Biogeosciences Discuss.,
https://doi.org/10.5194/bg-2018-31-AC1, 2018

Biogeosciences
Discussions

Interactive comment on "Experimental assessment of the sensitivity of an estuarine phytoplankton fall bloom to acidification and warming" *by* Robin Bénard et al.

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Received and published: 25 April 2018

Referee 1 comments: The manuscript by Benard et al. describes the results from a mesocosm experiment that was designed to investigate the responses of a natural phytoplankton community to warming and acidification. The authors observed a clear stimulation of phytoplankton growth by temperature whereas acidification had no or only a minor effect. Although many experimental studies have been conducted in recent years to investigate phytoplankton responses to warming or to acidification little is known about combined effects. The data provided by this study are thus potentially valuable and interesting. However, important information is lacking in the current ver-

sion and need to be included and discussed to improve the value of this manuscript. The set-up of the experiment was designed to keep pH in the acidified mesocosms constant, yielding a decrease of pH after the bloom. This differs to the natural environment where a phytoplankton bloom can substantially modify (increase) pH. It also differs to the earlier mesocosms experiments that the authors reference in their discussion. I suggest that the authors discuss implications of the differences in the set-up of experiments.

Author's response to general comments: We thank the reviewer for the thorough evaluation of the manuscript and the insightful comments. The comment on our experimental approach is very appropriate and, as suggested, a new paragraph was added where its implications are discussed (see below). The experimental protocol where pH and pCO2 are kept constant during the full experiment is indeed different to what would happen in nature where changes in photosynthesis and respiration during the bloom development would affect theses parameters. The main reason why these conditions were kept constant was to allow us to precisely measure and maximise inference of the effects of pH and pCO2 on different processes (e.g. phytoplankton photosynthesis in this paper, and dimethylsulfide production in a companion paper to be submitted) taking place during all phases of the bloom.

The following section has been added in the new version of the manuscript: 4.5: Implications and limitations During our study, we chose to keep the pH constant during the whole experiment instead of allowing it to vary with changes in photosynthesis and respiration during the bloom phases. This approach differs from previous mesocosm experiments where generally no subsequent CO2 manipulations are conducted after the initial targets are attained (Schulz et al. 2017 and therein). Keeping the pH and pCO2 conditions stable during our study allowed us to precisely quantify the effect of the changing pH/pCO2 on the processes taking place during the different phases of the bloom. Such control was not exercised in two of our mesocosms (i.e. the drifters). In these two mesocosms, the pH increased from 7.9 to 8.3 at 10°C, and from 7.9 to 8.7 at

15°C. Since the buffer capacity of acidified waters diminishes with increasing CO2, the drift in pCO2 and pH due to biological activity would have been even greater in the more acidified treatments (Delille et al., 2005; Riebesell et al., 2007). Hence, allowing the pH to drift in all mesocosms would have likely ended in an overlapping of the treatments where acidification effects would have been harder to detect. Thus, our experiment could be considered as an intermediate between strictly controlled small scale laboratory experiments and large scale pelagic mesocosm experiments in which only the initial conditions are set. By limiting pCO2 decrease under high CO2 drawdown due to photosynthesis during the bloom phase, we minimise confounding effects of pCO2 potentially overlapping in association with high biological activity in the mesocosms. Hence, the experimental conditions could be considered as extreme examples of acidification conditions, due to the extent of pCO2 values studied. However, the absence of OA effects on most biological parameters measured during our study, even under these extreme conditions, strengthens the argument that the phytoplankton community in LSLE is resistant to OA.

Referee comment: Nutrient concentration and irradiance are main factors controlling phytoplankton growth in seawater. The authors should asses how these factors may have affected cell growth and primary production. This includes: 1. Add a drawing of the set-up and placement of mesocosms and treatments within the container. Containers often bear the risk of self-shading, which would need to be considered.

Author's response: We thank the reviewer for pointing that out. Light is indeed an important driver of phytoplankton photosynthesis and growth. As requested, the following information has been added in the text as well as a new figure:

Modification (line 88) Old sentence: Each enclosure is sealed with a Plexiglas cover allowing the transmission of 90

New sentence: The mesocosms exhibit opaque walls and all lie on the same plane level as not to shade each other. Light penetrates the mesocosms only through a sealed

Plexiglas circular cover at their uppermost part. The cover allows the transmission of 90

New figure (1): Mesocosm setup

In terms of the impact of nutrient concentrations on cell growth and primary production, we discuss this further: please refer to the specific comment about line 211 below.

