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Interactive comment on "Shifts in the microbial community in the Baltic Sea with increasing CO_2" *by* K. J. Crawfurd et al.

K. J. Crawfurd et al.

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Biogeosciences Discuss., doi:10.5194/bg-2015-606-RC1, 2016 Interactive comment (in bold, italic) on "Shifts in the microbial community in the Baltic Sea with increasing CO2" by K. J. Crawfurd et al.

Also supplied as pdf with Figs embedded in text

We thank the reviewers for taking the time to review this manuscript and for the pertinent and constructive comments they have raised. Wherever possible we have incorporated their suggestions and if not I hope that we have clearly explained our reasoning.

Anonymous Referee #1 My main criticisms here are that when I look at the figures, to me it seems as if overall shifts in the microbial community structure (or more precisely

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changes in the abundances of selected plankton members) do actually change in all the mesocosms but that changes are more pronounced in the 'high CO2' treatments. This means that 1. the title is misleading, and 2. parts of the interpretation and discussion of the data are misleading. However, this fact is not discussed at all in this manuscript.

1. We understand Reviewer's point of view and changed the title into "Shifts in the size structure of the microbial community in the Baltic Sea with increasing CO2". 2. We clarify in the Discussion of the revised manuscript that the extent of temporal dynamics differed for the high fCO2 mesocosms (and not the dynamics itself).

3. Unfortunately, the authors omit any discussion of factors other than the two that they investigated. For example, what about the changing temperature during the experiments; it ranges from 8-15 deg C, but this isn't discussed anywhere.

These variables affect the overall dynamics and not specifically the differences between the mescoosms (although we cannot exclude co-stressor effects by other variables on top of CO2 enrichment for the negative impact particularly – e.g. Pico III and Nano I). We make a statement in the Discussion of revised manuscript.

4. They also omit any discussion about any differences between the mesocosms and the surrounding waters and what the differences could mean (as far as I can see from the Suppl. figures, there are differences).

We chose not to include them in the main figures for 2 reasons. Firstly they are not directly comparable, this location is subject to water movement and during the experiment distinctly different water masses with different physical and biological signatures moved into the surrounding water. Secondly, and due to the previous reasoning, including the phytoplankton abundances in the surrounding waters makes the figures more difficult to read. Occassionally the abundances are much greater than in the mesocosms and it is then harder to discern differences between the mesocosms (see Supplementary Table S1 and Fig S1). Overall, microbial temporal dynamics are largely comparable, with a few exceptions: i.e., phytoplankton Nano I and II show much higher

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abundances in the outside water whilst all the picoplankton abundances are lower in the surrounding waters. We will add a description on the outside water microbial dynamics in the Results section of the revised manuscript, and add discussion on what may have caused the differences.

5. Further, I find it stunning that the total phytoplankton doesn't vary that much over the different mesocosm treatments (Fig. 1). The authors do not acknowledge or discuss this anywhere.

Total phytoplankton is numerically dominated by Synechococcus, making up to 74% of total (as explained in section 3.1), and Synechococcus especially did not show large variations between the fCO2 treatments (mesocosms).

6. The authors mention that no nutrients were added to the mesocosms to "resemble the natural bottom-up environmental conditions." Although I can understand why the authors did not add nutrients, however, I doubt that this resembles the natural environmental conditions over a period of six weeks. By enclosing the water masses, the 'natural' nutrient supply, which is either horizontally or vertically, is cut off. But this discussion might also have to be carried out across the different companion manuscripts on these mesocosm experiments submitted to the special issue in BG. But no matter how this discussion turns out, the authors should discuss it in this manuscript. Maybe it is reason, for example, why the start and end abundances of the experiments are sometime quite similar while changes happened in between.

Reviewer is correctly stating that we have not discussed lateral and vertical transport of nutrients, but the summer situation is largely driven by regenerative nutrient supply (Kuosa, 1991). The summer situation is one with vertical stratification and low nutrient concentrations resulting in small-sized phytoplankton dominance that are typically well-controlled by grazing and viral lysis (Kuosa, 1991 and demonstrated by our results). We will specify this more clearly in the Discussion of the revised manuscript. We also measured nutrients outside the mesocosms and found that nitrate, the limiting

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nutrient, was at similar concentrations inside and outside the mesocosms. Phosphate did increase outside the mesocosms but only after day 25. Silicate was higher and more variable outside the mesocosms.

