

RESPONSE TO REVIEWER COMMENTS

Reviewer comments in italics; author responses to bold

Reviewer #1:

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Two size cohorts of hard clams, oysters, scallops, and mussels, were grown with and without macroalga Ulva in two CO₂ treatments. The results show higher growth rates of bivalves in presence of Ulva, with a small benefit in the high CO₂ treatment. Increased bivalve growth rates in the presence of Ulva was attributed to the increase in saturation state caused by Ulva.

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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 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 CO₂.

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We thank the reviewer for their feedback.

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1. The authors attribute what is a substantial biological response by bivalves in the presence of Ulva and high CO₂ 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.

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We appreciate the reviewer's perspective on this point. First, we note that small changes in saturation state, even when saturated, can be biologically important and significant. In prior studies Barton et al (2012, Limnol, Oceanogr) saw that survival of early life stage Pacific oysters were correlated with $\Omega_{\text{aragonite}}$ even in the saturated range of values and that small change made substantial differences. Similarly, the growth of early life stage bivalves used in the present study (*Mercenaria mercenaria* and *Argopecten irradians*) was assessed under three concentrations of CO₂ (280, 390, and ~780 ppm) and significant differences in growth were observed between 280 and 390 ppm CO₂ which often corresponded to small changes in $\Omega_{\text{aragonite}}$ (<0.1 units) within the

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saturated ranged (Talmage and Gobler, 2010 PNAS, 2011, PLOS One). We note that we did refer to these examples in the discussion of the manuscript.

5 We agree with the reviewer's suggestion that plots of saturation states against the growth would be important to examine. Therefore, as suggested by the reviewer, for this revision we have made plots for every experiment showing growth rates of each species as a function of aragonite and calcite saturation state for each treatment on the x-axis. We have placed the resulting regression statistics in tables as supplements to the manuscript (new Table S10), with references to the table throughout the manuscript. To summarize these findings, there were strong positive and
10 significant ($p < 0.05$) correlations between shell length-based growth and saturation states of aragonite and calcite for all species and size classes, save for the single *Mytilus edulis* experiment. In at least half of the experiments, there was a strong positive correlation and significant ($p < 0.05$) correlation between tissue and shell weight-based growth and the saturation states of calcite and aragonite, with several additional results approaching significance ($p < 0.07$).

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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 since the last water change (every 3 days). It means that the bivalves mostly experienced the same saturation state across high CO₂ treatments, regardless of the 0.04 change that would have occurred over 3 days.

25 We thank the review for this comment, as it motivated us to dig deeper into the data we had already collected to discover that, in fact, the bivalves mostly experienced different saturation states within the *Ulva* treatments across the experiments. *Ulva* is capable of the rapid uptake of nutrients, which were added after every water change. Within 24 hr of each water change, pH values within containers with *Ulva*, regardless of CO₂ concentration, were higher than in the
30 containers without *Ulva*, meaning bivalves mostly experienced higher saturation states during experiments. We have provided plots to show this and now make reference to these new plots within the manuscript (Figures S2-S3).

*b. Seawater chemistry was highly variable. According to the authors, Ulva changes carbonate chemistry via CO₂ 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 CO₂ uptake via primary production by Ulva is presumably removed with continuous bubbling with treatment concentrations of air/CO₂ gas mix (P9). However, pCO₂ is quite variable across treatments and experiments, indicating that the method used for bubbling did not actually bring the system (treatments + biology) into
40 equilibrium. For example, within one experiment, the standard error in pCO₂ reported in Table S1 is up to 200 μatm (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_2 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
5 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.

We agree with the reviewer. There was variance in the chemistry during our experiments. Prior to starting any experiment, out vessels filled with seawater only were bubbled at a constant rate
10 which created a very stable system at full equilibrium for many days. Once biological organisms were introduced, however, as the reviewer correctly stated, the individual vessels were no longer in a simple abiotic equilibrium, but rather represented dynamic ecosystems with full complement of living organisms (*Ulva*, bivalves, phytoplankton added as food, microbial communities) undergoing all of the biological processes that influence carbonate chemistry (uptake and release
15 of ions, shell formation, shell dissolution, etc). One would not expect such systems to be in any kind of equilibrium. Furthermore, they each represented true biological replicates with a different set of bivalves, a different fronds of *Ulva*, different phytoplankton cells added and our variance in our reporting of our carbonate chemistry was based on replicate vessels (i.e. ecosystems) with $n=4$. Thus, these were true biological replicates representing the cumulative
20 effects of the whole experimental ecosystem (i.e. *Ulva*, bivalves, phytoplankton added as food, microbial communities, uptake and release of ions, etc) on the chemistry. While there is variance generated by these communities, the presence of *Ulva* consistently caused the calcium carbonate saturation states within experimental vessels to be statistically significantly higher than vessels without them. In addition, in this revision, we demonstrate in the new Figures S2-S3, pH changes
25 occurred with 24 of each water change, showing that, indeed, the chemistry was dynamic and varied in these living, biological systems, but that the presence of *Ulva* had a rapid and discernable effect on pH and carbonate chemistry.

We are uncertain as to how we were being ‘deceptive’ by creating both summary supplementary
30 tables given they are presenting all of our data making it available for everyone to read. While abiotic systems bubbled with CO_2 generally have consistent alkalinity, alkalinity can be affected by multiple biotic and abiotic processes associated all living organisms within each experimental vessel: Respiration, photosynthesis, shell dissolution, calcification, nitrate uptake, phosphate
uptake, ammonium uptake, microbial degradation, etc.

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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.

40 The DuraFET III used in the present study were calibrated with a seawater pH standard, as per Dickson (1993), and compared to measurements made spectrophotometrically using *m*-cresol (Dickson et al., 2007). Both methods yielded pH measurements that were identical and never

5 significantly different. We agree with the reviewer's concerns here and have added this information to the manuscript, which is now stated on P4, L3-7. Measurements of pH were used in the calculation of carbonate chemistry, which is stated on P4, L15-17. We have also provided the day-by-day pH values from experiments to illustrate the effects of nitrate uptake by *Ulva* (Figures S2-S3).

10 *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 CO₂ have provided more food to the bivalves and therefore contributed to their growth.*

15 We applaud the reviewer's skepticism as this is a core element of the review process and it forced us to generate additional plots and analyses of our experiments that provided evidence that is substantially more robust than our original submission. As the reviewer requested, we have shown that the growth rates of bivalves are significantly correlated with the saturation states of two forms of calcium carbonate (new Table S10) and that calcium carbonate saturation states were always significantly higher within the treatments with *Ulva*, two key data sets supporting the hypothesis that improved conditions for calcification was the key factor driving trends observed in this study. The reviewer has provided an alternative hypothesis but one that must be rejected given the data from our study. If high CO₂ led to the proliferation of algal cells, and thus more food for the bivalves, one would expect bivalve growth to increase. However, our results showed bivalve growth actually decreased growth under elevated CO₂. That being said, we agree with the reviewer's point that differences in algal cells within treatments could impact the growth of bivalves. Therefore, following the reviewer's line of reasoning, for this revision we have enumerated final algal cell densities within experimental vessels for all treatments. To summarize these findings, there were no significant differences in microalgal cell counts across any treatment within individual experiments. In addition to updating Tables S1 to show these results, a new table with this data has been created for this revision and added to the supplementary materials (Tables S13).

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

35 Yes, nutrients were added to all experiments and vessels. This point is specified on P3, L20-22. Despite the plausibility that the microalgae could have influenced the growth of bivalves, analyses of phytoplankton cell densities within each treatment and experiment rule out this possibility as there were no significant difference in algal cell counts across treatments within individual experiments. Table S1 has been updated to show these results and a table has been created and will be added to the supplementary materials (Table S13).

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.*

We do not believe or suggest that macroalgae alone can mitigate ocean acidification, but merely that primary productivity and/or nitrate assimilation by macroalgae may provide a temporal and/or spatial refuge for bivalves and other calcifying organisms as has been stated and concluded in prior studies. Given the scale, this may be particularly relevant to bivalves in an aquaculture setting with macroalgae grown in copious quantities in close proximity to bivalves potentially providing a “chemical resilience”. As per the reviewer’s comments, we have significantly scaled back this discussion for the revised version of this manuscript.

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

We have believe the title aptly describes the paper given the linear relationships between saturation states and the growth of bivalves and the < 24 h increases in pH associated with the presence of *Ulva* during experiments.

Abstract: remove p-values

We have removed the p-values from the Abstract.

- 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.

This was done since vulnerability of bivalves to acidification can be size-dependent. We have added this information to 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).*

We agree with the reviewer and have specified that daytime primary productivity increases pH and saturation states of aragonite, which provides a temporal refuge from acidification.

5 - *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.*

Nutrients were added to all experiments, Ulva or not, for the reason that the reviewer states. This point is specified on P3, L20-22.

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- *P3, L 24: how can 'ad libitum' food supply be exactly 4×10^4 cells mL⁻¹ d⁻¹?*

For the bivalves used in the present study, the rate of 4×10^4 cells mL⁻¹ d⁻¹ of the specified microalgae is an amount that is more than sufficient for the growth of said bivalves, regardless of size as per Helm MM, Bourne N, Lovatelli A (2004). Hatchery Culture of Bivalves: A Practical Manual. Rome, Italy: Food and Agriculture Organization of the United Nations (FAO), which we reference in this revision. In addition, we have demonstrated in this revision that there were always excess algal cells at the end of experiments (more than the amount present on day one), providing the direct evidence that this was indeed, *ad libitum* feeding.

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- *Report on assumptions of ANOVA (i.e., do residuals exhibit a normal distribution? was this tested?)*

In this revision we have reported on the assumptions of the ANOVA tests. In order to ensure that our data met the assumptions of the ANOVA (normality and equal variance), all data were log transformed before ANOVA were performed. We have added these details to the Methods section and have update the supplementary materials to reflect these changes.

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30 - *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).*

A single disk of Ulva was added per container, which we include in the Methods. In terms of weight, the amount of Ulva added to 1 L and 8 L containers was consistent with the benthic coverage of Ulva in Shinnecock Bay based on several years of benthic trawl data as well as other estuarine regions (Liu et al., 2015; Sfriso et al., 2001) and thus, yes, it was scaled to the size of the vessels. This point is specified in the Methods on P5, L4-8.

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- *Tables in supplement: check consistency of * with $p < 0.05$*

40 **We have changed the text within the supplementary materials to make consistent use of asterisks for significant results.**

- Please report the actual p-values in the text since the tables are in supplemental files.

We have changed the text to reflect the actual p-values within the Results section.

5 - *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 CO₂ and low CO₂ treatments. If the authors are reporting the effect of Ulva only at high CO₂, 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).*

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In this text, we reference Fig. 3 which demonstrates the increased shell length-based growth in the presence of *Ulva* (by 42%). The reference to Table S4 shows the ANOVA results, not the percent difference. The point being illustrated here is that growth increased in the presence of *Ulva* by a certain percent, which, by way of Two-way ANOVA, was found to be significant. We agree that with the reviewer that the 42% increase may not be the same within elevated and ambient CO₂ treatments, and have changed the text to separate the percent increase between the two CO₂ treatments.

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- *I was expecting the *Ulva* results in the Results section. It's not critical, but a small point of confusion.*

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We had to not include *Ulva* growth results in the Results section since it is not related to the primary goals of the study and since our previous studies have already reported on enhanced growth in *Ulva* incubated under elevated CO₂. We have added the mean response of *Ulva* as a supplementary figure for this revision and refer to this at the end of the results.

