7401. Why do you not make relationships between the dinospores and different groups of eukaryotes, since they are only infecting dinoflagellates?

We believe that Amoebophryidae parasite only dinoflagellates. Ciliates could be other potential host, but this has never been clearly demonstrated so far. As stated into the text (page 7403), we had positive probe signal in ciliate cells, but we think that this could be due to grazing of ciliates on parasites rather than an effective infection, as no mature infection have been observed.

In a preliminary phase of the study Spearman correlations between other eukaryotic cells (diatoms, heterotrophic and autotrophic nanoflagellates) and dinospores were tentatively sought for station 27, where the highest concentration of dinospores was observed. Correlations were lower than 0.5, with low significant values (p > 0.05). However, since diatoms, heterotrophic and autotrophic nanoflagellates are not supposed to be host of Amoebophryidae, we decided not to include these analyses in the final version of the manuscript. A sentence has been added to the test to clarify this point.

Discussion

Page 7404, line 15. When reading the discussion, authors have data on dinoflagellates, and from each group of eukaryotes. This data described in Christaki et al. 2010, correspond to the same cruise? If this is the case, why authors do not include this data in the present study? Here, the use of published data it would be justified because the

purpose of the present study is different to the one of the Christaki et al. 2010.

The eukaryotic groups considered in the manuscript by Christaki et al (previewed for the same special issue) are the heterotrophic nanoflagellates and the ciliates. The reason why we did not use these data is explained in the previous answer.

Page 7404, line 20. Authors say: the presence of other potential hosts of dinospores, overlooked during this study, could explain the high abundances of dinospores recorded at station 27. My question is: who could be other potential hosts?

One of the hypotheses that we claimed to explain the highest abundance of dinospores suggests that a high infectivity success of Amoebophyridae on the nano- and picoplanktonic dinoflagellates (page 7404) occurred at stations 27. On filters used for dinospore quantification we did not see small infected dinoflagellates, but this could have been due to technical problems. The fixative (paraformaldehyde) could have disrupted some cells (e.g. small naked flagellates), the observed area of the filter could have been too small, the actual volume of filtered water could have been insufficient as only 50 ml was filtered at station 27. For all these technical reasons we cannot exclude that small dinoflagellates could have been infected by Amoebophyridae. Unfortunately the infection of the small dinoflagellates community cannot be verified since net samples used for analyses of host biodiversity at stations A, B, C were collected with a 60 µm-mesh size net-tow. In addition other larger or more fragil organisms (copepods, ciliates, etc.) could have been infected by Amoebophyridae. If so, these infections could be another source of dinospores.

This part of the discussion was rewritten and better explained in the new version (see also answers to the first reviewer)

Page 7405, lines 21-23. Authors say that the encounter between dinospores and hosts can be triggered by physical factors and production of attractive allelopatic molecules. Could authors mention an example from their own experience or buy others, or is only a guess?

An example on turbulence has been cited (Llaveria et al., 2010). For allelopatic molecules, this is a still an hypothesis to test.

Page 7407, lines 10-11. Authors say that they cannot exclude that early stages of dinospores observed inside dinoflagellate simply resulted from the feeding activity of dinoflagellates. This idea is also repeated in page 7408, line 20. Then are you sure that all observed dinospores inside dinoflagellates shown in Fig. 3 are infecting or were eaten?

See comment above

Technical corrections

In order do not confuse Stations ABC with the way figures are marked, I propose that authors will name all figures with no capital letters as is shown almost always in the text.

We use the style proposed in the majority of Biogeoscience issues. However whenever the figure are mentioned, the word 'station' is used to avoid misinterpretations.

The reference Christaki et al in preparation for Biogeosciences Discussion, please check authors. I know that Vaqué is not in there.

Corrected