

## ***Interactive comment on “The ability of macroalgae to mitigate the negative effects of ocean acidification on four species of North Atlantic bivalve” by Craig S. Young and Christopher J. Gobler***

**Anonymous Referee #1**

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Two size cohorts of hard clams, oysters, scallops, and mussels, were grown with and without macroalga *Ulva* in two CO<sub>2</sub> treatments. The results show higher growth rates of bivalves in presence of *Ulva*, with a small benefit in the high CO<sub>2</sub> treatment. Increased bivalve growth rates in the presence of *Ulva* was attributed to the increase in saturation state caused by *Ulva*.

The study is an interesting approach to study the potential benefit of *Ulva* on growth of multiple bivalve species, in the context of aquaculture management with ocean acidification. The strength of this study is that the experiment was conducted on multiple

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species, two size classes, and there are multiple growth metrics with consistent results. The weakness of this study is the seawater chemistry and the conclusions drawn from the data. The results are intriguing and merit further exploration of why bivalves exhibited enhanced growth in the presence of *Ulva*. As not all factors were controlled in this experiment (e.g. unknown effect of algae and mussels on seawater chemistry, independently and by treatment), this study provides results to further develop specific hypotheses as to why these trends were observed. In its current form, I am not convinced by the conclusion that *Ulva* alters seawater chemistry which in turn causes increased bivalve growth under high CO<sub>2</sub>.

Main concerns:

1. The authors attribute what is a substantial biological response by bivalves in the presence of *Ulva* and high CO<sub>2</sub> to a very MINOR increase in saturation state over time (only 0.04!). A lot of emphasis is placed on statistical comparisons of saturation state across treatments, probably because the change is so small but offers an attractive explanation. However, a statistically significant difference in a carbonate chemistry parameter across treatments does not mean that it is biologically relevant. The authors do not discuss if the magnitude of change in growth is realistic for a 0.04 change in saturation state (perhaps some summary plot showing growth metrics of each species by treatment, with aragonite saturation state of each treatment on the x-axis, would provide insight). However, Comment #2 explains why the sampling design is insufficient to characterize seawater chemistry in this experiment in the first place.
2. The seawater chemistry sampling design and measurements are not sufficient to describe how organisms contributed to seawater chemistry or what they actually experienced.
  - a. Water was only sampled at the start and end of the experiment, despite multiple water changes during the closed-system experiment. If the changes in saturation state come from cumulative effect of nitrate assimilation by *Ulva*, this is in fact a change that

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occurred since the last water change (every 3 days). It means that the bivalves mostly experienced the same saturation state across high CO<sub>2</sub> treatments, regardless of the 0.04 change that would have occurred over 3 days.

b. Seawater chemistry was highly variable. According to the authors, Ulva changes carbonate chemistry via CO<sub>2</sub> uptake (decreasing DIC; P9, L11-22) and/or nutrient uptake (increasing TA, estimated at 10-20  $\mu\text{mol/kg}$ ; P9,L29). During the experiment, the effect of CO<sub>2</sub> uptake via primary production by Ulva is presumably removed with continuous bubbling with treatment concentrations of air/CO<sub>2</sub> gas mix (P9). However, pCO<sub>2</sub> is quite variable across treatments and experiments, indicating that the method used for bubbling did not actually bring the system (treatments + biology) into equilibrium. For example, within one experiment, the standard error in pCO<sub>2</sub> reported in Table S1 is up to 200  $\mu\text{atm}$  (based on N=2, start and end samples?). TA also varied substantially, even across treatments without Ulva, and TA did not always increase in the presence of Ulva (Table S1, this is masked by Table 1 which somewhat deceptively summarizes treatments across all experiments). For example, TA was 230  $\mu\text{mol/kg}$  less in the CO<sub>2</sub> treatment compared to control in the experiment for *Mercenaria mercenaria*, even without Ulva. The authors do not describe why all their measurements are so variable and inconsistent in what they define as a well-controlled system. It is unclear if SE refers to a start and end sampling, which again is not a relevant design if the authors think that biological processes contribute to changes in seawater chemistry.

c. Chemistry was calculated using pH that was measured by a Durafet but no information on calibration and quality control was provided. It is unclear how and where the daily pH measurements are used.

d. For all of the above reasons, I am not convinced that photosynthesis or nitrate assimilation by Ulva increased saturation state which then enhanced growth of bivalves (as claimed on P11, L29-30). Unless the authors can clarify these points, alternative hypotheses should be discussed. For example, could proliferation of algal cells in high CO<sub>2</sub> have provided more food to the bivalves and therefore contributed to their growth?

