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Interactive comment on "Major constrains of the pelagic food web efficiency in the Mediterranean Sea" *by* L. Zoccarato and S. Fonda Umani

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âĂć This MS aims at presenting an overview of the trophic efficiency of the microbial food web along the Mediterranean Sea. The study is based mostly on previous data, but some new data are also included (although there is no clear distinction between both data sets). ANSWER: Thank you so much for your suggestion, we change the text: Part of these results were already published: Gulf of Trieste (Fonda Umani et al., 2012); bathypelagic experiments during the trans-Mediterranean VECTOR cruise (Fonda Umani et al., 2010); surface experiments during the same cruise (Di Poi et al., 2013) and unpublished results from OBAMA cruise (see the following 2.1 Studied areas).

âĂć The approach used is the dilution technique of Landry and Hassett (1982), which has been modified to also account for the grazing of nanoplankton on picoplankton (presumably prokaryotes, although this is not clear in the text). ANSWER: We are aware that within picoplankton prokaryotes dominate but we cannot exclude the presence of some picoeukaryotes.

âĂć Here we find one major problem of the study. The authors claim, and have published previously, that by truncating the food web at the 10 μm level the rates obtained with a dilution grazing experiment will be those of heterotrophic nanoflagellates on smaller prey. First of all, nanoflagellates would not be included in the bottles according the authors definitions. ANSWER: Probably we were not clear enough and also improperly used English but as it was stated in the introduction (pag. 4367, line 15) in this study we considered nanoplankton in the range 2-10 μm. To be more precise we changed the text as following: "NP-Dilutions experiment. Twelve liters of seawater were collected at the surface and in the meso-bathypelagic layers, pre-filtered immediately through a 200 μm mesh and then filtered through a 10 μm mesh to remove larger predators."

âĂć Even ignoring this mistake, the rates obtained by this procedure would only be accurate if the removal of the > 10 μm fraction had no consequences on the grazers and prey. This is not the case because grazers and prey within this fraction are usually under a strong grazing control from microzooplankton. By removing this group (microzooplankton) the rates of nanoflagellates on other prey are simply not representative of actual rates. ANSWER: We are well aware that microzooplankton can strongly control heterotrophic nanoplankton so the measured ingestion rates of nanoplankton in these experiments are far to be realistic. Indeed we state at page 4378 lines 27 of the early version "it has to be kept in mind that ingestion rates of MZP and HNF were the maximum potential rates for these consumers since they are actively grazed by higher trophic level consumers in the natural contest". In the new ms version we better explicated the concept as follows: "We are aware that results of MZP dilution experiments include the effect of viral lysis (Parada, 2007; Fonda Umani et al., 2010; Di Pol et al.,

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2013) and the mortality due to NP predation (e.g. Stoecker et al., 2013). To partially solve this latter problem we performed parallel experiments to estimate the predation of NP alone. We can expect three different models of interaction: i) only NP graze on picoplankton, therefore the ingestion rates calculated in NP experiments are the same obtained in the MZP experiments; ii) MZP grazing on NP reduces the ingestion calculated for NP alone; iii) MZP directly feed on picoplankton, and consequently ingestion rates obtained for MZP experiments are higher than for NP experiments (Fonda Umani and Beran, 2003)."

âĂć Another major problem of the study is the lack of clear hypotheses and a concise statement differentiating the conclusions of the work from previous ones obtained from the same/similar dataset. ANSWER: We thanks the reviewer for this observation and change the aims as following: "To test the hypothesis that picoplankton, and particularly heterotrophic prokaryotes, are pivotal in sustaining not only NP but also MZP energy requirements over a wide range of trophic conditions, we compared the results of more than 80 dilution experiments (Landry and Hasset, 1982) carried out in the entire Mediterranean Sea."

âĂć Added to this is the clumpy mixing of data from several seasons and locations, including in the same bag data from surface and from bathypelagic zones. These approximations only make sense when the data set is extensive enough, which is not the case here. ANSWER: The data from several seasons and locations were used to compare different trophic conditions which can be found at different sites and different season at the same site (C1). The major aims of our work was to demonstrate that picoplankton biomass is important for microzooplanzton not only in mesotrophic and oligotrophic conditions but also in eutrophic and eutrophied conditions. Data from mesopelagic and bathypelagic zones were used to test the hypothesis that also in ultra-oligotrophic conditions some carbon fluxes toward upper trophic levels may be possible.

