

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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Referee comments #2: The manuscript of Bénard and collaborators reports on an experiment that has been conducted using indoor mesocosms (2.6 m³) to test for the effect of ocean acidification and warming on the development of a fall phytoplankton bloom in the Lower St. Lawrence Estuary. The experiment setup comprised 2 sets of 6 mesocosms installed in two temperature-controlled containers, that were filled with seawater sieved onto 250 microns. In one container, the water temperature was raised by 5 °C compared to the mesocosms installed in the other container (10 vs. 15 °C). A gradient approach (no replicates) has been considered for pCO₂/pH cov-

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ering a range of pH from 7.2 to 8.6. The experiment lasted 13 days and covered the development of a bloom and its decline. Major conclusions of this study are that pCO₂ has no effect on all measured parameters and processes while increasing temperature led to a faster build-up of chlorophyll and higher particulate primary production rates. Overall, this is a very well written manuscript that deals with an important topic. The introduction is well documented and shows that while this topic is of great importance, a fair amount of studies has already been conducted, including studies using in situ mesocosms in various environments. Although I would like to ultimately recommend this manuscript for publication in BG, I am concerned by 3 major aspects of this work and would like the authors to answer these comments.

Author’s response: We would like to thank the reviewer for the general evaluation of the manuscript and the insightful comments. We will further discuss the following comments from the reviewer.

Referee comment: Realism. The authors clearly mention that the surface mixed-layer pCO₂ is strongly modulated by biological productivity, yet they decided to run an experiment during which a bloom is produced and where carbonate chemistry has been maintained as constant. This would be acceptable if well explained and discussed, but the problem is that “control” mesocosms were actually not controlled (consider changing their name. . .) and pH was left increasing while the bloom was forming to (what I consider to be) very high and potentially unrealistic pH (?) values of 8.6. In situ pH was apparently close to 7.8, these “control” mesocosms appear to me as “perturbed”! Besides this major concern, I have to admit I do not understand how carbonate chemistry was controlled. The authors mention that “acidification” was carried out over day -1. On that day, I actually also observe a sudden increase of pH for the “controls”, pH8 and pH7.8. . . How did that happen? Naturally? Why was the increase in pH much higher in the controls than for the other mesocosms. Obviously, some information is missing here. Do you know the reason why pH decreased so fast between day -4 and day -3?

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Author's response: First, following this comment, the "Controls" have been more appropriately renamed "Drifters" to clearly show that the pCO₂ in these specific mesocosms was not controlled. We noted that Reviewer #1 also pointed out the shortcomings in the discussion of the different approaches to control pH during this type of experiment.

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.

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To further clarify how the acidification and pH treatments were controlled, the following phrases have been added.

Addition (line 112): To attain initial targeted pH, CO₂-saturated artificial seawater was precisely added via an automatic delivery system to mesocosms M1 (7.4), M3 (7.6), M5 (7.2), M7 (7.4), M8 (7.2), and M10 (7.6). Mesocosms M2 (8.0), M4 (7.8), M6 (Drifter), M9 (8.0), M11 (Drifter) and M12 (7.8) were gently mixed to allow the outward degassing of the supersaturated CO₂. Once the mesocosms had reached their target pH, the automatic system controlled the sporadic addition of CO₂-saturated water to refrain the pH from rising. Only the "Drifters" were not controlled throughout the experiment.

Referee comment: Timing. The second concern I have is related to the division of the experiment in 2 phases. Phase 1 corresponds to the development of the diatom bloom extended up to the depletion of nitrate (day 0 to 4) and Phase 2 corresponds to the declining phase of the bloom in the absence of detectable nitrate. Except that this is not really true since temperature increased the speed at which chl_a built-up and nutrients were consumed (this is not really mentioned in 3.2). At 15°C, except for 1 mesocosm, nitrate was exhausted already on day 2 while at 10°C, NO₃ in most mesocosms were actually exhausted on day 4. My point is that since T modified the timing of the bloom (and its decline), it does not seem correct to me to consider fixed periods. The build-up of chl_a and all related statistical analyses should be conducted at 15°C between day 0 and 2, and all tests related to the decline of the bloom between day 3 and 13. Would that change some of your results?