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- *P5, L35: report tests of ANOVA assumptions, report p-values that are corrected for multiple comparisons.*

For this revision we have specifically reported on the use of Shapiro-Wilk test to test for normality, in addition to an equal variance test, both of which are built into SigmaPlot. We performed log transformations of the data to ensure that they passed both tests and will update the supplementary materials to reflect this change. We have also changed the text within the manuscript to state what assumptions were made for ANOVA.

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- *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.*

We thank the reviewer for pointing this out. We have removed the reference to Unsworth et al. (2012).

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- Discussion should include information about the magnitude of the beneficial effect of *Ulva* under high CO₂.

5 **We agree with the reviewer. For this revision, we state the percent increase in growth rate of the bivalves in the discussion.**

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

10 **We agree with including the sample size and have added an asterisk next to the parameters that were measured but not the ones that were calculated and explain what the asterisk represents in the table legend.**

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

15 **We have indicated the definition of the error bars within the figure captions, and have placed significant differences within the figures, specifically the main treatment effects (CO₂ and *Ulva*).**

Reviewer #2:

20 *“The ability of macroalgae to mitigate the negative effects of ocean acidification on four species of North Atlantic bivalve” This paper evaluates the effect of the presence of the macroalga *Ulva rigida* on the growth of four North Atlantic bivalve species, *Mercenaria mercenaria*, *Crassostrea virginica*, *Argopecten irradians* and *Mytilus edulis*. The authors have used small and larger sizes of three out of four species, specifically the three obtained from hatcheries. The pCO₂ levels the bivalves are exposed to are high, but conceivable for estuarine systems. The authors claim that “saturation states for calcium carbonate (Ω) were significantly higher in the presence of *Ulva* under both ambient and elevated CO₂ delivery rates ($p < 0.05$)”, and that “alkalinity was increased by the presence of *Ulva*”. This might be statistically significant, but as alkalinity actually decreases (or is similar) in some treatments (small *Mercenaria*, large *Mercenaria* control pH, small *Crassostrea*, large *Crassostrea* low pH) it would be interesting to see the relationship between these parameters and growth directly and visually.*

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We agree with the reviewer’s assessment. We agree with the reviewer’s suggestion that plots of saturation states against the growth would be important. As suggested by the reviewer here, for this revision, we have made plots showing growth metrics of each species by treatment, with aragonite saturation state of each treatment on the x-axis. We have placed the resulting statistics in tables as supplements to the manuscript (new Table S10), with references throughout the manuscript. To summarize these findings, there were strong positive and significant ($p < 0.05$) correlations between shell length-based growth and saturation states of aragonite and calcite for all species and size classes, save for the single *Mytilus edulis* experiment. In at least half of the experiments, there was a strong positive correlation and significant ($p < 0.05$) correlation between tissue and shell weight-based growth and the saturation states of calcite and aragonite, with several additional results approaching significance ($p < 0.07$). Regarding alkalinity, we note that it

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is affected by many processes and while nitrate uptake will increase alkalinity, other processes may decrease it and that prior research has definitively demonstrated that saturation states for calcium carbonate are the key factor dictating the effects of acidification on bivalves.

5 *In treatments with Ulva additions, one would expect the variability in pH to be higher due to respiratory activity and production. However, the average pH is higher but the variability in pH seems similar to treatments without Ulva. In fact, I would expect the algal-addition treatments to have a fluctuating pH and the control treatments to be stable, which could arguably have caused the differences. However the authors do not discuss this and the tables do not show these differences in*
10 *variability of pH. Was the pH fluctuating on a day-night scale in the Ulva treatments? Or was the gas flowrate so high this was not discernable, and what causes the variability in the control treatments?*

The reviewer is correct that the treatments with *Ulva* had more variability in pH but did, on average, have higher pH levels. For this revision, we have added plots (Figures S2-S3) showing the changes in pH over time for the *Ulva* treatments to demonstrate that there is variability but that the pH rose in these treatments after each water change, likely due to the uptake of nitrate and the assimilation of CO₂.

20 *The nutrient and algae addition to the vessels might cause different nutrient concentrations in the treatments, with Ulva taking up nutrients while they remain suspended in the control vessels, which could have influenced results.*

25 **We agree with the reviewer that additions of nutrients and algae might cause different nutrient concentrations within treatments, and that *Ulva* may alter nutrient concentrations. One sign of different nutrient levels effecting the bivalves would be via higher levels of phytoplankton in vessels without *Ulva* which could yield more growth in the bivalves. However, for this revision we added the enumerations of phytoplankton concentrations, which were found to be similar and not significantly different between treatments across all experiments.**

30 **Importantly, we also note that any differences in nutrients among the vessels would occur in an ecosystem setting as well with more nitrate assimilation and removal and thus an increase in alkalinity in times and places where there is more *Ulva*. Hence, any differences on this front would be realistic in an ecosystem setting.**

35 *It is unclear what time of the year the experiments have been done (presumable summer due to hatchery times), and how the results might vary in other seasons (i.e. when *Ulva* is not productive).*

40 **The reviewer's presumption is correct as the experiments occurred throughout summer 2017, which is the peak growing season for bivalves and *Ulva*. We targeted this season specifically for that reason, although it should be noted that within the collection site of *Ulva*, the macroalgae appear during the early days of spring, and persists into the end of the fall months, which is beyond the time that our experiments were concluded. During period when *Ulva* grows more**

slowly (spring and fall) it would be expected that its growth would be slower and its ability to mitigate acidification would be lower.

5 *The various sizes and the amount of different species of bivalves used in this study make it an interesting read, even though it is not entirely clear what causes the beneficial effect of the presence of *Ulva* (its effect on the carbonate chemistry, nutrient concentration or something else).*

10 **We agree with the reviewer that the exact cause of the increased bivalve growth in the presence of *Ulva* is not entirely known. We believe the new regression analyses we have included that depicted the significant linear relationships between calcium carbonate saturation states and bivalve growth across all treatments in six of seven experiments makes the carbonate chemistry angle more convincing. Similarly, our newly included daily pH data that shows pH levels were consistently higher within 24 h of each water change in treatments with *Ulva*. Further, our inclusion of phytoplankton density data showing there are no differences among treatments**
15 **indicate this was not a driver of the findings.**

Specific comments: Methods P.3, line 9 “light intensity ($\sim 200 \mu\text{mol photons m}^{-2} \text{s}^{-1}$)”, how does this compare to ambient conditions?

20 **Light intensity used in all experiments was set to mimic ambient light intensity where *Ulva* grows in near shore regions. We have added this text to the manuscript to specify this.**

P.3, line 23: Isochyrsis should be Isochrysis

25 **We have made the suggested change.**

P.4, line 17: “some estuarine environments” – representable for the environments of the study organisms and their origin?

30 **Yes. For example, Wallace et al. (2014) observed $p\text{CO}_2$ concentrations exceeding 2,000 μatm in Jamaica Bay, NY, USA, which hosts the bivalve and macroalgae species used in the present study.**

35 *P.4, line 32-33: “Well-pigmented, circular sections of *Ulva* ($\sim 3.5 \text{ cm}$ and $\sim 7 \text{ cm}$ for experiments in small containers and large vessels, respectively”. These small containers were 1L, while the large vessels had a volume of 8L. The biomass of *Ulva* however, is 2x as large for the larger volume, which does not respect the ratio biomass/water volume. The authors state that the weight was consistent with the benthic coverage in Shinnecock Bay, would that mean that the 8L vessels had 2x the diameter of the small containers and would water volume not be more important than surface in this case? Or was there more than 1 disk per container (p.5., line 23 states “disks”)? This section is a bit unclear.*

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The amount of *Ulva* used was based on tissue weight, not tissue surface area, and the amount of *Ulva* added to 1 L and 8 L containers was consistent with the benthic coverage of *Ulva* in

Shinnecock Bay based on several years of benthic trawl data as well as other estuarine regions (Liu et al., 2015; Sfriso et al., 2001). This point is specified in the Methods on P5, L4-8.

5 Considering the 2-dimensional nature in which interactions of the bivalves and the macroalgae would occur, it made more sense to base the weight of macroalgae used on the surface area of the container, and not necessarily the volume.

P.5, line 16-17: “with discrete and continuous measurements of pH, dissolved oxygen, and temperature”, which measurements were discrete and which continuous?

10 We measured pH and temperature discretely and dissolved oxygen continuously. We have changed the text to specify this difference.

*Results P.6, lines 19-20: “For the larger-sized cohort of *M. mercenaria* (5.00 ± 0.41 mm), Ω_{calcite} and $\Omega_{\text{aragonite}}$ were significantly higher in treatments containing *Ulva* and significantly lower in high CO₂ treatments” Throughout the manuscript’s result section this way of describing the differences between high CO₂ / *Ulva* treatments is confusing. In the highCO₂+*Ulva* treatment the Ω_{calcite} is actually lower than the control-*Ulva* treatment (as expected), however from the text it appears at a first glance that all *Ulva* containing treatments are higher, the sentences might be clarified to prevent confusion.*

20 We intended to specify that Ω_{calcite} and $\Omega_{\text{aragonite}}$, although significantly lower under elevated CO₂ concentrations in general, were significantly higher in the presence of *Ulva* in both ambient and elevated CO₂ treatments. We agree with the reviewer that the sentence structure used throughout the manuscript may cause confusion and have changed the text to separate any significant differences in Ω_{calcite} and $\Omega_{\text{aragonite}}$, be it under elevated CO₂ conditions, or in the presence of *Ulva*.
25 We have also included references to the respective figures that show Ω_{calcite} and $\Omega_{\text{aragonite}}$, which would make it clear that Ω_{calcite} and $\Omega_{\text{aragonite}}$ are lower under elevated CO₂, but higher in the presence of *Ulva* in both ambient and elevated CO₂ treatments.

*Discussion Could the fact that *Mytilus* seems less sensitive to addition of *Ulva* be related to the more “natural” (no hatchery) origin of the juveniles and their exposure to environmental fluctuations vs. the more stable hatchery conditions?*

This is a good point raised by the reviewer. The area within Shinnecock Bay where *Mytilus* were collected is well-flushed and not prone to significant decreases in dissolved oxygen or pH or
35 increases in pCO₂. In addition, *Mercenaria* and *Argopecten* within the hatchery at Stony Brook University in Southampton are exposed to similar environmental conditions that are found in the collection sites in Shinnecock Bay from which these original broodstock came. We have clarified the recent origin of the broodstock used in experiments in the methods.

40 *If the presence of algae buffered the carbonate chemistry (p.9, line 23) and this is the mechanism for enhanced growth, this should be visible when $\Omega_{\text{calcite/aragonite}}$ is plotted vs. growth. However, the saturation state with *Ulva* is still considerably below 1 in the highCO₂ treatments and the SD is high.*

5 **This was an excellent suggestion by the reviewer and for this revision, we have included regression of Ω_{calcite} and $\Omega_{\text{aragonite}}$ vs. growth which in nearly all cases provided significant correlations. While the Ω is below 1 in many cases, prior studies have shown bivalves can grow, albeit slower, under such conditions (Talmage and Gobler 2010, 2011).**

Did the authors measure nutrients at the end of the incubations? It would be interesting to explore their theory that through Ulva presence “the nitrogen assimilation effects on alkalinity outweighed the effects of photosynthetic consumption of DIC” (p.9, line 33)

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Nutrient concentrations were not measured.