âĂć A recurrent problem in the text is also the incorrect use of the term trophic efficiency, here defined as the ingestion rate divided by the biomass of grazers. This is not a measure of efficiency; it is an approximation to the standing stock of one prey consumed per day. ANSWER: We thanks the reviewer for the comment. We are sorry for the misunderstanding and we change at page 4374 the point 2.5 as following: “2.5 Elaborations The ingestion efficiencies of MZP and NP were calculated for each prey by dividing the ingestion rate by the corresponding preys’ potential production estimated respectively in the MZP and NP dilution experiments. Potential production is considered a good proxy for primary production (Calbet and Landry, 2004) We change also the results (and the figures) as following: Total MZP ingestion efficiency (as the ratio between Ingestion (I) and Potential Production (PP) on total preys) for each dilution experiments are reported in Figure 5a. In the graph we reported also the bisector, which indicates a 1:1 ratio. In oligotrophic and meso-eutrophic conditions the ratio was very close to the balance between I and PP. In eutrophied conditions there is a prevalence of PP over I, with the exception of two points that correspond to February 2001 and August 2000 experiments. These experiments were carried out at the end of a diatom bloom. Total NP ingestion efficiency is reported in Figure 5b with the indication of the bisector. As a general rule PP overcomes I rates or the ratio was very close to 1, with the relevant exceptions of four meso-eutrophic points and one in oligotrophic conditions. . . . NP ingestion efficiency was generally low (Fig. 7b), and particularly at low PP values. At high PP ingestion exceeded PP in two mesopelagic experiments, the two characterized by high stock biomass; while in the most bathypelagic experiments (VIERA) PP largely overcame ingestion.”

âĂć Added to this point is the lack of ecological meaning of the results; most times the microzooplankton grazing surpassed by far the standing stocks and potential production of prey. Should we allow something for settling, especially under eutrophic conditions? ANSWER: Thank you for your remark, which probably is due to the difficult to read Figure 3 and 4 and to the wrong interpretation we gave to ingestion efficiency. Now we present new graphs were the ingestion efficiencies are properly computed. Now we hope it will be easier to see that only in few cases the ingestion rates overcame the potential production and we provided also reliable explanations in the discussion: For microzooplanzton "In eutrophied conditions PP in most of the cases overcome the ingestion, thus justifying the possible export. The cases in which I exceeded PP corresponded to the end of diatom blooms therefore indicating the role of top down control in removing the produced biomass." For nanoplankton "The few recorded cases of ingestion exceeding potential production were no more observed in the parallel MZP experiments, suggesting that the potential high ingestion rates of NP were reduced by MZP grazing over NP."

âĂć Finally, and I hope this is just an omission of the methods, no controls without nutrients seems to have been prepared. Therefore, no actual estimate of in situ phytoplankton growth is possible. ANSWER: We thank the reviewer for this note, we omitted that in the methods so we change the text as follow: "To estimate in situ phytoplankton growth rate several, but not all, incubations were conducted with and without the addition of nutrients (5 μM NaNO3 and 1μM KH2PO4). Differences between the two estimated growth rates were not significant (Wilcoxon test p-value = 0.65)."

Detailed comments: âĂć The introduction fails in properly addressing the need for the study. It jumps between subjects without a logical flow that drives the reader to consistent hypotheses. There are also some inconsistencies; for instance, classic food web cannot predominate in meso- and eutrophic conditions if microzooplankton are targeted as major grazers in upwelling and coastal areas. These two statements are presented one after the other in the text. ANSWER: We completely rewrote the introduction to improve the logical flow of the topics, please see the new manuscript.

âĂć I really doubt the grazing by microzooplankton is scarcely estimated in the literature. Regarding the data sets used, there are two major problems, besides the ones already indicated: 1) the cruises encompassed mostly summer and spring months; however, data on winter and fall is presented. 2) Why not to include other studies from other authors in the area? Are perhaps their results contradicting the major conclusions here? ANSWER: We aimed to compare different trophic conditions that can be found at the same site in different seasons. In the Mediterranean Sea there are very few results of dilution experiments and all of these were made using the standard method (chl a). At the best of our knowledge there are no published results on experiments that specifically consider all possible preys.

âĂć Were the bottles turned upside down to avoid settling of organisms? What does natural light conditions mean? The technique is highly influenced by light levels. ANSWER: We better specify the incubation conditions in the methods section as follow: The second set of dilution (T24) was incubated at in situ temperature for 24 hours on the deck (or on the shore) in 600 L tanks with a circulation of sea-water. Flowing water maintained in movement the bottles that, at any rates, were turned upside each 3 - 4 hours. As regard to light condition, the incubations bottles in the 600 L tanks were covered by almost 0.5 m of water, thus light corresponds to the one present at the sampling depth.

âĂć When were the chlorophyll samples taken? Moreover, how can it be that 1 up to 5L was filtered if the bottles were 0.5-2L? ANSWER: We better specify the sampling for chlorphyll a in the methods section as follow: “Chlorophyll a samples were collected from the same Niskin bottles sampled for the dilution experiments by filtering on board from 1 L up to 5 L of seawater through. . .”