Author's response: Dividing the experiment into phases allows the disentanglement of the potential impacts caused by different processes and conditions occurring during different phases of a bloom, essentially its development and its decline. This is a strategy commonly used in published studies that delve into the impacts of OA on bloom dynamics. We previously considered different division criteria for the experiment (day of nitrate exhaustion, maximum Chl *a* concentration, averaged day of nitrate exhaustion) and ultimately opted for the averaged day of nitrate exhaustion as to mark the end

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of the nutrient-rich development phase. This would allow comparisons with numerous mesocosm experiments that also divide their experiment using fixed periods. However, in many of those studies, the distinction between the phases was sharply defined and timing was not such a significant factor. In our study, the onset, peak Chl a buildup and decline of the blooms, showed variation, and overall timing of the blooms was different between temperature treatments. Thus, we agree with the reviewer's suggestion to modify the phase criteria and suggest to take it one step further to strengthen the inference of treatment effects. Assigning phase durations based on differential Chl a buildup between temperature treatments as the reviewer suggests (Phase I: days 0-4 at 10°C, and days 0-2 at 15°C) would exclude some data from mesocosms that are still in the growth phase from the analyses of that phase. For example, M3 and M5 maximum Chl a concentrations are attained on day 7, and M7 maximum Chl a concentration is achieved on day 4. Therefore, we suggest modifying the phases for each mesocosm as follow: Phase I (day 0 to day of maximum Chl a concentration) and Phase II (day after maximum Chl a concentration to day 13). By doing so, all the analyses on Phase I will be constrained to the Chl a accumulation phase for each mesocosm, while Phase II will be an accurate representation of the individual declining phases. This modification does not change the global narrative or conclusions of the manuscript but does carry a few modifications in the statistical outputs. The absence of acidification effects is still valid for all parameters measured, as they stand currently, except for picocyanobacterial abundance at 15°C during Phase II which shows a negative linear trend with increasing pCO₂ using the new phase criteria. We already suggest in the paper that potential heightened grazing pressure could counteract the stimulating effect of increased CO₂ availability on picocyanobacteria, and this is still valid. With regards to the temperature effects, the differences on the mean concentrations of Chl a would no longer be significant in either phases. However, the accumulation rate of Chl a, a parameter that better defines bloom development, is still significantly higher at 15°C, reflecting the faster accumulation of Chl a. The temperature effects on particulate primary production during the specific phases are no longer apparent, however our initial

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conclusion that the PP is not affected over the full duration of the experiment remains valid. This will strengthen the conclusion that only the timing of the bloom development is affected by temperature, with negligible effects on the other parameters. Since we had already processed data in this manner and final figures can be swiftly produced to reflect the changes in the statistical analyses, we are confident that these modifications can bolster the paper and its findings.

Referee comment: Grazing. I regret that potentially the most exciting result of this experiment suggesting that pCO₂ "positive" effects on phytoplankton were actually masked by significant increases in micro-grazing is not more developed. I understand the politics behind the publication of papers from a joint experiment, it would just bring much more value to your paper if these results were incorporated and discussed. Top-down control is very often neglected in these OA-OW experiments. . .

Author's response: We agree with this comment. The impact of the different treatments on zooplankton abundance will be discussed in a companion paper by colleagues.

Minor comments

L217: concentrations were AR: "Concentrations where" changed to "concentrations were"

L225: "suggesting a faster loss of pigments. . .". Not really convinced by that. . . Is the slope different? AR: Following the changes made with regards to phase criteria and ensuing statistical analyses, this section would be adjusted as follows:

Old section (line 223-226): During Phase II, we observed no significant effect of increasing pCO₂ on the mean Chl a concentrations at the two temperatures tested. Nevertheless, during that phase, the mean Chl a concentrations decreased from 18.2 ± 0.9 μg L⁻¹ at 10 °C to 12.4 ± 0.7 μg L⁻¹ at 15 °C, suggesting a faster loss of the pigments following the depletion of NO₃⁻.

New sentence: During Phase II, we observed no significant effect of increasing

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pCO₂, nor temperature, on the mean Chl a concentrations following the depletion of NO₃.

L230: "The strong correlation" I do not understand this sentence. How a correlation can suggest anything about importance? AR: The sentence has been removed.

Figure 1a: label pHT in situ, why in situ? AR: All pHT are measured at 25°C and are computed to the temperatures of the mesocosms. The label "pHT in situ" meant that the pHT is calculated at the in situ temperature of each mesocosm. Therefore, for mesocosms M1–M6 the pHT is computed at 10°C, while for mesocosms M7–M12 the pHT is computed at 15°C. To avoid confusion, we changed the label to "pHT".

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