The ability of macroalgae to mitigate the negative effects of ocean acidification on four species of North Atlantic bivalve

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Abstract. Coastal ecosystems can experience acidification via upwelling, eutrophication, riverine discharge, and climate change. While the resulting increases in $p\text{CO}_2$ can have deleterious effects on calcifying animals, this change in carbonate chemistry may benefit some marine autotrophs. Here, we report on experiments performed with North Atlantic populations of hard clams (*Mercentaria mercenaria*), eastern oysters (*Crassostrea virginica*), bay scallops (*Argopecten irradians*), and blue mussels (*Mytilus edulis*) grown with and without North Atlantic populations of the green macroalgae, *Ulva*. In 6 of 7 experiments, exposure to elevated $p\text{CO}_2$ levels ($\sim 1,700 \mu\text{atm}$) resulted in depressed shell- and/or tissue-based growth rates of bivalves compared to control conditions (~~$p < 0.05$~~) whereas rates were significantly higher in the presence of *Ulva* in all experiments. ~~$p < 0.05$~~ . In many cases, the co-exposure elevated $p\text{CO}_2$ levels and *Ulva* had an antagonistic effect on bivalve growth rates whereby the presence of *Ulva* under elevated $p\text{CO}_2$ levels significantly improved their performance compared to the acidification only treatment. ~~$p < 0.05$~~ . Saturation states for calcium carbonate (Ω) were significantly higher in the presence of *Ulva* under both ambient and elevated CO_2 delivery rates (~~$p < 0.05$~~) and growth rates of bivalves were significantly correlated with Ω in six of seven ~~6 or 7~~ experiments. Collectively, the results suggest that photosynthesis and/or nitrate assimilation by *Ulva* increased alkalinity, fostering a carbonate chemistry regime more suitable for optimal growth of calcifying bivalves. This suggests that large natural and/or aquacultured collections of macroalgae in acidified environments could serve as a refuge for calcifying animals that may otherwise be negatively impacted by elevated $p\text{CO}_2$ levels and depressed Ω .

25

1 Introduction

The continued delivery of CO_2 into surface oceans is expected to cause significant shifts in pools of inorganic carbon by the end of this century, with projected increases in CO_2 and HCO_3^- and decreases in CO_3^{2-} and the saturation states of calcite (Ω_{calcite}) and aragonite ($\Omega_{\text{aragonite}}$) (Feely et al., 2009; Meehl et al., 2007). Beyond the delivery of CO_2 via the combustion of fossil fuels, upwelling, riverine discharge, eutrophication-accelerated microbial respiration all represent strong sources of CO_2 into coastal zones (Cai et al., 2011; Feely et al., 2008; Melzner et al., 2013; Salisbury et al., 2008; Wallace et al., 2014). Eutrophication-enhanced respiration in coastal zones can lead to the accumulation of respiratory CO_2 that can

exceed concentrations projected for the end of the century ($>2,000 \mu\text{atm}$), as well as result in the undersaturation of aragonite ($\Omega_{\text{aragonite}} < 1$; Cai et al., 2017; Wallace et al., 2014).

5 Calcifying organisms are highly vulnerable to the projected shifts in the various pools of total dissolved inorganic carbon (DIC), with the deleterious effects of ocean acidification being well-documented for corals (Hoegh-Guldberg et al., 2007; Kleypas et al., 1999), coralline algae (Gao and Zheng, 2010; Martin and Gattuso, 2009), and bivalves (Barton et al., 2012; Gazeau et al., 2007; Talmage and Gobler, 2011). Acidification-induced reductions in Ω_{calcite} and $\Omega_{\text{aragonite}}$ can result in lowered survivorship and inhibited growth for larvae and juvenile stage bivalves (Gobler et al., 2014; Green et al., 2009; Talmage and Gobler, 2011; Waldbusser et al., 2015a). Since bivalves provide numerous ecosystem and economic services (Newell, 2004), and elevated $p\text{CO}_2$ is a common occurrence in many coastal ecosystems (Feely et al., 2008; Salisbury et al., 2008; Wallace et al., 2014), it is important to understand how other co-occurring estuarine life will respond to high $p\text{CO}_2$ conditions and may, in turn, effect acidification-vulnerable organisms such as bivalves.

15 Contrary to the negative effects of increased CO_2 on calcifying organisms, previous studies have shown that some photosynthetic organisms, such as seagrasses (Koch et al., 2013; Palacios and Zimmerman, 2007), phytoplankton (Fu et al., 2012; Hattenrath-Lehmann et al., 2015), and macroalgae (Olischläger et al., 2013; Young and Gobler, 2016) may benefit from a high CO_2 environment. Such photosynthetic autotrophs may also have the capacity to buffer carbonate chemistry, potentially alleviating the harmful effects of excessive CO_2 on calcifying organisms. For example, prior studies have observed that ~~daytime primary~~ productivity within seagrass meadows can increase pH and $\Omega_{\text{aragonite}}$ which, under future acidified conditions, may provide temporal refuge for calcifying animals (Garrard et al., 2014; Hendriks et al., 2014; ~~Unsworth et al., 2012~~). Given the significant global declines in seagrass (Orth et al., 2006; Short et al., 2011; Waycott et al., 2009), as well as the overgrowth of seagrass beds by macroalgae (McGlathery, 2001; Valiela et al., 1997), it is plausible macroalgae may more commonly provide similar ecosystem services. While future increases in CO_2 may promote the growth of fast-growing, macroalgae such as *Ulva* (Björk et al., 1993; Olischläger et al., 2013; Young and Gobler, 2016, 2017) and could, in turn, could provide chemical resilience for calcifying organisms in acidified environments (Anthony et al., 2013; Wahl et al., 2017), such interactions have yet to be fully explored.

25 Recent studies have demonstrated that populations of *Ulva rigida* from Northwest Atlantic coastal waters experience enhanced growth under elevated CO_2 concentrations (Young and Gobler, 2016, 2017). While past studies have suggested that macroalgae may buffer carbonate chemistry to the benefit of bivalves (Anthony et al., 2013; Wahl et al., 2017), no study has assessed how *Ulva*, a common macroalga known to undergo enhanced growth under acidified and eutrophic conditions, may affect bivalves under CO_2 -enhanced conditions. The objective of this study, therefore, was to assess how elevated $p\text{CO}_2$ and the presence of *Ulva* influences the growth and survival of seven cohorts of ~~small- and large-sized of~~ juvenile bivalves indigenous to North Atlantic, including hard clams (= northern quahogs; *Mercenaria mercenaria*), eastern oysters (*Crassostrea virginica*), bay scallops (*Argopecten irradians*), and blue mussels (*Mytilus edulis*). Small- and large-sized individuals of bivalves were assessed for three species given the effects of ocean acidification can be size- and species dependent for juvenile bivalves (Talmage and Gobler, 2011; Waldbusser et al., 2010). Each bivalve cohort was

grown with and without elevated CO₂ levels as well as with and without *Ulva*. Growth and survival of the bivalves were quantified along with carbonate chemistry within experimental vessels.

2 Methods

5 2.1 Experimental design

Seven experiments were performed to assess the effects of elevated pCO₂ and the presence of *Ulva* on the growth and survival of *M. mercenaria*, *C. virginica*, *A. irradians*, and *M. edulis*. Experiments using smaller bivalves (1 – 5 mm) were performed in 1 L polycarbonate vessels, while experiments with larger bivalves (20 – 21 mm) were performed in larger, 8 L polycarbonate vessels. All containers were acid washed (10% HCl) and liberally rinsed with deionized water prior to use. The experimental vessels were placed in an environmental control chamber set to a consistent temperature (~21°C), light intensity (~200 μmol photons m⁻² s⁻¹) and duration (14 h: 10 h light:dark cycle). The light intensity and duration/photoperiod were set to mimic ambient conditions observed at the *Ulva* collection sites during the time of collection (see below). Containers were filled with filtered (0.2μm polysulfone filter capsule, Pall[®]) seawater and were randomly assigned, in quadruplicate, to one of four treatments: a control with ambient CO₂ concentrations (~400 μatm) without *Ulva*, a treatment with ambient CO₂ levels that received *Ulva*, a treatment with elevated CO₂ concentrations (~1700 μatm) without *Ulva*, and a treatment with elevated CO₂ and *Ulva*, resulting in 16 experimental containers. Two additional containers were filled with filtered seawater and bubbled in a manner identical to the ambient or elevated CO₂ treatments (described below) and were used to obtain initial dissolved inorganic carbon measurements. Continuous dissolved oxygen (DO) measurements were made using HOBO optical DO sensors (Onset[®]) in additional parallel vessels with and without *Ulva* added at the same levels used in experimental vessels and bubbled identically to experimental vessels. All experimental containers for each experiment received nutrient additions (50μM nitrate, 3 μM phosphate) at the beginning of the experiment, as well as after each twice weekly water changes (details below) to ensure nutrient replete growth of *Ulva*. The nutrient and CO₂ concentrations used during experiments were within the range of concentrations present in US East Coast estuaries (Baumann and Smith, 2017; Baumann et al., 2015; Wallace et al., 2014; Wallace and Gobler, 2015), and were used during prior experiments that involved *Ulva* from Shinnecock Bay, NY, USA (Young and Gobler, 2016, 2017). Across all experiments, bivalves were fed a mixture of *Isochrysis galbana* and *Chaetoceros muelleri* at rate known to be *ad libitum* (4 x 10⁴ cells mL⁻¹ d⁻¹; Helm et al., 2004) (~~Gobler et al., 2014~~). Microalgal cultures were maintained in exponential phase growth in f/2 media using standard culturing conditions (Helm et al., 2004).

To deliver dissolved gases, each experimental vessel was aerated via a 3.8 x 1.3 cm air diffuser (Pentair) connected to a 1 mL, polystyrene serological pipette inserted to the bottom of each vessel and connected via Tygon tubing to an air source. Containers were subjected to ambient (~400 μatm) and elevated (~1700 μatm) CO₂ concentrations via a gas proportionator system (Cole Parmer[®] Flowmeter system, multitube frame) that mixed ambient air with 5% CO₂ gas (Talmage and Gobler, 2010). Gases were mixed and delivered at a flow rate of 2500 ± 5 mL min⁻¹ through gang valves into the serological pipettes that fit through an opening in the plexiglass used to cover the experimental containers, turning over

the volume of the experimental containers >1000 times daily. Bubbling began two-to-three days prior to the start of each experiment to allow CO₂ concentrations and carbonate chemistry to reach a state of equilibrium. Experiments persisted for ~two weeks. Measurements of pH within containers were made daily with a Honeywell DuraFET III ion-sensitive field-effect transistor-based (ISFET) solid-state pH sensor (± 0.01 pH unit, total scale), which was calibrated with a seawater pH standard (Dickson, 1993). Measurements of pH made with the DuraFET were compared to measurements made spectrophotometrically using *m*-cresol purple (Dickson et al., 2007), and were found to be nearly identical and never significantly different. Discrete water samples were collected at the beginning and conclusion of experiments to directly measure DIC within each experimental vessel in each treatment (n=4 per treatment). The DIC samples were preserved using a saturated mercuric chloride (HgCl₂) solution and stored at ~4°C until analysis. Samples were analyzed by a VINDTA 3D (Versatile INstrument for the Determination of Total inorganic carbon) delivery system coupled with a UIC Inc. coulometer (model CM50170). During the coulometric analysis, all carbonate species were converted to CO₂ gas by the addition of excess hydrogen to the sample and the evolved CO₂ gas was subsequently carried into the titration cell of the coulometer. The gas then reacted quantitatively with an ethanolamine-based reagent to generate hydrogen ions, which were titrated with coulometrically-generated OH⁻, and CO₂ was measured by integrating the total change required to titrate the hydrogen ions (Johnson et al., 1993). Final Σ Total alkalinity, $\Omega_{\text{aragonite}}$, Ω_{calcite} , $p\text{CO}_2$, and concentrations of HCO₃⁻, CO₃²⁻ and OH⁻ (Tables 1 and S1) were calculated from measured levels of DIC, pH, temperature, and salinity, as well as the first and second dissociation constants of carbonic acid in seawater (Millero, 2010) using the program CO2SYS (<http://cdiac.ornl.gov/ftop/co2sys/>), representing the replicates for each treatment (n=4). For quality assurance, levels of DIC and pH within certified reference material (provided by Dr. Andrew Dickson of the University of California, San Diego, Scripps Institution of Oceanography; batches 158, 159 = 2044, 2027 $\mu\text{mol DIC kg seawater}^{-1}$, respectively) were measured during analyses of every set of samples. The analysis of samples continued only after complete recovery (99.8 ± 0.2 %) of certified reference material was attained. Actual mean $p\text{CO}_2$ and pH values were 350 μatm and 8.00, respectively for ambient conditions, and 1750 μatm and 7.38, respectively, for elevated CO₂ conditions, values within the range found seasonally in some estuarine environments (Baumann and Smith, 2017; Baumann et al., 2015; Wallace et al., 2014; Wallace and Gobler, 2015). Two-way ANOVAs and post-hoc tests were used to assess significant differences in carbonate chemistry among experimental vessels with the main treatment effects being $p\text{CO}_2$ (ambient or elevated) and the presence of *Ulva* within SigmaPlot 11.0.