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After all, nutrients were added and this would benefit *Isochrysis* spp. (spelling error on P3,L23) and *Chaetoceros* spp.

3. The extensive discussion (e.g. last four paragraphs) on macroalgae/seagrass benefits to bivalves detracts from the discussion of the results of this study, and makes the authors appear biased towards the hypothesis that macroalgae will mitigate ocean acidification (e.g., their interpretation of Unsworth et al 2012 on P11,L17, comments below). The ability for seagrass and macroalgae to chemically buffer ocean acidification (e.g., P12, L1-2) is not a fact, and needs to be considered in the context of the greater coastal environment that the habitat is in (e.g., freshwater inputs, upwelling, water residence time, etc., e.g. see Cyronak et al 2018 "Short-term spatial and temporal carbonate chemistry variability in two contrasting seagrass meadows: implications for pH buffering capacities"). The authors do not discuss the fact that their experiment was conducted in a closed system. It is unrealistic to conclude that a minute impact on alkalinity by Ulva (if verified, see comment 1 & 2) would mitigate ocean acidification in an open system. For these reasons, extrapolating these results to field applications should not take up more than a paragraph, and the authors should only do so if all of the issues with seawater chemistry can be sufficiently resolved.

Minor comments:

Title: based on the issues with seawater chemistry, this title may need to be revised

Abstract: remove p-values

Introduction:

- Half of this study has to do with large vs. small bivalves but the significance of this is not mentioned in the introduction. Please add the motivation for this in the Introduction.

- P2, L18: specify that pH and saturation state in seagrass meadows provide \*temporal\* refuge from acidification (as pH also declines below background seawater pH at night or in winter seasons).

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Methods:

- Were nutrients added to vessels without Ulva as well? If not, the presence of Ulva is confounded with presence of nutrients which could influence the growth of Isochrysis and Chaetoceros and therefore the food supply by treatment.
- P3, L 24: how can 'ad libitum' food supply be exactly  $4 \times 10^4$  cells mL<sup>-1</sup> d<sup>-1</sup>?
- Report on assumptions of ANOVA (i.e., do residuals exhibit a normal distribution? was this tested?)
- P4, L34: add # of circles of algae added to each vessel. Was this scaled by container volume for small (1 L) and large bivalves (8 L)? If Ulva changes seawater chemistry in a consistent way, this data can be used to explore that (e.g., weight to volume and magnitude change in seawater chemistry).

Results:

- Tables in supplement: check consistency of \* with  $p < 0.05$ .
- Please report the actual p-values in the text since the tables are in supplemental files.
- I don't understand how ANOVA results are used to make statements like "When in the presence of Ulva, shell length-based growth was significantly increased by 42% (Two-way ANOVA;  $p < 0.05$ )" when it is unlikely that the % change is the same in high CO<sub>2</sub> and low CO<sub>2</sub> treatments. If the authors are reporting the effect of Ulva only at high CO<sub>2</sub>, then the statistics should come from the Tukey post-hoc comparison. Authors should also report on the interaction of the two-way ANOVA (significant or not).
- I was expecting the Ulva results in the Results section. It's not critical, but a small point of confusion.
- P5, L35: report tests of ANOVA assumptions, report p-values that are corrected for multiple comparisons.

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Discussion:

- P11, L16-19: this statement is incorrect. Unsworth et al 2012 is a theoretical modeling study. Model results were then applied to coral calcification rates that came from laboratory-based experiments. The authors themselves state that the results from the modeling need to be field tested.
- Discussion should include information about the magnitude of the beneficial effect of Ulva under high CO<sub>2</sub>.

Table 1: indicate which parameters were measured, and sample size (N).

Figures: define error bars and indicate when there are significant differences among groups

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