âĂć One-way ANOVA test is inappropriate to identify clustering. Actually, it is evident in figure 2 that there is no clustering, but rather a gradual distribution of the data. ANSWER: We change the description of Figure 2 in the results to better explain what we did: “Figure 2 shows the biomass of all primary producers at the surface assessed per each sampling event. We arbitrarily divided the increasing biomass values into three major groups: the first one with values for total autotrophic fraction < 6.44 μg C L-1 that we consider representative of oligotrophic conditions (mean chl a 0.22 mg L-1); the second one that can be consider meso-eutrophic with an autotrophic total carbon < 61.93 μg C L-1 and mean chl a of 0.60 mg L-1 and the last one which can be considered very eutrophic (or eutrophied) with biomass largely exceeding 100 μg C

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L-1 and mean chl a of 2.60 mg L-1. Groups presented significant differences among them (one-way Kruskal–Wallis test was highly significant, p-value < 0.0001)."

âĂć Please, discriminate between autotrophic and heterotrophic nanoplankton (NP) The use of acronyms is excessive and at times annoying. ANSWER: We are sorry for the confusion we made in the introduction speaking about autotrophic and heterotrophic nanoplankton. We fix that in the rewritten introduction. We intentionally do not want to discriminate between autotrophic and heterotrophic nanoplankton since it has been demonstrated the large diffusion of mixotrophic behaviour (see Mitra et al. 2014 and references therein). To ascribe the estimated nanoplanktonic grazing only to heterotrophic nanoflagelles would lead to an overestimation of single consumer ingestion rate and to an underestimation of the nanoplanktonic fraction exhibiting heterotrophic feeding behaviour.

âĂć How can it be that small flagellates constitute a fraction of microphytoplankton (MPP)? Page. 4375 line 20. ANSWER: We are sorry, there was a misunderstanding and we change the sentence as follow: "MPP represented only a small fraction of this biomass and mainly because of the presence of small organisms other than diatoms."

âĂć Figures 3 and 4 are too small. Moreover, it does not make sense that the normalized values are much higher than 1. This would mean that the entire biomass of prey is removed several times per day, which could only be under high production rates (several doublings per day) or under a community recession scenario. I do not think none of the above is the case here. ANSWER: We change Figure 3 and 4, now they show only the ingestion rates on different preys in each experiment. The 'normalized' values (what we called ingestion efficiency) were wrongly calculated on the standing stocks while now they are calculated on the potential production of preys. As pointed out above, only in few cases ingestion rates overcame the potential production and we gave reliable explanation for that.

âĂć Where there any saturation responses found during the dilution grazing experi-

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ments? How were the data processed in those cases? ANSWER: No, in our results we never found any saturation responses.

âĂć Figure 5 curve adjustment does not make any sense at all. ANSWER: We thanks the reviewer for this criticism, we removed those curve adjustment and provided 4 functional response models that have been tested on the scatterplot. In methods "The relations between ingestion rates and available biomasses of each kind of prey were investigated for MZP and NP. The functional responses of the ingestion rates over a wide range of prey concentrations were examined against four common models: (For the equations see the attached new version of the manuscript). where I and C0 are ingestion rate and biomass estimated in each dilution experiment, α and β are constant and represent respectively the maximum rate of ingestion and the rate at which I changes in relation with C0. The values for α and β that minimize the residual sum-of-squares in each equation (4, 5, 6 and 7) were computed with the Nonlinear Least Squares function implemented in the stats package of R. Only fitting models whose parameters were significant (p-values < 0.05) were kept and compared by the analysis of variance (ANOVA) and by the maximum likelihood to the same data (with the Akaike information criterion – AIC, and the Bayesian information criterion - BIC) to evaluate the fitting quality of the models. We also discussed the quality of the fitting and provided possible ecological explanations."

âĂć Fig. 9. Add biomasses in the figure. What does bottom mean? ANSWER: We added the mean biomass for each class of organisms in the graph. We change 'bottom' with 'Meso-bathypelagic'.

âĂć The discussion needs to be more concise to answer the questions highlighted in the introduction and should avoid recapitulation of the results. There are many non-sense sentences and arguments in the discussion. For instance, in page 4382 we read that ". . .while in eutrophic conditions PP in most of the cases overcome the ingestion. MZP reached the saturation threshold in the kinetic curves and we might hypothesise an export of biomass from primary producers that can sink or be transferred up to higher trophic levels". Where does this surplus of biomass come from? ANSWER: We rewrote the discussion avoiding result recapitulation and focussing the debate on questions related with the aim of the paper.

âĂć Please, revise English grammar and usage of punctuation marks. END OF REVIEW ANSWER: The new ms version was checked for the English.

Please see the posted new version of the manuscript.

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