7. The discussion section could benefit from a bit more 'discussion' rather than just the listing of other articles. How do the results you present actually fit into the literature and what does it mean for your data when other studies have shown certain effects (also see comments below).

We will rework the Discussion accordingly Reviewer's comment.

8. Several sentences and paragraphs are quite lengthy and not easy to understand (all the way to not understandable at all).

We have carefully checked the manuscript and shortened / clarified where we thought necessary (as Reviewer is not specifically mentioning section), and we also ask another native English speaking colleague to read the manuscript for clarity.

9. The introduction is lengthy and repetitive in some places and could be condensed.

We do not agree with Reviewer that the Introduction is lengthy, but we realize it is repetitive at times. We removed redundancies while securing readability.

10. Throughout the manuscript, the numbers of the mesocosms are used, e.g. M1 or M3. This is very confusing especially in the discussion. Could be exchanged for LOW1 or HIGH2 or something that designates a treatment to that number, especially since M1 and M5 seem to be replicates, as well as M6 and M7, and M3 and M8.

The notation used is consistent across all manuscripts in this special issue and the mesocosms with their mean fCO2 are presented in Fig. 1. However, we see Reviewer's point and will include a Table, as well as specify better at the start of the Results section as well as in the Discussion which mesocosms are LOW and HIGH. See Attachment1:Table 1

11. what are the ‘failed’ experiments, are they ‘samples lost’? or outliers?

Failed experiments include very low cell abundance samples, complicating proper analysis (and consequently results) of the diluted series, as well as results displaying a positive slope rather than a negative slope for apparent growth rates versus fraction natural water (thus where the dilution does not result in a reduction in mortality). An explanation is now given in the text (M&M section 2.3). We also make reference to paper by Kimmance & Brussaard 2010 describing such issues, as well as Stoecker et al. 2015 which suggest potential causes for positive regressions.

12. - p2, l3: salinity in the Baltic Sea ranges from near-freshwater to near-full seawater, I wouldn’t necessarily call it extremely low salinity implying a negative effect, especially since it varies a lot throughout the Baltic Sea.

Reviewer is correct. However, during our study salinity was around 5.7 only. We have deleted this sentence and provided the information about the sampling location in the next sentence (stating also there the actual salinity).

13. - p2, l6: “We examined the effects of ocean acidification in the microbial community during. . .” Do you mean on the community structure or on the carbon export or on primary production rates? Please specify in the abstract.

We specified this in the Abstract of the revised manuscript, making clear we examined effects on microbial community structure.

14. - p2, l25: the threats don’t face the marine ecosystems but the marine ecosystems face the threats

Thank you, we corrected this.

15. - p3, l2-9: This paragraph doesn’t fit here and disrupts the flow. I would place it to where you describe your experiments.

We have amended the Introduction and moved this information with additional clarifi-

cation to M&M section, and to paragraph concerning general effect of lower salinity on pH buffer capacity in Discussion.

16. - p3, l10-12: reads awkwardly, split into two sentences

We split into 2 sentences.

17. - p3 l26- p4, l3: this is repetitive

The Introduction is revised and we modified repetitive sections.

18. - p4, l20: which key knowledge, it's not clear from this sentence

Altered.

19. - p4, l24: delete the 'top-down control'

We rephrased this sentence.

20. - p5 and following: the experimental set-up could be made much clearer, maybe use a sketch for this

We follow the general overview paper in this same special issue and make reference to this paper (by Paul et al. 2015), describing the experimental set-up very well (also with figures). We rephrased the sentence making reference to Paul et al. paper in order to clarify this better.

21. - p5, l23: nitrate, phosphate, silicate and ammonium are per definition (dissolved) inorganic nutrients

Agreed and we altered accordingly.

22. - p7, l1-2: Pico III and Pico I do not have comparable cell sizes; one is about 1 micrometer and the one is about 2.9 micrometer in diameter, maybe you meant Pico II and III?

