

## ***Interactive comment on “Effects of wastewater treatment plant effluent inputs on planktonic metabolic rates and microbial community composition in the Baltic Sea” by Raquel Vaquer-Sunyer et al.***

**Anonymous Referee #1**

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### Comments

The authors tested the effect of the wastewater treatment plant (WWTP) effluent inputs on Baltic Sea planktonic communities in 4 experiments. They did so sampling seawater during winter, spring, summer and autumn and observing the effect of different WWTP addition to natural communities. They observed that nitrogen-rich DOM inputs increased BP and decreased PP. This trend will drive to an increase of carbon consumption and shift the ecosystem toward heterotrophy. Although the experiment was well performed and that the authors analysed several variables, I had the feeling that

C1

the paper was written in a hurry, sometimes with lack of precision and details. However, the study and the results obtained here are strongly recommended to be available for scientific community. Then, I recommend this manuscript to be published with minor revisions.

Detailed comments: Introduction 65: For non-specialists in WWTP, I think that you should explain why you are talking about the Chesapeake Bay and its discharge limit. Is it a bay that receives important WWTP effluent at the US scale? Comparable with the study area? 58-73: You should explain a little bit more how is the WWTP effluent in the Baltic Sea. What is its average discharge? TN, DIM, DON, DOM concentrations? Do any treatment have been implemented in order to reduce the WWTP discharges into the Baltic Sea?

Methods 87-90: Why did you choose to sample during the four seasons? Does the effluent discharge more during winter due to rainfall and enhance the WWTP inputs on Baltic Sea? 93-96: Did you check your WWTP after being filtered and frozen if some organism remains into your sample (as some bacteria may be smaller than 0.2  $\mu\text{m}$ ) using for example flow cytometry? 102-103: In my opinion, the third treatment is unclear. 103-105: I am not sure that I understood how did you do CD. You said that CD consisting of seawater diluted with milli-Q to have the same portion of community that 1:10, 1:15 and IN. Does that mean that you made the same “dilution” than the WWTP treatment but instead of using WWTP, you used milliQ and you made a CD of 1:10 (100mL of milliQ for 1000mL of seawater), another of 1:5 (200mL of milliQ for 1000mL of seawater) and another as IN (?)? If this is the way you made, I don't understand how do you have just one CD... 122-137: In this paragraph, you didn't explain how did you do dark and light incubation, how many replicates did you have. You just said “bottles were incubated at the in situ temperature... during one week”. Confinement methods prone to error as you might exclude zooplankton, enhance trophic interactions within the bottle and so on. Most of the incubations realized to estimate changes in DO in order to determine NCP and CR lasts between 12-48h. One week of incubation is a

C2

lot. Do you think that the community inside your bottles after 3-4 days of incubation is representative of in situ community that may receive once an amount of DON or DIN 3-4 days earlier? You did not talk in this paragraph about the confinement effect and the effect of 7-days incubation. I think that it is really important and that you should take time to write about it and give some criticism of your estimates. 130-133: You explained that incubations were illuminated by artificial light with a mean PAR of 1373.2  $\mu\text{W}/\text{cm}^2$ . Why did you choose this amount of light? Is it representative of the daily PAR that Baltic community receives at 2m depth? Does the illumination was constant during light exposition or does it increase until reaching a maximum natural irradiance and then decrease? The light hours range for the summer experiment is about the double of light for winter experiment. Do you think that GPP is comparable in summer and in winter experiment? Would it not be better to express GPP per hour and not per day? 134-137: I understood that you estimated NCP this way: DOmin1-DOmin0, DOmin2-DOmin1, DOmin3-DOmin2, ... DOmin1440-DOmin1439. Then, you had NCPmin1, NCPmin2, NCPmin3, ..., NCPmin1440 and you sum it to have it per day. Is it right? Or did you make it directly DOmin1440-DOmin0=NCP24h? According to the calculation that you made, did you compare it with the other way? Is it similar? 141: Can you explain what is the killed control please? 140-142: It is not specified during the 60min incubation the temperature and irradiance received by the samples.

Results 211-245: In the Metabolic rates results section, I missed to read more about statistics. For example, does GPP was significantly different between each treatment for each season? The same for CR and NCP. 223: I think you wanted to write "GPP decreased with Chl a content, it increased with DOC concentration" in reference with Table 3.

Discussion 345-348: I don't agree with this part of your discussion. I really don't see from which results you concluded that DOM significantly increased BP and decreased NCP, GPP and CR. Where are the statistical tests showing that GPP, NCP and CR were significantly different from control and CD? I understand that in BP results (247-260),

### C3

you observed a tendency to increase with higher addition of effluent (nothing significant I guess), that you observed significant differences in BP for different sampling day, treatments, interaction between sampling day and treatment but there is no mention in this paragraph that BP was significantly higher at the end of the experiment with effluent addition... Furthermore you observed no significant differences between treatments for spring and winter experiments. I don't see in this results section any mention of "BP was significantly higher under effluent addition than control and CD at the end of the experiment" For GPP, NCP and CR, you even didn't show any statistical test in the results that can lead you to this conclusion. In Figs. 2, 4 and 5, we can see that the responses are different according to the season, that responses aren't linear along the experiment (response different at day 3 than at day 6) but you didn't talk about that in the result section and we missed that. Looking to these figures, I don't agree with the general conclusion that NCP, GPP and CR were suppressed by DOM addition, but if some statistical tests show me the inverse ok... but I need to see it! And, did you consider that the "good" response is at day 7? If it is so, why? Why not at day 3? 354: recent references? 359: Correct "deceased" by "decreased" 358-360: Did BGE increase and decrease significantly? R2, p? If not, you should specify it too. 360-363: The example that you presented from the Bothnian Bay didn't seem really significant... Can you find other references showing significant increase of BGE with nutrient addition? 368-371, 381-393: Again, I disagree with the conclusion of a reduction of PP with effluent addition and that planktonic community in this region will shift toward heterotrophy. Either you have to improve your results showing statistical tests that can insure your conclusion that in general planktonic metabolism decreased under effluent addition or remove it. 432-434: There are more references about it and you should add few of them... not only yours àž 436: Use the same bibliography style than previously (Vaquer-Sunyer et al. 2015)

Conclusion 441: "DOM from WWTP effluent is nitrogen-rich." Remove this sentence. 442-461: Same thing than previously, I disagree with your conclusion.

### C4

Table 1 Nutrients formulas should be written correctly with subscript and superscript.  
Add C/N ratio to know if sampled communities were N limited or not.

Table 2 Idem (nutrient formulas)

Table 3 R2 not R2

Figs. 1, 2, 4, 5, 6 For each figure, it could be better to have the four season plots with the same axis range in order to compare the seasonal variation of each variable.

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C5