Referee comment: Please give absolute values for irradiance instead of

Author's response: We acknowledge that light intensity has the potential to affect phytoplankton response to acidification and warming. Light intensity varied between days over the duration of the experiment (see new figure below). We cannot exclude the possible interaction of light intensity with acidification or warming, or the effect the varying intensity has on the dynamics of primary production. However, as all mesocosms were subjected to the same fluctuating natural irradiance, and that pCO2 and temperature were the only factors changing between the treatments, we limited our interpretation to these two parameters.

New sentence (line 112): Incident light was variable during our experiment, with only few sunny days (Fig. 2).

New figure (2): Irradiance

Referee comment: It is important to consider that net primary production was measured. Hence, responses to warming and acidification may not only be related to photosynthetic production but also to respiration processes. Please discuss.

Author's response: We agree with the reviewer. The text was modified accordingly (Line 394).

Old sentence: The warming-induced decrease in carbon fixation measured during Phase II may thus result from an increase in respiration by the nitrogen-limited diatoms.

New sentence: The warming-induced decrease in carbon fixation measured during

Phase II may thus result from an increase in respiration by the nitrogen-limited diatoms during periods of darkness of the incubations.

Referee comment: Given that the authors did not add nutrients to the natural seawater, the strong increase in biomass (from 10 to up to 30 μg/L Chl a in one day) after incubation is very surprising. What could have limited phytoplankton growth in situ? Please discuss.

Author's response: The following phrases were added to address this phenomenon (line 302). New sentence: In situ nutrient conditions prior to the water collection were favourable for a bloom development. Based on previous studies, in situ phytoplankton growth was probably limited by light due to water turbidity and vertical mixing at the time of water collection (Levasseur et al. 1984). Grazing may also have played a role in keeping the in situ biomass of flagellates low prior to our sampling. However, a natural diatom fall bloom was observed in the days following the water collection in the adjacent region (Ferreyra, pers. comm.). The increased stability within the mesocosms, combined with the reduction of the grazing pressure (filtration on 250 μm) likely contributed to the fast accumulation of phytoplankton biomass.

Referee comment: There was a strong drop in pH prior to the acidification treatment on day -3. What may have been the reason for this drop?

Author's response (AR): The following modifications have been made to address the pH drop / pCO2 rise at the onset of the experiment.

Modification (line 200) Old sentence: The pH remained relatively stable throughout the experiment in the pH-controlled treatments, but decreased slightly during Phase II by an average of -0.14 $\pm$ 0.07 units relative to the target pHT (Fig. 1a).

New sentence: Following the filling of the mesocosms, the pHT in all mesocosms decreased from an average of 7.84 to 7.53. Throughout the rest of the experiment after treatments were applied, the pH remained relatively stable in the pH-controlled

treatments, but decreased slightly during Phase II by an average of -0.14 $\pm$ 0.07 units relative to the target pHT (Fig. 1a).

Addition (line 294) New sentence: The onset of the experiment was marked by an increase of pCO2 on the day following the filling of the mesocosms. This phenomenon often takes place at the beginning of such experiments when pumping tends to break phytoplankton cells and larger debris into smaller ones. We attribute the rapid fluctuations in pCO2 to the release of organic matter following the filling of the mesocosms with a stimulating effect on heterotrophic respiration, and hence CO2 production.

Specific comments:

Line 87: add diameter and height of mesocosms AR: Line 87 has been modified as follow: (note that the dimensions of the mesocosms are now also presented in the new figure 1):

Old sentence: The mesocosms are cylindrical with a cone-shaped bottom within which mixing is achieved using a propeller fixed near the top.

New sentence: The mesocosms are cylindrical (2.67 m $\times$ 1.40 m) with a cone-shaped bottom within which mixing is achieved using a propeller fixed near the top (Fig. 1).