2.2 Assessing the effects of elevated $p\text{CO}_2$ and *Ulva* on juvenile bivalves

The macroalgae used for this study were collected from Shinnecock Bay, NY, USA, (40.85° N, 72.50° W) during low tide. Permission to access this area and collect macroalgae and *M. edulis* was received from the Southampton Town Trustees, Southampton, NY, USA, who hold jurisdiction over Shinnecock Bay. Large, well-pigmented, robust fronds of *Ulva* were collected and transported to the Stony Brook Marine Science Center in seawater-filled containers within 15 minutes of collection. Previously, ITS sequencing and microscopy was used to determine that the species of *Ulva* that

dominated Shinnecock Bay in summer and fall was *Ulva rigida* (Young and Gobler, 2016, 2017) and microscopic examinations during this study indicated this was the species used in all experiments presented here. We refer to the algae simply as *Ulva* throughout the study due to the plastic nature of the macroalgal taxonomic nomenclature, as well as the high similarity of ITS sequences among species of *Ulva* (Hofmann et al., 2010; Kirkendale et al., 2013).

5 Well-pigmented, circular sections of *Ulva* (~3.5 cm and ~7 cm for experiments in small containers and large vessels (described below), respectively, with one disk per container were cut from the larger thalli with care taken to avoid the outer, potentially reproductive region of the algae (Wallace and Gobler, 2015). The weights of *Ulva* used in experiments relative to the vessels was consistent with the benthic coverage of *Ulva* in Shinnecock Bay (~8 g m⁻²; Gobler and Young, unpublished benthic trawl data) and other estuarine regions (Liu et al., 2015; Sfriso et al., 2001). Experimental disks of *Ulva* 10 were extensively rinsed with filtered (0.2 μm) seawater and spun in a salad spinner to remove debris and epiphytes with this step being repeated multiple times. *Ulva* samples were weighed on a Scientech ZSA 120 digital microbalance (± 0.0001 g) to obtain initial wet weight in grams. All samples were kept in 100 mL 0.2 μm filtered seawater-filled containers after spinning and weighing to prevent desiccation prior to use in experiments.

15 Small and large cohorts of *Mercenaria mercenaria* (~1 mm and ~5 mm, respectively) and *Argopecten irradians* (~5 mm and ~20 mm, respectively) used during experiments were spawned at the Stony Brook Marine Science Center of Stony Brook University hatchery (40.89° N, 72.44° W) using broodstock from Shinnecock Bay-derived broodstock collected 1–2one-to-two months prior to spawning and were exposed to environmental conditions (salinity, dissolved oxygen, pH) similar to their collection site.–Small and large cohorts of *Crassostrea virginica* (~2 mm and ~20 mm, respectively) used during experiments were produced by hatcheries within the Cornell Cooperative Extension of Southold shellfish hatchery, 20 NY, USA (40.04° N, 72.39° W) using broodstock from the Peconic Estuary, NY, USA. Cohorts of small juvenile *Mytilus edulis* (~5 mm) used during experiments were collected from Shinnecock Bay, NY, USA during low tide (40.84° N, 72.50° W). Experiments using smaller bivalves (1 – 5 mm) were performed in 1 L polycarbonate vessels with 20 individuals per vessel, while experiments with larger bivalves (20 – 21 mm) were performed in larger, 8 L polycarbonate vessels with five individuals per vessel.

25 Experiments began with the introduction of bivalves, *Ulva*, and nutrients into experimental vessels, with discrete and continuous measurements of pH and temperature, and continuous measurements of dissolved oxygen, and temperature made as described above throughout experiments. At the beginning of each experiment, 20 individuals from each bivalve cohort were set aside to obtain initial measurements of shell length (defined here as distance from umbo to furthest ventral margin), tissue weight, and shell weight. Bivalve dimensions were determined via digital calipers and digital images with the 30 two approaches producing nearly identical and not statistically different measurements. Captured images of bivalves were analyzed using ImageJ, with the scale of each image individually calibrated. Every three to four days, a complete water change was performed for all containers using water bubbled in 20-L carboys with gas mixtures for ambient and elevated CO₂ treatments as described above to ensure bivalves were exposed to their respective CO₂ concentrations.–Once weekly, *Ulva* disks from each container were removed, rinsed, spun in the salad spinner, weighed, and returned to the vessels.

Additionally, every week, bivalves were collected on a 500 μm sieve, transferred to a petri dish, and measured for length with any mortality noted. Mortality rates were very low (always $<10\%$) and did not differ among treatments. At the conclusion of experiments, final pH, temperature, and salinity measurements were made and final water samples for DIC analysis were collected and analyzed as described above. Additionally, 50 mL samples were removed from each container to assess final cell concentrations of phytoplankton provided for food (*I. galbana* and *C. muelleri*); which were preserved with Lugol's iodine (5%) solution and enumerated via microscopy (Tables 1 and S1).

At the conclusion of experiments, measurements of shell length for bivalves within the experimental containers as well as individuals set aside for initial measurements were made, and growth (expressed as mm d^{-1}) was determined from the changes in shell dimensions during the experiment. Tissue and shell weight were obtained by weighing bivalves after drying at 60°C for 72 hr, combusting them at 450°C for 4 hr, and weighing them again. Growth (expressed as mg d^{-1}) was determined by comparing the initial and final dry and combusted weights of individuals from each replicated vessel. Specifically, tissue weight was determined by subtracting the combusted weight from the dry weight, while shell weight was determined by subtracting the tissue weight from the dry weight. Two-way ANOVAs were performed using within SigmaPlot 11.0 to assess significant differences in growth rates based on shell length, tissue weight, shell weight, and survival during experiments, where the main treatment effects were $p\text{CO}_2$ (ambient or elevated), and the presence of *Ulva*. All data were log transformed prior to Two-way ANOVA to ensure that the assumptions of equal variance and normality were met. Normality was tested via the use of Shapiro-Wilk tests, which are built into SigmaPlot 11.0. If significant differences were detected, a Tukey Honest Significant Difference (Tukey HSD) test using R 3.4.0 within RStudio 1.0.143 was performed to identify specific differences among treatments. Finally, linear regression models of shell length-, tissue weight-, and shell weight-based growth rates with Ω_{calcite} and $\Omega_{\text{aragonite}}$ were performed created using R-® software (version: 3.4.0; <http://www.r-project.org>).

3 Results

3.1 *Mercenaria mercenaria*

For the cohort of smaller juvenile *M. mercenaria* (1.34 ± 0.24 mm), Ω_{calcite} and $\Omega_{\text{aragonite}}$ were significantly lower in treatments with elevated CO_2 (Two-way ANOVA; $p < 0.001$ for both, Fig. 1; Tables S2-S3) and significantly higher in treatments containing *Ulva* (Two-way ANOVA; $p = 0.002$ and $p = 0.007 < 0.05$, respectively for both; Fig. 1; Tables S2-S3). Growth of the small *M. mercenaria* based upon shell length, ~~tissue shell~~ weight, and ~~shell tissue~~ weight was highly sensitive to increases in $p\text{CO}_2$ as well as the presence of *Ulva*. When exposed to elevated CO_2 conditions, shell length-, ~~tissue shell~~ weight-, and ~~shell tissue~~ weight-based growth rates were 49%, 664%, and 4166% lower, respectively, when compared to their counterparts in ambient CO_2 treatments (Two-way ANOVA; $p < 0.001$, $p < 0.001$, and $p = 0.038$, respectively ~~5 for all~~; Fig. 1; Tables S4-S6). In contrast, shell length-, shell weight-, and tissue weight-based growth rates all growth rates were significantly higher in the presence of *Ulva* (Two-way ANOVA; $p = < 0.006$, $p = 0.011$, and $p = 0.008$, respectively ~~5~~; Fig. 1; Tables S4-S6) with growth based on shell length, tissue weight, and shell weight being 1528%, 3729%, and 4732% higher,

respectively, within elevated CO₂ treatments, and 10%, 25%, and 30%, respectively, within ambient CO₂ treatments (Fig. 1). Multiple comparison tests revealed that *Ulva* often mitigated the negative effects of elevated CO₂ on hard clams. For example, length-based growth in elevated CO₂ treatments with *Ulva* was significantly higher than elevated CO₂ treatments without *Ulva* (Tukey HSD; $p \leq 0.0445$; Table S7). However, this was not observed for tissue and shell weight based growth (Tukey HSD, $p > 0.05$; Tables S8-S9). Linear regressions between shell length-based growth rates and $\Omega_{\text{aragonite}}$ and Ω_{calcite} showed strong, significant positive correlations with $\Omega_{\text{aragonite}}$ and Ω_{calcite} across all treatments ($R^2=0.79$; $p < 0.001$, and $R^2=0.79$; $p < 0.001$, respectively; Table S10). There were also strong, positive significant correlations between shell weight-based growth and $\Omega_{\text{aragonite}}$ ($R^2=0.53$; $p=0.001$; Table S10) and Ω_{calcite} ($R^2=0.53$; $p=0.002$; Table S11). For tissue weight-based growth, there were also positive significant correlations with $\Omega_{\text{aragonite}}$ and Ω_{calcite} that approached significance ($R^2=0.30$; $p=0.052$ and $R^2=0.30$; $p=0.054$, respectively; Tables S10-S11).

