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## ***Interactive comment on “Picoplankton community structure before, during and after convection event in the offshore waters of the southern Adriatic Sea” by M. Najdek et al.***

### **Anonymous Referee #1**

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This article is a multidisciplinary study. The seasonal evolutions of the dynamics of heterotrophic bacteria in the south Adriatic Pit are studied in the frame of biogeochemistry, phytoplankton and water mass changes. Heterotrophic bacteria are studied through multiple approaches to investigate its physiology and biodiversity. Interpretation of the evolutions of abundances, but also production using both tools (thymidine and leucine) as well as metabolic capacities using Biolog ecoplates, and DGGE techniques for biodiversity, are made in particular in relation with LIW intrusions and a winter convection episode. The study of such simultaneous and varying parameters related to heterotrophic bacterial activity and diversity are scarce, particularly when it is fully analyzed according to regional circulation and water masses. This paper is interesting

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and should be published. I have, however, many detailed comments that should help to improve the ms.

detailed comments

Page 17861 lines 10-20. A scheme showing main events of circulation (with NIG cyclonic and anticyclonic) in the vicinity of both stations p 300 and P 1200 should help the reader.

Line 28 Correct 'autotrophs'

page 17962 line 24. The frequency of sampling should be given also in this paragraph, event if we have the response on figure 2. If not the paragraph 3.1 is not easy to understand. Were ctd casts made on the same time of the day for all surveys?

p 17963. line 3/4 What are the absolute depth of p 1200 and P300 and what were the deeper layers, i.e., are they far from the bottom. Is P1200 in the deeper part or centre of the SAP?

line 6. Were nutrients analyzed on board without preliminary fixation? What was the technique used for NH<sub>4</sub>? What were the reproducibility and detection limit levels for these nutrients?

Line 10, Because then in the text two water categories were divided according the zero level of Chl a (p 17866 line 17, page 17867 line 11), it should be interesting also to present its detection limit considering a 500 ml volume of water filtered.

line 22. Did the authors examine HNA and LNA groups?

p 17864 lines 5/6. As there were some events of high activities, did the author check for isotopic dilution and specific labeling for both Leu and Tdr techniques?

line 8/9 Instead of writing "finished with 100% TCA" , the final concentration of TCA should be given.

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line 17. Details how many carbon source per family of molecules presented figure 9. What is the final concentration of carbon in this Biolog plate?

line 23. It is not clear if each time point of reading is considered in the AWDC formula or only the absorbance of the triplicates when max values were reached. It is not clear how the percentage substrate utilization (fig 9 B C D) and their corresponding error bars are calculated. Figure 9B is too small, mostly impossible to read.

page 17866 line 11 Check writing style for units. is it really  $\mu\text{g L}^{-1}$ , and not  $\mu\text{g L}^{-1}$ ?

line 24 The first time a ' $\pm$ ' is cited, the authors should indicate if it is for introducing se or sd. It is not necessary to indicate so much digits when unnecessary (for instance for T it should be  $18 \pm 4$ , or  $17.6 \pm 4.2$  instead of  $17.61 \pm 4.20$  °C. Check in the whole text. The worst is page 17888 lines 15-20 ( $35.64 \pm 43.10$  pM h<sup>-1</sup>).

lines 21 24. 'differed significantly... significantly lower...' Add in M&M sections tools used for statistics (comparison of averages, it seems).

line 25. What is the threshold used for separating water masses affected by LIW or SAW? A criteria of salinity? And when a station where only a part of the water column is influenced by the LIW is the data of the whole water column in the 'LIW' category? or divided in two parts? This is important to understand how the averages per type of water mass is calculated, and then compared.

p 17867. line 4. A table should indicate PL depth at all seasons and stations because in the figure 4, it is not easy to guess the 'zero' level' on a log chl scale. It is important, again, because then averages are also compared within and without PL layers. line 23 'HB correlated significantly with Chla and negatively with din po4 and Sio4'. Is it simply an indirect effect of depth? Are these relations still valid when considering only euphotic zone?

Line 24. 'SYN Pro and pEu were detected only in PL'. Is there a particular reason that it should not be systematically the case?

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p 17868. This paragraph is confusing. It is not always very clear to which data corresponds to the averages. Sometimes PL layers, sometimes DCM, sometime Chl a rich layers...

p 17869 lines 1/2. This sentence is not clear. Does Leu and Tdr B correlate with HB on one hand, and with Chl a on the other hand, but for a different set of data?

page 17871 line 10. I cannot not consider that a convection episode, bringing phytoplankton cells in the dark column layer, is extending the 'productive' layer. Phytoplankton in the dark do not make photosynthesis anymore. In addition, due to the dilution effects, integrated data (per m<sup>-2</sup>) should stay the same. At least the term "productive" should be more explicitly defined: Bringing new carbon, labile carbon in the twilight zone etc ...

p 17873 line 6. PHP is expressed here in carbon units. The authors should add conversion factors used for Leu and Tdr in the M&M sections. In addition, does the values cited here come from Tdr or leu data? Again, it is not clear from which data averaged vales are calculated: whole water column? both stations?

Lines 18-21. Indicate where are the statistics showing this.

Line 25 discussion on L/T. According table S2, only LeuC (specific activity per cell) and L/T are higher in P300, so not absolute rates. The authors argue that presence of Syn should increase the L/T ratio, but line 15 they said that 20 is a balanced ratio. There is not many papers reporting simultaneous Leu and Tdr measurements and more comparison with literature should be done. Are there other reference reporting low values of L/T as they get (<1)? What are the physiological or technical reasons for such low L/T values?

P 17875 line 4. According table S2 Tdr is nit higher at st 1200 in March. Why a low L/T would mean more active bacteria?

Line 15. Sentence unclear "since these two layers matched also in tdr regulation of

bacterial function by the same factors”

Table 1. As the authors transformed some data by using  $\log(x+1)$  do they keep all the “zero” data in their regressions (for chlorophyll for instance).

Figure 4 and Figure 2 have both an interruption of their depth scale but not at the same level, so it is hard to compare trends for the reader.

Figure 4. Why abundances are not homogeneous too along the water column in February, during the convection event?

Check units in whole text and tables. For DIN for instance,  $\mu\text{M}$  or  $\text{mol l}^{-1}$  but not  $\mu\text{M l}^{-1}$ . For fluxes for instance,  $\mu\text{M h}^{-1}$  or  $\mu\text{ mol l}^{-1} \text{ h}^{-1}$  but not  $\mu\text{M l}^{-1} \text{ h}^{-1}$

Figure 5. What are the thresholds for separation of LIW and SAW water masses? Does box plots include all seasons and depths? all stations?

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