Line 99ff: add total duration of the experiment to the description AR: Line 100 has been modified as follow:

Old sentence: The water was collected at 5 m depth near Rimouski harbour (48° 28' 39.9" N, 68° 31' 03.0" W) on the 27th of September 2014. In situ conditions were: salinity = 26.52, temperature = 10 °C, nitrate (NO3-) = 12.8 $\pm$ 0.6 μmol L-1, silicic acid (Si(OH)4) = 16 $\pm$ 2 μmol L-1, and soluble reactive phosphate (SRP) = 1.4 $\pm$ 0.3 μmol L-1. The same day (indicated as day -5 hereafter), the water was filtered through a 250 μm mesh while simultaneously filling the 12 mesocosm tanks by gravity with a custom made 'octopus' tubing system.

New sentence: The water was collected at 5 m depth near Rimouski harbour (48°

28' 39.9" N, 68° 31' 03.0" W) on the 27th of September 2014 (indicated as day -5 hereafter), and the experiment lasted until the 15th of October 2014 (day 13). In situ conditions were: salinity = 26.52, temperature = 10 °C, nitrate (NO3-) = 12.8 ± 0.6 μmol L-1, silicic acid (Si(OH)4) = 16 ± 2 μmol L-1, and soluble reactive phosphate (SRP) = 1.4 ± 0.3 μmol L-1. On day -5, the water was filtered through a 250 μm mesh while simultaneously filling the 12 mesocosm tanks by gravity with a custom made 'octopus' tubing system.

Line 104: give value for initial pCO2 AR: Line 104 has been modified as follow: Old sentence: The initial in situ temperature of 10 °C was maintained in the twelve mesocosms for the first 24 h (day -4).

New sentence: The initial pCO2 was 623 ± 7 μatm and the in situ temperature of 10 °C was maintained in the twelve mesocosms for the first 24 h (day -4).

Line 113ff: Add total amount of volume sampled from the mesocosms each day AR: The following was added to line 116: "Total amount of volume sampled every day was 24 liters or less."

Line 166: Was the no replicate incubation? Was the error within treatment assessed? AR: There was no replication of incubations. The number of bottles to handle was already quite extensive and the maximum capacity of our incubators had been reached. We chose to adopt the strategy of an increased number of treatments (mesocosms), which, even with reduced replication, allows greater power to characterise the functional relationships between OA parameters and organism or ecosystem response (Riebesell et al. 2011 (Guide to Best Practices in Ocean Acidification)). However, we conducted independent measures of particulate PP, dissolved PP, as well as total PP every day in all the mesocosms allowing us to verify that the fractions measured in particulate and dissolved Pp reliably added up to the total PP.

Line 171: Give irradiance values AR: The daily irradiances are now presented in Figure B.

Line 202: pCO2 was 1340 ± 150 μatm on day -3; why was the value so high? AR: The pCO2 doubled after the filling of the mesocosms most probably due to an increase in CO2 production following the release of organic matter and the increase in heterotrophic respiration. We conclude that the filling of the mesocosms tends to break phytoplankton cells and larger debris into smaller ones, with a stimulating effect on bacteria. Refer to additions on lines 200 and 294.

Line 211:' The three nutrients displayed a similar temporal depletion pattern following the development of the phytoplanktonic bloom.' I disagree the nutrients in the warm treatments were clearly reduced much faster. AR: Right. We meant that the general pattern was similar between the three nutrients (nitrate, soluble reactive phosphate and silicic acid) within each of the mesocosms, however we agree that clarity could be added here. We rephrased this part of the results section:

Old sentence: The three nutrients displayed a similar temporal depletion pattern following the development of the phytoplanktonic bloom.

New sentences: Within individual mesocosms, concentrations of nitrate, silicic acid and soluble reactive phosphate displayed similar temporal patterns following the development of the phytoplankton bloom. Overall, nutrient depletion was reached within 5 days in all mesocosms at 10°C, exception made of the drifter which became nutrient-deplete by day 3. Nutrient depletion was reached slightly earlier within the 15oC mesocosms, all of them displaying exhaustion within 3 days of the experiment. Accordingly, bloom development and primary production within each mesocosm were eventually limited by the supply in nutrients, irrespective of the temperature or pH treatment.

Line 217: 'Chl a concentrations where below 1 μg L-1 just after the filling of the mesocosms, and averaged 5.9 ± 0.6 μg L-1 on day 0' If Chla increased that much regardless of treatment; light limitation or exclusion of zooplankton probably had a major influence of phytoplankton development and should be considered in more depth. AR: Water for our experiments was collected near shore where turbidity is high in this part of the St.