Yes, we thank Reviewer for noting this and have corrected accordingly.

23. - p7, l4: was this conversion factor used for all organisms or just the Synechococcus? There are studies that clearly show that the carbon density changes with cell volume with the density being lower at higher volumes (see Verity et al. 1992 L&O or Menden-Deuer and Lessard 2000 L&O) If the same conversion factor was used for all organisms, this would likely bias the results significantly

We have recalculated applying conversion factors of 237 fg C μm-3 (Worden et al.2004) and 196.5 fg C μm-3 for pico- and nano-sized phytoplankton (Garrison et al. 2000), respectively according to Mojica et al. 2015 and will use the revised Figures in the manuscript. However the overall dynamics are the same. See Attachment 2: Fig.1

24. - p7, l11: why not use the term total prokaryotes, because this is what it actually is, and not the heterotrophic prokaryotes which clearly should not include Synechococcus or other photoautotrophic organisms (the 10% argument is not correct here in my opinion).

Synechoccus makes up for around 10% of the total prokaryotes in our study, but we understand Reviewer's comment and changed 'heterotrophic prokaryotes' into 'prokaryotes' (and thus also HP into prokaryotes).

25. - p7, l15: final concentrations of what, molar? micromolar? micrograms per kg?

It is a final concentration of the commercial stock, which does not have a specified unit. This is the common way of expressing these final concentrations, but we moved 'commercial stock' directly following 'final concentration of' to improve understanding.

26. - p11, l19: the RËĘ2 is 0.49 in the figure and the regression line doesn't seem like it would be 0.98 either

Actually it is 0.98 and the figure was incorrect; we appologize for the confusion and thank the Reviewer for noting. We have replaced it with the correct one. See Attachment 3:Fig.2

27. - p11, l22-25: this part is hard to read. Please rephrase.

We rephrased and split into separate sentences.

28. - p12, l3: wasn't the start of the mesocosm day -5 or day 0 rather than day 13?

We altered the text to make it clearer that we referred to the bloom period.

29. - p12, l5: the decline following what?

Should be the 'following decline', which we now corrected.

30. - p12, l20: there is no such thing as net abundance; you can have net rates but not net abundances (also check throughout the manuscript)

Reviewer is correct and we deleted 'net'.

31. - p12/13: "This may have stimulated the gross growth in M3 as compared to M1 (day 19; Fig. 3b) for a longer period in the high fCO2 mesocosms, this accompanied by higher losses at low CO2 resulted in a positive correlation of net growth rates with fCO2 (Fig. 3e, RËĘ2 = 0.71) and almost 2-fold higher net abundances at day 21 (Fig. 3a) correlating with fCO2 (Fig. 3h, RËĘ2 = 0.84)." I have honestly no idea what this sentence means. It is unnecessarily long and confusing. Further, the 'net abundances',please see comment above, and

We reduced the length of the sentence by splitting it into two and corrected according to Reviewer's comment.

maybe either CO2 or fCO2 treatments could be used consistently throughout the manuscript

Noted and we have altered this to fCO2 throughout (in agreement with the general overview paper by Paul et al. (2015).

32. - p13, l13-17: unnecessarily long and confusing sentence

We understand Reviewer's concern and have split the sentence into two sentences to improve readability.

33. - p14, l25: what are “CO2 days”?

Sentence was indeed confusing and we have clarified it now.

34. - p18, l17-19: How do the authors infer a bacterial production rate of about 0.6 dËĘ-1 when grazing is about 0.3-0.5 dËĘ-1? Is that due to a net positive growth in bacterial abundance? If so, it would be good to mention here. Otherwise the reader might assume steady state as I did here.

Indeed this is due to a positive net growth rate, as stated in the preceding sentence. For clarity we changed the terminology to ‘gross growth rates’ now. Furthermore, we have moved the actual rate information to the Results to accommodate also Reviewer’s comment to include less results and more discussion. We added more discussion on the estimated gross growth rate in comparison to bacterial production rates measured by others (Hornick et al., this issue).