For the larger-sized cohort of *M. mercenaria* (5.00 ± 0.41 mm), Ω_{calcite} and $\Omega_{\text{aragonite}}$ were significantly higher in treatments containing *Ulva* (Two-way ANOVA; $p=0.002$ and $p < 0.001$, respectively; Fig. 2; Tables S2-S3) and significantly lower in high CO₂ treatments (Two-way ANOVA; $p < 0.001$ for both $p < 0.05$ for both; Fig. 2; Tables S2-S3). Larger *M. mercenaria* responded to elevated CO₂ conditions and the presence of *Ulva* in a manner similar to that of the smaller clams. Under elevated CO₂ concentrations, shell length-, shell weight-, and tissue weight-based, and shell-based growth rates were significantly lower (by 45%, 3022%, and 2230%, respectively) relative than the ambient CO₂ treatments (Two-way ANOVA; $p=0.010 < 0.05$, $p=0.010$, and $p < 0.001$, respectively; Fig. 2; Tables S4-S6). In the presence of *Ulva*, however, shell length-, shell weight-, and tissue weight-based length-, tissue-, and shell-based growth rates were significantly higher (by 1026%, 2146%, and 2033%, respectively, in elevated CO₂ treatments, and by 21%, 18%, 162%, respectively, in ambient CO₂ treatments) relative to treatments that did not receive *Ulva* (Two-way ANOVA; $p \leq 0.003$, $p=0.006$, and $p=0.009$, respectively; Fig. 2; Tables S4-S6). Across all treatments, shell length- and tissue weight-based growth rates were positively correlated with $\Omega_{\text{aragonite}}$ ($R^2=0.45$; $p=0.006$ and $R^2=0.44$; $p=0.013$, respectively; Table S10) and Ω_{calcite} ($R^2=0.45$; $p=0.006$ and $R^2=0.44$; $p=0.013$, respectively; Table S11). For shell weight-based growth, there were positive, nearly significant correlations with $\Omega_{\text{aragonite}}$ and Ω_{calcite} that approached significance ($R^2=0.28$; $p=0.063$ and $R^2=0.28$; $p=0.063$, respectively; Tables S10-S11).

3.2 *Crassostrea virginica*

During the experiment with the cohort of small *C. virginica* (2.45 ± 0.41 mm), Ω_{calcite} and $\Omega_{\text{aragonite}}$ were significantly higher in treatments containing *Ulva* (Two-way ANOVA; $p=0.025$ for both; Fig. 3; Tables S2-S3) and significantly lower in treatments receiving elevated CO₂ (Two-way ANOVA; $p < 0.001$ for both $p < 0.05$ for both; Fig. 3; Tables S2-S3). Growth rates of small *C. virginica* were sensitive to elevated CO₂ concentrations and the presence of *Ulva*. Length-, tissue-, and shell weight-based growth rates were 63%, 78%, and 145% lower, respectively, when exposed to elevated CO₂ concentrations compared to control treatments (Two-way ANOVA; $p \leq 0.0115$, $p=0.006$, and $p=0.012$, respectively; Fig. 3; Tables S4-S6). When in the presence of *Ulva*, shell length-based growth was significantly increased by 2442% and 55% in elevated and

ambient CO₂ treatments, respectively (Two-way ANOVA; $p \leq 0.0405$; Fig. 3; Table S4), but tissue and shell weight-based growth were not significantly different than the control (Two-way ANOVA; $p \geq 0.319$ and $p = 0.946$, respectively). Linear regressions showed across all experimental vessels, there were strong significant positive correlations between shell length-, tissue weight-, and shell weight-based growth and $\Omega_{\text{aragonite}}$ ($R^2 = 0.26$; $p = 0.044$, $R^2 = 0.53$; $p = 0.003$, and $R^2 = 0.39$; $p = 0.013$, respectively; Table S10) and Ω_{calcite} ($R^2 = 0.26$; $p = 0.045$, $R^2 = 0.53$; $p = 0.003$, and $R^2 = 0.39$; $p = 0.013$, respectively; Table S11).

For the larger juvenile *C. virginica* (24.92 ± 0.89 mm), Ω_{calcite} and $\Omega_{\text{aragonite}}$ were significantly higher in treatments containing *Ulva* (Two-way ANOVA; $p < 0.001$ for both; Fig. 4; Tables S2-S3) and significantly lower in treatments receiving elevated CO₂ (Two-way ANOVA; $p < 0.001$ for both) (Two-way ANOVA; $p < 0.05$ for both; Fig. 4; Tables S2-S3). Growth responses for the larger *C. virginica* differed from the smaller-sized juveniles. Shell length-based growth was 167% and significantly lower under elevated CO₂ concentrations relative to the control and significantly higher (by 23% and 450% in ambient and elevated CO₂ treatments, respectively 72%) in the presence of *Ulva* relative to the control (Two-way ANOVA; $p \leq 0.001$ and $p = 0.006$, respectively 5; Fig. 4; Table S4). While shell weight-based and tissue weight-based growth were not significantly altered by elevated CO₂ or the presence of *Ulva*, there was an antagonistic, interactive effect between both variables whereby the co-exposure to elevated CO₂ and *Ulva* yielded growth rates higher than would have been predicted by growth rates within the individual treatments (Two-way ANOVA; $p \leq 0.0245$; Fig. 4; Tables S5-S6). Consistent with this finding, shell length-based growth in elevated CO₂ treatments with *Ulva* was significantly higher than in elevated CO₂ treatments without *Ulva* (Tukey HSD; $p \leq 0.0325$; Table S7). There was a strong positive correlation between shell length-based growth and $\Omega_{\text{aragonite}}$ ($R^2 = 0.66$; $p = 0.002$, respectively; Table S10) and Ω_{calcite} ($R^2 = 0.66$; $p = 0.002$, respectively; Table S11) but not for ~~There were no significant correlations between tissue and shell weight-based growth and $\Omega_{\text{aragonite}}$ or Ω_{calcite} (Tables S10-S11).~~

3.3 *Argopecten irradians*

For the cohort of small *A. irradians* (4.73 ± 0.59 mm), Ω_{calcite} and $\Omega_{\text{aragonite}}$ were significantly higher in treatments containing *Ulva* (Two-way ANOVA; $p < 0.001$ for both; Fig. 5; Tables S2-S3) and significantly lower in treatments with elevated CO₂ (Two-way ANOVA; $p < 0.05$ for both; Fig. 5; Tables S2-S3) ($p < 0.001$ for both). The growth of small juvenile *A. irradians* was altered by pCO₂ and, to a lesser extent, the presence of *Ulva*. Shell length-, tissue weight-, and shell weight-based growth rates All measurements of growth were significantly reduced by exposure to elevated CO₂ concentrations (Two-way ANOVA; $p < 0.001$, $p = 0.023$, and $p = 0.041$, respectively 5; Fig. 5; Tables S4-S6). Specifically, growth rates based on shell length, tissue weight, and shell weight were 26%, 40%, and 43% lower, respectively, when exposed to elevated CO₂ compared to ambient CO₂ treatments (Fig. 5). Shell length-based growth was significantly higher (by 10% and 29% in ambient and elevated CO₂ treatments, respectively 5%) in the presence of *Ulva* relative to treatments that did not receive *Ulva* (Two-way ANOVA; $p = 0.007$; Fig. 5; Table S4). In contrast, tissue and shell weight-based growth were not significantly affected by the presence of *Ulva* (Two-way ANOVA; $p \geq 0.27405$ and $p = 0.637$, respectively; Fig. 5; Tables S52-S63). Shell length-based growth within elevated CO₂ treatments with *Ulva* was significantly higher than in the elevated

CO₂ treatments without *Ulva* (Tukey HSD; $p \leq 0.0115$; Table S7). There were no significant differences in shell or tissue weight-based growth among any treatments (Tukey HSD; $p > 0.05$ for all; Tables S8-S9). Comparisons within individual treatments showed that shell length-based growth within elevated CO₂ treatments without *Ulva* was significantly lower than the elevated CO₂ treatments with *Ulva* (Tukey HSD; $p \leq 0.011$; Table S7). Linear regressions showed strong For all treatments, there were significant positive correlations between shell length-, tissue weight-, and shell weight-based growth of smaller scallops and $\Omega_{\text{aragonite}}$ ($R^2=0.56$; $p=0.001$, $R^2=0.36$; $p=0.018$, and $R^2=0.47$; $p=0.004$, respectively; Table S10) and Ω_{calcite} ($R^2=0.56$; $p=0.001$, $R^2=0.36$; $p=0.018$, and $R^2=0.47$; $p=0.004$, respectively; Table S11).

For the larger cohorts of juvenile *A. irradians* (21.08 ± 1.06 mm), Ω_{calcite} and $\Omega_{\text{aragonite}}$ were significantly lower in treatments exposed to high CO₂ and significantly higher in treatments containing *Ulva* (Two-way ANOVA; $p < 0.0015$ for both; Fig. 6; Tables S2-S3). The growth rates of larger *A. irradians* based on shell length and tissue weight were significantly reduced under elevated CO₂ concentrations by 32% and 105%, respectively (Two-way ANOVA; $p < 0.001$ and $p = 0.019$, respectively; Fig. 6; Tables S4 and S6) while shell weight-based growth was not (Two-way ANOVA; $p \geq 0.55305$; Table S5). Growth rates based on shell length and tissue weight were significantly increased in the presence of *Ulva* by 1646% and 16449%, respectively, in elevated CO₂ treatments, and by 60% and 16%, respectively, in ambient CO₂ treatments (Two-way ANOVA; $p \leq 0.016$ and $p = 0.032$, respectively; Fig. 6; Tables S4 and S6) while shell weight-based growth was not (Two-way ANOVA; $p \geq 0.39005$; Table S5). There was a strong positive correlation between shell length-based growth of larger scallops and $\Omega_{\text{aragonite}}$ ($R^2=0.74$; $p=0.001$, respectively; Table S10) and Ω_{calcite} ($R^2=0.74$; $p=0.001$, respectively; Table S11). There were no significant correlations between but not for tissue and shell weight-based growth and $\Omega_{\text{aragonite}}$ or Ω_{calcite} (Tables S10-S11). Comparisons within individual treatments showed that shell length-based growth within elevated CO₂ treatments without *Ulva* was significantly lower than the elevated CO₂ treatments with *Ulva* (Tukey HSD; $p < 0.01$; Table S7).

3.4 *Mytilus edulis*

During the experiments with *M. edulis* (4.87 ± 0.92 mm), Ω_{calcite} and $\Omega_{\text{aragonite}}$ were significantly higher in treatments containing *Ulva* (Two-way ANOVA; $p=0.017$ and $p=0.020$, respectively; Fig. 7; Tables S2-S3) and significantly lower in treatments exposed to high CO₂ (Two-way ANOVA; $p < 0.0015$ for both; Fig. 7; Tables S2-S3). Growth rates of *M. edulis* based on shell length, tissue weight, and shell weight were all not significantly changed by exposure to elevated CO₂ concentrations (Two-way ANOVA; $p=0.149$, $p=0.210$, and $p=0.439$, respectively > 0.05 ; Fig. 7; Tables S4-S6). In contrast, shell length-, tissue weight-, and shell weight-based growth all growth-measurements were significantly higher in the presence of *Ulva* (Two-way ANOVA; $p=0.045$, $p=0.047$, and $p=0.024$, respectively $p < 0.05$; Fig. 7; Tables S4-S6). Specifically, in the presence of *Ulva*, growth based on shell length, tissue weight, and shell weight was 1624%, 3025%, and 4544% higher, respectively, in elevated CO₂ treatments, and 28%, 19%, and 36%, respectively, in ambient CO₂ treatments relative to treatments that did not receive *Ulva*, regardless of CO₂ concentration (Fig. 7). There were no significant

~~correlations between shell length, tissue and shell weight based growth~~ Mussel growth rates were not correlated with ~~and~~ $\Omega_{\text{aragonite}}$ or Ω_{calcite} (Tables S10-S11).

3.5 *Ulva* and microalgae

5 ~~_____~~ Across all experiments, ~~t~~he growth of *Ulva* was found to be, on average, significantly higher by 20% when exposed to elevated CO₂ concentrations (One-way ANOVA; $p=0.043$; Fig. S1; Table S12). ~~Under elevated CO₂ conditions, *Ulva* growth increased by ~20% (Fig. S1).~~ Concentrations of *Isochrysis galbana* and *Chaetoceros muelleri* were not significantly different between any treatment ~~across all~~ in any experiments (Two-way ANOVA; $p<0.05$ for all; Table S12). On average, final cell concentrations within treatments were ~~~905,000~~ $\sim 95,000$ cells mL⁻¹ (Tables 1 and S1).