Lawrence Estuary. We thus attribute the rapid response observed at the beginning of our incubations to an increase in light availability. The presence of high nutrient levels in the water at the beginning of the experiment also suggests that light intensity in the upper mixed layer was too low to allow the development of the bloom near the dock where the water was collected. Please refer to modifications made for line 302.

Line 327: The citation of Bach et al 2017 is not accurate as that study didn't determine carbon fixation AR: The citation of Bach et al., 2017 has been removed.

Figure 3: Wasn't the Chl a accumulation (day 0 to Chl a max) not much higher in the warm control? AR: Figure 3 y-axis has been adjusted.

Figure 5 g, f: same axis labelling different figure. . .please check. AR: Figure 5g, e, f labelling has been verified. Figure 5j, k, l label has been modified from "Chl a-normalized PP (μmol C (μg Chl a)-1 d-1)" to: "Chl a-normalized PD (μmol C (μg Chl a)-1 d-1)".

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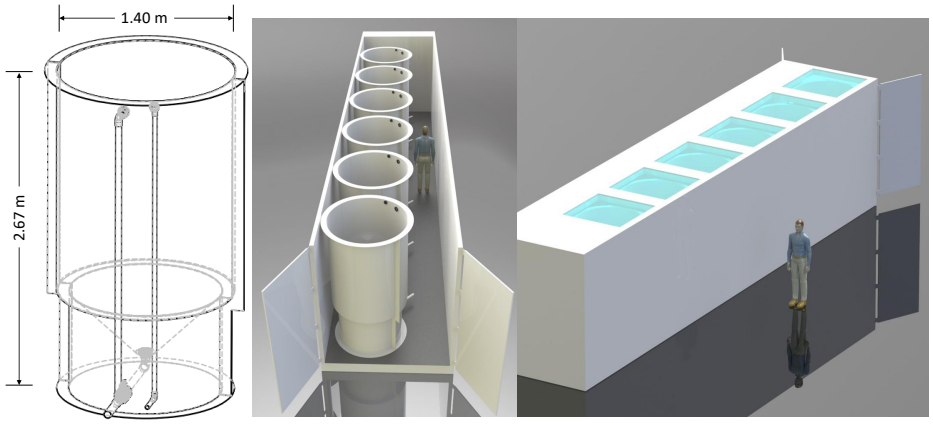
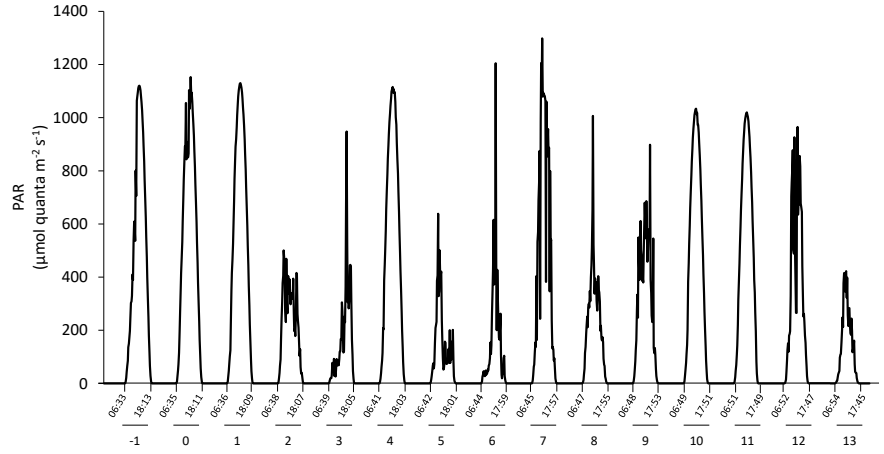


Fig. 1.


PAR
(µmol quanta m-2 s-1)
1400
1200
1000
800
600
400
200
0
06:33
18:13
06:35
18:11
06:36
18:09
06:38
18:07
06:39
18:05
06:41
18:03
06:42
18:01
06:44
17:59
06:45
17:57
06:47
17:55
06:48
17:53
06:49
17:51
06:51
17:49
06:52
17:47
06:54
17:45
-1
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1
2
3
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8
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Fig. 2.