35. - p18, l27: “Also Pico II showed positively correlated net growth rates with CO2 enrichment, but somewhat later into phase I (days 12-17) due to reduced losses.” Awkward start of the sentence, maybe: “Net growth rates of Pico II correlated positively with CO2 enrichment. . .. .”

We thank Reviewer for the improvement and have corrected accordingly.

36. - p20, l25-28: these are mainly results and then one other article is mentioned; but what does this now mean for your data? Do you think that TEP production was a factor regulating the abundances in your study? the actual discussion is missing

We realize the sentence on TEP was a bit of a stand-alone and we chose to delete the sentence as it no longer fits the reworked discussion of these results.

37.- p21, l9-11: This comes out of the blue. How did you examine this?

This is examined using mytomycin C which induces prophage to go into the lytic state. It is explained in the M&M section 2.3. and we have also added this to the Results

section in the revised manuscript.

38. - p22, l2: ". . . has a very different physiology,. . ." different from what?

We meant different from picoeukaryotes. We clarified this now in the text.

39. - p22, l17: DOC could have come also from sloppy feeding?

We added this option to the discussion on the topic.

40. - p23, l17: do you mean remineralization of organic matter rather than nutrients?

Yes thanks, we apologize for the mistake an altered the text accordingly.

41. - p23, l22: "multiple other factors"? Please name them here.

We now rephrased the sentence and specifically named SST and stratification.

42. - Fig. 2: Instead of calling it the 'total prokaryotic phytoplankton', just call it what it is, the Synechococcus population

We have altered the figure accordingly.

43. - Fig. 2: How is the p<0.1 indicated? Is it possibly also the category 'p>0.05'? - What are the black dots here and in other plots (and I don't mean the single asterisks)?

The black dots are the p<0.1 indicators. We have clarified the legend accordingly.

44. - I don't see any 'f' in this figure (and some of the following figures).

True, this was our mistake as not all figures have failed experiments (f). We have deleted this from those figure legends.

45.- Fig. 2: panel b here and in following figures: I understand why the authors want to present the data together, but the plots are really obscured this way and it makes it hard for the reader to discern any data from them. I would suggest to split them into two panels

We have split panel b into two panels for clarity

46.- Fig. 2: Here and following figures, what do you mean by “otherwise no data is a zero”?

We referred to assays with true zero rates. We understand the misunderstanding and now indicate true zeros (thus not failed assays) with a “0” and made this clear in the figure legends.

47. - Fig. 6: The legend says that linear regression statistics are provided in the plot, however, I couldn’t see a p value.

We apologize as this was a left-over from an earlier version. We show only the r2, and made this clear in the legends of the revised manuscript.

48.- Fig. 10: the grazing rate and lysis rate are both loss rates; nevertheless, one of them is presented on a negative scale while the other is presented on a positive scale. I find it confusing.

We agree with Reviewer and have changed the figure now such that all loss rates are presented on a negative scale. See Attachment 4: Fig 3

49. - Suppl. Table S1: What are the units here?

They are abundances per milliliter; we have edited this.

50. - Suppl. Table S2: What are the units here?

They are rates per day; we have edited this.

51. - Fig. S1 and S2 legends: the upper layer is mentioned here but the measurements are from the total water mass, i.e. 0.3-17 m rather than 0.3-10 m

We corrected the figure legend.

52. - Fig. S2: panel f is missing

We corrected the figure legend.

53. - Fig. S3 and S4 please use the proper symbol for micromol and not umol

We corrected accordingly.

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Kimmance, S. A. and Brussaard, C. P. D.: Estimation of viral-induced phytoplankton mortality using the, in Manual of Aquatic Viral Ecology, pp. 65–73., 2010.

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Mojica, K. D. A., van de Poll, W. H., Kehoe, M., Huisman, J., Timmermans, K. R., Buma, A. G. J., van der Woerd, H. J., Hahn-Woernle, L., Dijkstra, H. A. and Brussaard, C. P. D.: Phytoplankton community structure in relation to vertical stratification along a north-south gradient in the Northeast Atlantic Ocean, Limnol. Oceanogr., 60(5), 1498–1521, doi:10.1002/lno.10113, 2015.