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4 Discussion

During this study, elevated CO₂ concentrations significantly reduced at least one or more growth measurements of ~~cohorts of~~ small- and large-sized ~~cohorts of~~ juvenile *Mercenaria mercenaria*, *Crassostrea virginica*, and *Argopecten irradians*, but not *Mytilus edulis*. The presence of *Ulva* significantly increased the growth of all cohorts of all bivalve species. Comparisons of individual treatments indicated that under elevated CO₂ concentrations, the addition of *Ulva* often significantly increased growth rates ~~for cohorts of~~ clams, scallops, and oysters ~~by 23 – 30%~~. Both $\Omega_{\text{aragonite}}$ and Ω_{calcite} were significantly higher in the presence of *Ulva* in all experiments under both high and low CO₂ regimes, despite the rapid turnover of dissolved gas pools in experiments (>1000 time per day), ~~and the growth rates of bivalves were significantly correlated with $\Omega_{\text{aragonite}}$ and Ω_{calcite} in treatment vessels for six of seven experiments.~~ Collectively, these findings provide insight regarding the ability of macroalgae such as *Ulva* to mitigate the deleterious effects of ocean acidification on bivalves, and, potentially, other calcifying organisms.

The negative effects of ocean acidification on the growth and survival of bivalves and other calcifying organisms have been well-documented. Consistent with prior studies that have gauged the response of juvenile bivalves to elevated CO₂ (Gazeau et al., 2007; Green et al., 2009; Talmage and Gobler, 2011), the results of the current study show decreased tissue growth as well as calcification in the form of shell length- and weight-based growth ~~under acidified conditions~~, a finding consistent with significantly lower $\Omega_{\text{aragonite}}$ and Ω_{calcite} in elevated CO₂ treatments. Early life-stage bivalve shells are composed partly or completely of aragonite, making them vulnerable to undersaturation of aragonite (Carriker, 1996; Stenzel, 1964; Talmage and Gobler, 2009). While the formation of calcium carbonate is thermodynamically favored when Ω exceeds 1.0, biotic aragonite is less crystalline than nonbiogenic aragonite (Weiss et al., 2002) and studies of early life stage Pacific oysters have suggested that a $\Omega_{\text{aragonite}}$ exceeding 1.6 may be required to yield successful growth and survival (Barton et al., 2012). Similarly, Talmage and Gobler (2010) found that increases in $\Omega_{\text{aragonite}}$ within the saturated range ($\Omega_{\text{aragonite}}$ increases from 2.9 to 3.3) significantly increased the growth of early life stage *M. mercenaria* and *A. irradians*, a finding suggesting that acidification since pre-industrial time ~~can~~ depresses the performance of these ~~individuals~~ species. In the current study, growth rates of bivalves exposed to *Ulva* under ambient $p\text{CO}_2$ frequently exceeded those of individuals grown

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under the same CO₂ delivery rate without *Ulva* as $\Omega_{\text{aragonite}}$ was significantly increased, on average from 1.91 to 2.16 (Table 1), with both levels being saturated but also being below the threshold that yielded maximal growth rates in early life stage bivalves for Talmage and Gobler (2010). Furthermore, even minor, yet sustained, increases or decreases in Ω_{calcite} and $\Omega_{\text{aragonite}}$ (<0.1 units) can result in significant changes in the growth of larval and juvenile bivalves (Barton et al., 2012; Talmage and Gobler, 2011), which was similarly observed in ~~some~~ many of the experiments in the present study. Within the present study, the presence of *Ulva* within elevated CO₂ treatments yielded, on average, shell length, tissue weight, and shell weight based growth rates that were 23%, 27%, and 30%, respectively, higher than in treatments without *Ulva*. Hence, the potential benefits of macroalgae to calcifying bivalves may be realized in both acidified and ‘normal’ conditions.

Acidification can have cascading negative physiological consequences for bivalves. In larval bivalves, high CO₂ depresses calcification, lipid content, RNA:DNA ratios, metamorphosis, and growth rates (Gobler and Talmage, 2013). The reduction in tissue weight-based growth under elevated CO₂ concentrations found during the present study is consistent with Beniash et al. (2010), who found significant declines in soft body mass of juvenile *C. virginica* maintained in hypercapnia (pH 7.5). Additionally, the same study and others (Gazeau et al., 2007; Matoo et al., 2013) have reported increased metabolic rates in bivalves exposed to elevated CO₂ levels. As suggested by Waldbusser et al. (2015b), decreasing $\Omega_{\text{aragonite}}$ increases the amount of energy spent by bivalves on shell formation which diverts energy away from maintaining homeostasis and other metabolic processes including those that contribute toward growth (Beniash et al., 2010; Waldbusser et al., 2015b).

Macroalgae can control carbonate chemistry in shallow ecosystems and, in turn, can affect the performance of carbonaceous organisms. A study by Anthony et al. (2013) found that within mixed assemblages of turf and fleshy macroalgae, the saturation state of aragonite increased during the daytime. Krause-Jensen et al. (2015) reported that macroalgae may provide a refuge for calcifying organisms. Specifically, within a subarctic fjord, macroalgae drove strong diel variability in pH and $\Omega_{\text{aragonite}}$, with *M. edulis* being found to grow in close association with macroalgae, even in tidal pools that became supersaturated and undersaturated between day and night cycles, respectively (Krause-Jensen et al., 2015). Additionally, Wahl et al. (2017) demonstrated that daytime increases in pH associated with the macroalgae *Fucus vesiculosus* provided a refuge against acidified conditions for *M. edulis*, with calcification rates of *M. edulis* increasing with increases in pH wrought by the algae. In the current study, *Ulva* yielded significantly increased $\Omega_{\text{aragonite}}$, Ω_{calcite} , and bivalve growth in all seven experiments performed. Furthermore, dissolved Dissolved oxygen levels were also high (> 7 mg L⁻¹) in all treatments and the growth rates of bivalves were often significantly higher in high CO₂ treatments with *Ulva* compared to those without. Furthermore, in ambient and elevated CO₂ treatments, the presence of *Ulva* significantly increased pH beyond levels observed in treatments without *Ulva*, sometimes often in as little as 24 hr, with those increases sustaineding the over duration of the experiments (Figs. S2-S3). Hence, it seems likely that the macroalgae buffered carbonate chemistry to the benefit of bivalves. While it is possible that photosynthetic activity by microalgae (*I. galbana* and *C. muelleri*) may have contributed to shifts in carbonate chemistry, there were no significant differences in microalgae cell concentrations in any treatment across all experiments where microalgae were enumerated (Two-way ANOVA; $p > 0.05$; Table S13). This

~~suggestings that microalgal contributions was to changes in carbonate chemistry; were minimal and overshadowed by relative to the photosynthetic activity by the macroalgae of *Ulva*.~~

Beyond photosynthesis, macroalgae may also alter carbonate chemistry via the uptake of nitrogenous nutrients. Specifically, the uptake of nitrate or ammonium by marine autotrophs results in an equimolar increase or decrease in total alkalinity, respectively (Brewer and Goldman, 1976; Goldman and Brewer, 1980; Talmage and Gobler, 2012), which occurs due to the production of OH⁻ and H⁺ to balance the uptake of nitrate and ammonium, respectively (Brewer and Goldman, 1976; Goldman and Brewer, 1980; Redfield et al., 1963). Given that 50 μM nitrate was added to all experimental vessels with *Ulva* to promote its growth during each experimental water change, it is possible that the assimilation of this nitrate by *Ulva* contributed to the average 10 – 20 μM increase in total alkalinity observed within treatments with *Ulva* (Two-way ANOVA; $p < 0.05$; Table 1; Tables S140-S154). Higher alkalinity seawater requires higher concentrations of CO₂ to reduce pH, thus resulting in smaller changes in $\Omega_{\text{aragonite}}$ and Ω_{calcite} . Given the rapid turnover of dissolved gasses in experimental vessels, it is possible the nitrogen assimilation effects on alkalinity outweighed the effects of photosynthetic consumption of DIC.

Prior studies have found that *Ulva* can experience enhanced growth (Björk et al., 1993; Olischläger et al., 2013; Young and Gobler, 2016) and outcompete other autotrophs (Young and Gobler, 2017) under elevated CO₂ concentrations. Hence, the dominance of *Ulva* and similar macroalgae in estuaries that experience seasonal acidification (Wallace and Gobler, 2015) could ultimately benefit bivalves and other calcifying organisms. In the present experiments, *Ulva* growth was, on average, ~20% higher under elevated CO₂ conditions. ~~(One-way ANOVA; $p < 0.05$; Fig. S1 and Table S12).~~ Furthermore, the presence of the macroalgae frequently transformed $\Omega_{\text{aragonite}}$ of elevated CO₂ treatments from undersaturated to nearly saturated (Tables 1 and S1) and often yielded growth rates of bivalves significantly greater than the elevated CO₂ treatments without *Ulva*. Had the dissolved gas pools within experimental vessels not been turned over rapidly via aeration, it is possible the effects of *Ulva* on the carbonate chemistry would have been even greater.

The benefits of *Ulva* and detriments of high CO₂ to the four bivalves studied differed by species. While every cohort displayed significantly enhanced growth in the presence of *Ulva*, scallops were the only species to experience significantly higher growth in the elevated CO₂ treatment with *Ulva* compared to the elevated CO₂ treatment with ~~out~~ *Ulva* for both the small and large juvenile cohorts. In contrast, for clams and oysters, only one of the two cohorts displayed this specific response. Early life stages of bay scallops have been consistently shown to be more vulnerable to acidification than the other bivalve species studied here (Stevens and Gobler, in revision; Talmage and Gobler, 2009, 2011). This may be due, in part, to its rapid growth and metabolism compared to other bivalves (Kennedy et al., 1996; Krauter and Castagna, 2001; Shumway and Parsons, 2006), traits that may also make it more likely to benefit from the improved carbonate chemistry wrought by the presence of *Ulva*. The resistance of *M. edulis* to elevated CO₂ concentrations contrasted with prior studies of European strains of this bivalve (Berge et al., 2006; Gazeau et al., 2007) but is consistent with prior cohorts of this species isolated from Shinnecock Bay, NY, USA (Stevens and Gobler, in revision). However, Thomsen et al. (2012) found that specific growth and calcification rates of juvenile *M. edulis* under acidified conditions were dependent ~~more~~ on food availability ~~than carbonate chemistry~~. Given that food was supplied *ad libitum* in the present study, it is possible that the

negative effects of elevated CO₂ concentrations on *M. edulis* may have been mitigated by adequate food availability as well as improved carbonate chemistry facilitated by *Ulva*.

Beyond the modification of carbonate chemistry, there are additional ecosystem benefits that may be provided by macroalgae. Macroalgal beds can serve as ~~an~~ nursery habitat for juvenile *Callinectes sapidus* (Wilson et al., 1990), as well as other decapods (Heck et al., 2003; Sogard and Able, 1991). Macroalgae can also serve as a refuge from predation for some juvenile and adult bivalves (Carroll et al., 2010). An additional potential benefit provided to bivalves by *Ulva* and other macroalgae is their ability to inhibit the growth of phytoplankton species that cause harmful algal blooms (HABs; Tang and Gobler, 2011; Tang et al., 2015) that can directly harm the bivalve species used in the present study (Gobler and Sunda, 2012; Leverone et al., 2006; Stoecker et al., 2008; Tang and Gobler, 2009). Furthermore, given its ability to rapidly assimilate and store nitrate and ammonium (Pedersen and Borum, 1997), *Ulva* can serve as a biofilter within eutrophic ecosystems (Hernández et al., 2002; Neori et al., 2003). Given that harmful algal blooms flourish in eutrophic zones (Anderson et al., 2008; Anderson et al., 2002), the mitigation of high nutrient conditions by *Ulva* may reduce the intensity of HABs, which may indirectly benefit bivalve species that are negatively impacted by the occurrence of such events. Finally, there is great precedent for the deployment of macroalgae as a principal component of integrated multi-trophic aquaculture systems whereby seaweeds are co-cultivated with aquacultured shellfish with the macroalgae often being harvested for profit (Neori, 2008; Nobre et al., 2010; Troell et al., 2009). Such an approach may be increasingly important for the protection of aquacultured bivalves in an increasing acidified ocean in the future.