Paul, A. J., Bach, L. T., Boxhammer, T., Czerny, J., Hellemann, D., Trense, Y., Nausch, M., Sswat, M., Riebesell, U., Road, M., Lismore, E. and Way, E.: Effect of elevated CO2 on organic matter pools and fluxes in a summer , post spring-bloom Baltic Sea plankton community,Biogeosciences , 1–60, 2015. Stoecker, D. K., Nejstgaard, J. C., Madhusoodhanan, R., Pohnert, G., Wolfram, S., Jakobsen, H. H., Šulčius, S. and Larsen, A. (2015), Underestimation of microzooplankton grazing in dilution experi-

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Worden, A. Z., J. K. Nolan, and B. Palenik. 2004. Assessing the dynamics and ecology of marine picophytoplankton: The importance of the eukaryotic component. Limnol.Oceanogr. 49: 168-179. doi:10.4319/lo.2004.49.1.0168

Please also note the supplement to this comment:
http://www.biogeosciences-discuss.net/bg-2015-606/bg-2015-606-AC2-supplement.pdf

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Interactive comment on Biogeosciences Discuss., doi:10.5194/bg-2015-606, 2016.

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*Table 1. fCO_2 concentrations (µatm) as an average for the duration of the experiment following CO_2 addition and specification of this CO_2 level as low, medium or high. *denotes mesocosms sampled for grazing and viral lysis assays*

Mesocosm	*M1**	*M5*	*M7*	*M6*	*M3**	*M8*
CO_2 Level	*LOW*	*LOW*	*INTERMEDIATE*	*INTERMEDIATE*	*HIGH*	*HIGH*
Mean fCO_2 (µatm) days 1-43	*365*	*368*	*497*	*821*	*1007*	*1231*

Fig. 1. Attachment 1

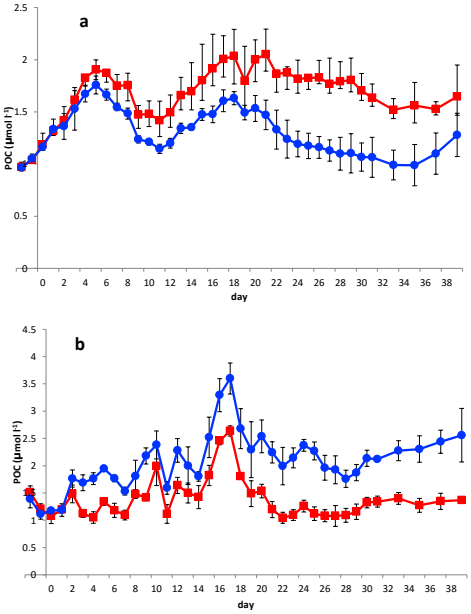

Fig.8. POC calculated from mean cell abundances assuming cells to be spherical and applying conversion factors of 237 fg C µm^{-3} (Worden et al.2004) and 196.5 fg C µm^{-3} for pico- and nano-sized plankton (Garrison et al. 2000), respectively according to Mojica et al. (2015). Error bars show one standard deviation. a) Temporal dynamics of Pico I and II b) Temporal dynamics of POC for all other eukaryotes ie. Pico III, Nano I and II.

Fig. 2. Attachment 2

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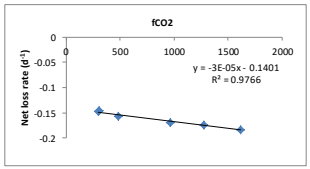

Fig. 3. Attachment 3

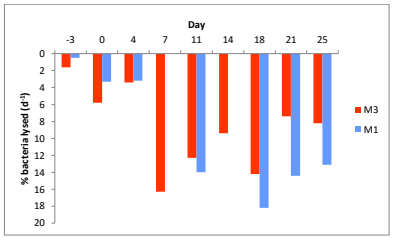

Fig. 3

Fig. 10. b) Viral lysis as percentage of HP standing stock in mescocosm M1 (low fCO_2, blue) and M3 (high fCO_2, red),

Fig. 4. Attachment 4

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