Despite the reported positive interactions between *Ulva* and the various species of bivalves in prior studies (Carroll et al., 2010; Heck et al., 2003; Sogard and Able, 1991; Wilson et al., 1990; this study), macroalgae can negatively impact bivalves and other calcifying organisms. Secondary metabolites released by *Ulva* can elevate mortality rates in the larval stages of bivalves (Diederich, 2005; Nelson et al., 2003), barnacles (Brock et al., 2007; Magre, 1974), crabs (Johnson and Welsh, 1985), and molluscs (Wang et al., 2011). *Ulva* can form “green tides” (Smetacek and Zingone, 2013) that, upon their collapse, can create hypoxic regions (Valiela et al., 1992) that can negatively affect benthic fauna (Viaroli et al., 2001). Furthermore, extensive coverage of bivalves by *Ulva* and the subsequent decomposition of the algae can also result in the accumulation of H₂S, which, when coupled with low dissolved oxygen, can depress the growth and survival of bivalves (Tyler, 2007). However, as pointed out by Wilson et al. (1990), the accumulation of secondary metabolites and decreased dissolved oxygen associated with the overgrowth of *Ulva* is often mitigated in high-flow areas, alleviating potential harm to the nearby organisms. Furthermore, it is plausible-likely that other macroalgae that are not known to negatively impact marine life provide similar buffering of carbonate chemistry (Anthony et al., 2013; Krause-Jensen et al., 2015; Wahl et al., 2017).

Numerous species of seagrass experience enhanced growth under elevated CO₂ concentrations (Beer and Koch, 1996; Palacios and Zimmerman, 2007; Zimmerman et al., 1997) and can buffer ocean acidification thus benefiting calcifying organisms (Garrard et al., 2014; Hendriks et al., 2014; ~~Unsworth et al., 2012~~). For example, a theoretical model by Unsworth et al. (2012) found that seagrass meadows increased can increase pH and $\Omega_{\text{aragonite}}$ by 0.38 and 2.9 units, respectively, which

~~has the potential to increase resulted in calcification by corals downstream of the seagrass by to be ~18% higher relative to environments without seagrass, should these benefits be realized in a natural setting.~~ However, this ecosystem service provided by seagrass meadows may be disrupted by eutrophication (Valiela et al., 1997) and acidification (Young et al., in press) of coastal ecosystems which ~~could may~~ favor the growth of macroalgae over seagrass (Valiela et al., 1997; Young et al., in revisionpress). ~~Fast growing, opportunistic macroalgae such as *Ulva* can inhibit the growth of seagrasses through shading (Valiela et al., 1997), competition for nutrients (Duarte, 1995), changes in the biogeochemical environment (Hauxwell et al., 2001), and under high CO₂ conditions (Young et al., in revision). *Ulva* has, however, been shown to serve as a nursery habitat for epibenthic invertebrates at densities comparable to *Zostera* meadows (Heck et al., 2003; Sogard and Able, 1991) in areas where there is a lack of the seagrass.~~ As seagrasses decline worldwide (Orth et al., 2006; Short et al., 2011), the ecosystem services provided by seagrasses, such as being nursery habitats or buffering against ocean acidification, may, in some cases, be provided by macroalgae, potentially benefiting calcifying organisms such as bivalves that had formerly depended on seagrass as a refuge habitat.

In conclusion, during this study photosynthetic activity and/or nitrate assimilation by *Ulva* increased $\Omega_{\text{aragonite}}$ and Ω_{calcite} and yielded enhanced growth of bivalves by mitigating the deleterious effects of elevated $p\text{CO}_2$. This benefit was not exclusive to acidified conditions, as evidenced by increased bivalve growth in the presence of *Ulva* within ambient CO₂ treatments. While macroalgae can have adverse effects on some larval-staged bivalves, the chemical resilience provided by the macroalgae, *Ulva*, along with other potential ecosystem benefits such as providing nursery habitat (Wilson et al., 1990), predation refuge (Carroll et al., 2010), and inhibiting the growth of harmful microalgae (Tang and Gobler, 2011; Tang et al., 2015) may, in some case, outweigh the negative effects. ~~Although seagrass meadows can also buffer carbonate chemistry to the benefit of bivalves and other calcifying organisms, their populations continue to display worldwide declines (Orth et al., 2006; Short et al., 2011).~~ Given that macroalgae tend to outcompete seagrass under high CO₂ conditions (Young et al., in revisionpress), the ability of macroalgae to provide ecosystem services similar to those of seagrass, particularly buffering carbonate chemistry, may be increasingly important for calcifying organisms in modern-day eutrophic, acidified estuaries, as well as within future, ocean acidification scenarios. Finally, the purposeful deployment of seaweeds in an aquaculture setting would seem to be a beneficial strategy for protecting bivalves against current and future acidification.

5 Author contributions

Conceived and designed the experiments: C.J.G., C.S.Y. Performed the experiments: C.S.Y. Analyzed the data: C.S.Y., C.J.G. Contributed reagents/materials/analysis tools: C.J.G. Wrote the manuscript: C.S.Y., C.J.G.

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5 7. References

- Anderson, D. M., Glibert, P. M., and Burkholder, J. M.: Harmful algal blooms and eutrophication: Nutrient sources, composition, and consequences, *Estuaries*, 25, 704-726, doi: 10.1007/BF02804901, 2002.
- Anderson, D. M., Burkholder, J. M., Cochlan, W. P., Glibert, P. M., Gobler, C. J., Heil, C. A., Kudela, R. M., Parsons, M. L., Rensel, J. E. J., Townsend, D. W., Trainer, V. L., and Vargo, G. A.: Harmful algal blooms and eutrophication: Examining linkages from selected coastal regions of the United States, *Harmful Algae*, 8, 39-53, doi: 10.1016/j.hal.2008.08.017, 2008.
- Anthony, K. R. N., Diaz-Pulido, G., Verlinden, N., Tilbrook, B., and Andersson, A. J.: Benthic buffers and boosters of ocean acidification on coral reefs, *Biogeosciences*, 10, 4897-4909, doi: 10.5194/bg-10-4897-2013, 2013.
- Barton, A., Hales, B., Waldbusser, G. G., Langdon, C., and Feely, R. A.: The Pacific oyster, *Crassostrea gigas*, shows negative correlation to naturally elevated carbon dioxide levels: Implications for near-term ocean acidification effects, *Limnol. Oceanogr.*, 57, 698-710, doi: 10.4319/lo.2012.57.3.0698, 2012.
- Baumann, H., Wallace, R. B., Tagliaferri, T., and Gobler, C. J.: Large natural pH, CO₂ and O₂ fluctuations in a temperate tidal salt marsh on diel, seasonal, and interannual time scales, *Estuar. Coast.*, 38, 220-231, doi: 10.1007/s12237-014-9800-y, 2015.
- Baumann, H., and Smith, E. M.: Quantifying metabolically driven pH and oxygen fluctuations in US nearshore habitats at diel to interannual time scales, *Estuar. Coast.*, 1-16, doi: 10.1007/s1223, 2017.
- Beer, S., and Koch, E.: Photosynthesis of marine macroalgae and seagrasses in globally changing CO₂ environments, *Mar. Ecol.-Prog. Ser.*, 141, 199-204, doi: 10.3354/meps141199, 1996.
- Beniash, E., Ivanina, A., Lieb, N. S., Kurochkin, I., and Sokolova, I. M.: Elevated level of carbon dioxide affects metabolism and shell formation in oysters *Crassostrea virginica*, *Mar. Ecol.-Prog. Ser.*, 419, 95-108, doi: 10.3354/meps08841, 2010.
- Berge, J. A., Bjerkeng, B., Pettersen, O., Schaanning, M. T., and Oxnevad, S.: Effects of increased sea water concentrations of CO₂ on growth of the bivalve *Mytilus edulis* L, *Chemosphere*, 62, 681-687, doi: 10.1016/j.chemosphere.2005.04.111, 2006.
- Björk, M., Haglund, K., Ramazanov, Z., and Pedersén, M.: Inducible mechanisms for HCO₃⁻ utilization and repression of photorespiration in protoplasts and thalli of three species of *Ulva* (Chlorophyta), *J. Phycol.*, 29, 166-173, doi: 10.1111/j.0022-3646.1993.00166.x, 1993.
- Brewer, P. G., and Goldman, J. C.: Alkalinity changes generated by phytoplankton growth, *Limnol. Oceanogr.*, 21, 108-117, doi: 10.4319/lo.1976.21.1.0108, 1976.
- Brock, E., Nylund, G. M., and Pavia, H.: Chemical inhibition of barnacle larval settlement by the brown alga *Fucus vesiculosus*, *Mar. Ecol.-Prog. Ser.*, 337, 165-174, doi: 10.3354/meps337165, 2007.

- Cai, W.-J., Hu, X., Huang, W.-J., Murrell, M. C., Lehrter, J. C., Lohrenz, S. E., Chou, W.-C., Zhai, W., Hollibaugh, J. T., Wang, Y., Zhao, P., Guo, X., Gundersen, K., Dai, M., and Gong, G.-C.: Acidification of subsurface coastal waters enhanced by eutrophication, *Nat. Geosci.*, 4, 766-770, doi: 10.1038/ngeo1297, 2011.
- 5 Cai, W.-J., Huang, W.-J., Luther, G. W., Pierrot, D., Li, M., Testa, J., Xue, M., Joesoef, A., Mann, R., Brodeur, J., Xu, Y.-Y., Chen, B., Hussain, N., Waldbusser, G. G., Cornwell, J., and Kemp, W. M.: Redox reactions and weak buffering capacity lead to acidification in the Chesapeake Bay, *Nat. Commun.*, 8, 369, doi: 10.1038/s41467-017-00417-7, 2017.
- Carriker, M. R.: The shell and ligament, in: *The Eastern oyster: Crassostrea virginica*, edited by: Kennedy, V. S., Newell, R. I. E., and Eble, A. E., Maryland Sea Grant College, University of Maryland System, 75-168, 1996.
- 10 Carroll, J. M., Peterson, B. J., Bonal, D., Weinstock, A., Smith, C. F., and Tettelbach, S. T.: Comparative survival of bay scallops in eelgrass and the introduced alga, *Codium fragile*, in a New York estuary, *Mar. Biol.*, 157, 249-259, doi: 10.1007/s00227-009-1312-0, 2010.
- [Dickson, A. G.: The measurement of sea water pH, *Mar. Chem.*, 44, 131-142, doi: 10.1016/0304-4203\(93\)90198-W, 1993.](#)
- [Dickson, A. G., Sabine, C. L., and Christian, J. R.: Guide to best practices for ocean CO₂ measurements, *PICES Special Publication*, 3, 191 pp., doi, 2007.](#)
- 15
- Diederich, S.: Differential recruitment of introduced Pacific oysters and native mussels at the North Sea coast: Coexistence possible?, *J. Sea Res.*, 53, 269-281, doi: 10.1016/j.seares.2005.01.002, 2005.
- ~~[Duarte, C. M.: Submerged aquatic vegetation in relation to different nutrient regimes, *Ophelia*, 41, 87-112, doi: 10.1080/00785236.1995.10422039, 1995.](#)~~
- 20 Feely, R. A., Sabine, C. L., Hernandez-Ayon, J. M., Ianson, D., and Hales, B.: Evidence for upwelling of corrosive "acidified" water onto the continental shelf, *Science*, 320, 1490-1492, doi: 10.1126/science.1155676, 2008.
- Feely, R. A., Doney, S. C., and Cooley, S. R.: Ocean acidification: Present conditions and future changes in a high-CO₂ world, *Oceanography*, 22, 36-47, doi: 10.5670/oceanog.2009.95, 2009.
- 25 Fu, F. X., Tatters, A. O., and Hutchins, D. A.: Global change and the future of harmful algal blooms in the ocean, *Mar. Ecol.-Prog. Ser.*, 470, 207-233, doi: 10.3354/meps10047, 2012.
- Gao, K., and Zheng, Y.: Combined effects of ocean acidification and solar UV radiation on photosynthesis, growth, pigmentation and calcification of the coralline alga *Corallina sessilis* (Rhodophyta), *Global Change Biol.*, 16, 2388-2398, doi: 10.1111/j.1365-2486.2009.02113.x, 2010.
- 30 Garrard, S. L., Gambi, M. C., Scipione, M. B., Patti, F. P., Lorenti, M., Zupo, V., Paterson, D. M., and Buia, M. C.: Indirect effects may buffer negative responses of seagrass invertebrate communities to ocean acidification, *J. Exp. Mar. Biol. Ecol.*, 461, 31-38, doi: 10.1016/j.jembe.2014.07.011, 2014.
- Gazeau, F., Quiblier, C., Jansen, J. M., Gattuso, J.-P., Middelburg, J. J., and Heip, C. H. R.: Impact of elevated CO₂ on shellfish calcification, *Geophys. Res. Lett.*, 34, L07603, doi: 10.1029/2006GL028554, 2007.
- 35 Gobler, C. J., and Sunda, W. G.: Ecosystem disruptive algal blooms of the brown tide species, *Aureococcus anophagefferens* and *Aureoumbra lagunensis*, *Harmful Algae*, 14, 36-45, doi: 10.1016/j.hal.2011.10.013, 2012.

- Gobler, C. J., and Talmage, S. C.: Short- and long-term consequences of larval stage exposure to constantly and ephemerally elevated carbon dioxide for marine bivalve populations, *Biogeosciences*, 10, 2241-2253, doi: 10.5194/bgd-9-15901-2012, 2013.
- 5 Gobler, C. J., DePasquale, E. L., Griffith, A. W., and Baumann, H.: Hypoxia and acidification have additive and synergistic negative effects on the growth, survival, and metamorphosis of early life stage bivalves, *PLoS ONE*, 9, e83648, doi: 10.1371/journal.pone.0083648, 2014.
- Goldman, J. C., and Brewer, P. G.: Effect of nitrogen source and growth rate on phytoplankton-mediated changes in alkalinity, *Limnol. Oceanogr.*, 25, 353-357, doi: 10.4319/lo.1980.25.2.0352, 1980.
- 10 Green, M. A., Waldbusser, G. G., Reilly, S. L., Emerson, K., and O'Donnell, S.: Death by dissolution: sediment saturation state as a mortality factor for juvenile bivalves, *Limnol. Oceanogr.*, 54, 1037-1047, doi: 10.4319/lo.2009.54.4.1037, 2009.
- Hattenrath-Lehmann, T. K., Smith, J. L., Wallace, R. B., Merlo, L. R., Koch, F., Mittelsdorf, H., Goleski, J. A., Anderson, D. M., and Gobler, C. J.: The effects of elevated CO₂ on the growth and toxicity of field populations and cultures of the saxitoxin-producing dinoflagellate, *Alexandrium fundyense*, *Limnol. Oceanogr.*, 60, 198-214, doi: 10.1002/lno.10012, 2015.
- 15 ~~Hauxwell, J., Cebrian, J., Furlong, C., and Valiela, I.: Macroalgal canopies contribute to eelgrass (*Zostera marina*) decline in temperate estuarine ecosystems, *Ecology*, 82, 1007-1022, doi, 2001.~~
- Heck, K. L., G., H., and Orth, R. J.: Critical evaluation of the nursery role hypothesis for seagrass meadows, *Mar. Ecol.-Prog. Ser.*, 253, 123-136, doi: 10.3354/meps253123, 2003.
- Helm, M. M., Bourne, N., and Lovatelli, A.: *Hatchery Culture of Bivalves: A Practical Manual*, Food and Agriculture Organization of the United Nations, 177 pp., 2004.
- 20 Hendriks, I. E., Olsen, Y. S., Ramajo, L., Basso, L., Steckbauer, A., Moore, T. S., Howard, J., and Duarte, C. M.: Photosynthetic activity buffers ocean acidification in seagrass meadows, *Biogeosciences*, 11, 333-346, doi: 10.5194/bgd-10-12313-2013, 2014.
- 25 Hernández, I., Martínez-Aragón, J. F., Tovar, A., Pérez-Lloréns, J. L., and Vergara, J. J.: Biofiltering efficiency in removal of dissolved nutrients by three species of estuarine macroalgae cultivated with sea bass (*Dicentrarchus labrax*) waste waters 2. Ammonium, *J. Appl. Phycol.*, 14, 375-384, doi: 10.1023/A:1022178417203, 2002.
- Hoegh-Guldberg, O., Mumby, P. J., Hooten, A. J., Steneck, R. S., Greenfield, P., Gomez, E., Harvell, C. D., Sale, P. F., Edwards, A. J., K., C., Knowlton, N., Eakin, C. M., Iglesias-Prieto, R., Muthiga, N., Bradbury, R. H., Dubi, A., and Hatziolos, M. E.: Coral reefs under rapid climate change and ocean acidification, *Science*, 318, 1737-1742, doi: 10.1126/science.1152509, 2007.
- 30 Hofmann, L. C., Nettleton, J. C., Neefus, C. D., and Mathieson, A. C.: Cryptic diversity of *Ulva* (Ulvales, Chlorophyta) in the Great Bay Estuarine System (Atlantic USA): introduced and indigenous distromatic species, *Eur. J. Phycol.*, 45, 230-239, doi: 10.1080/09670261003746201, 2010.
- Johnson, D. A., and Welsh, B. L.: Detrimental effects of *Ulva lactuca* (L.) exudates and low oxygen on estuarine crab larvae, *J. Exp. Mar. Biol. Ecol.*, 86, 73-83, doi: 10.1016/0022-0981(85)90043-7, 1985.
- 35 Johnson, K. M., Wills, K. D., Butler, D. B., Johnson, W. K., and Wong, C. S.: Coulometric total carbon dioxide analysis for marine studies: maximizing the performance of an automated gas extraction system and coulometric detector, *Mar. Chem.*, 44, 167-187, doi: 10.1016/0304-4203(93)90201-X, 1993.

- Kennedy, V. S., Newell, R. I. E., and Eble, A. E.: The Eastern oyster: *Crassostrea virginica*, College Park, MD, Maryland Sea Grant College, 1996.
- Kirkendale, L., Saunders, G. W., and Winberg, P.: A molecular survey of *Ulva* (Chlorophyta) in temperate Australia reveals enhanced levels of cosmopolitanism, *J. Phycol.*, 49, 69-81, doi: 10.1111/jpy.12016, 2013.
- 5 Kleypas, J. A., Buddemeier, R. W., Archer, D., Gattuso, J.-P., Langdon, C., and Opdyke, B. N.: Geochemical consequences of increased atmospheric carbon dioxide on coral reefs, *Science*, 284, 118-120, doi: 10.1126/science.284.5411.118, 1999.
- Koch, M., Bowes, G., Ross, C., and Zhang, X.-H.: Climate change and ocean acidification effects on seagrasses and marine macroalgae, *Global Change Biol.*, 19, 103-132, doi, 2013.
- Kraeuter, J. N., and Castagna, M.: *Biology of the Hard Clam*, Elsevier Science, New York, NY, 2001.
- 10 Krause-Jensen, D., Duarte, C. M., Hendriks, I. E., Meire, L., Blicher, M. E., Marbà, N., and Sejr, M. K.: Macroalgae contribute to nested mosaics of pH variability in a subarctic fjord, *Biogeosciences*, 12, 4895-4911, doi: 10.5194/bg-12-4895-2015, 2015.
- Leverone, J. R., Blake, N. J., Pierce, R. H., and Shumway, S. E.: Effects of the dinoflagellate *Karenia brevis* on larval development in three species of bivalve mollusc from Florida, *Toxicon*, 48, 75-84, doi: 10.1016/j.toxicon.2006.04.012, 2006.
- 15 Liu, X., Li, Y., Wang, Z., Zhang, Q., and Cai, X.: Cruise observation of *Ulva prolifera* bloom in the southern Yellow Sea, China, *Estuar. Coast. Shelf Sci.*, 163, 17-22, doi: 10.1016/j.ecss.2014.09.014, 2015.
- Magre, E. J.: *Ulva lactuca* L. negatively affects *Balanus balanoides* (L.) (Cirripedia Thoracica) in tidepools, *Crustaceana*, 27, 231-234, doi: 10.1163/156854074X00758, 1974.
- Martin, S., and Gattuso, J.-P.: Response of Mediterranean coralline algae to ocean acidification and elevated temperature, *Global Change Biol.*, 15, 2089-2100, doi: 10.1111/j.1365-2486.2009.01874.x, 2009.
- 20 Matoo, O. B., Ivanina, A. V., Ullstad, C., Beniash, E., and Sokolova, I. M.: Interactive effects of elevated temperature and CO₂ levels on metabolism and oxidative stress in two common marine bivalves (*Crassostrea virginica* and *Mercenaria mercenaria*), *Comp. Biochem. Physiol. A.*, 164, 545-553, doi: 10.1016/j.cbpa.2012.12.025, 2013.
- McGlathery, K. J.: Macroalgal blooms contribute to the decline of seagrass in nutrient-enriched coastal waters, *J. Phycol.*, 25, 37, 453-456, doi: 10.1046/j.1529-8817.2001.037004453.x, 2001.
- Meehl, G. A., Stocker, T. F., Collins, W. D., Friedlingstein, P., Gaye, A. T., Gregory, J. M., Kitoh, A., Knutti, R., Murphy, J. M., Noda, A., Raper, S. C. B., Watterson, I. G., Weaver, A. J., and Zhao, Z.-C.: *Global Climate Projections*, Cambridge University Press, Cambridge, 747-845, 2007.
- Melzner, F., Jörn, T., Koeve, W., Oschlies, A., Gutowska, M. A., Bange, H. W., Hansen, H. P., and Körtzinger, A.: Future ocean acidification will be amplified by hypoxia in coastal habitats, *Mar. Biol.*, 160, 1875-1888, doi: 10.1007/s00227-012-1954-1, 2013.
- 30 Millero, F. J.: History of the equation of state of seawater, *Oceanography*, 23, 18-33, doi: 10.5670/oceanog.2010.21, 2010.
- Nelson, T. A., Lee, D. J., and Smith, B. C.: Are “green tides” harmful algal blooms? Toxic properties of water-soluble extracts from two bloom-forming macroalgae, *Ulva fenestrata* and *Ulvaria obscura* (Ulvophyceae), *J. Phycol.*, 39, 874-879, 35 doi: 10.1046/j.1529-8817.2003.02157.x, 2003.

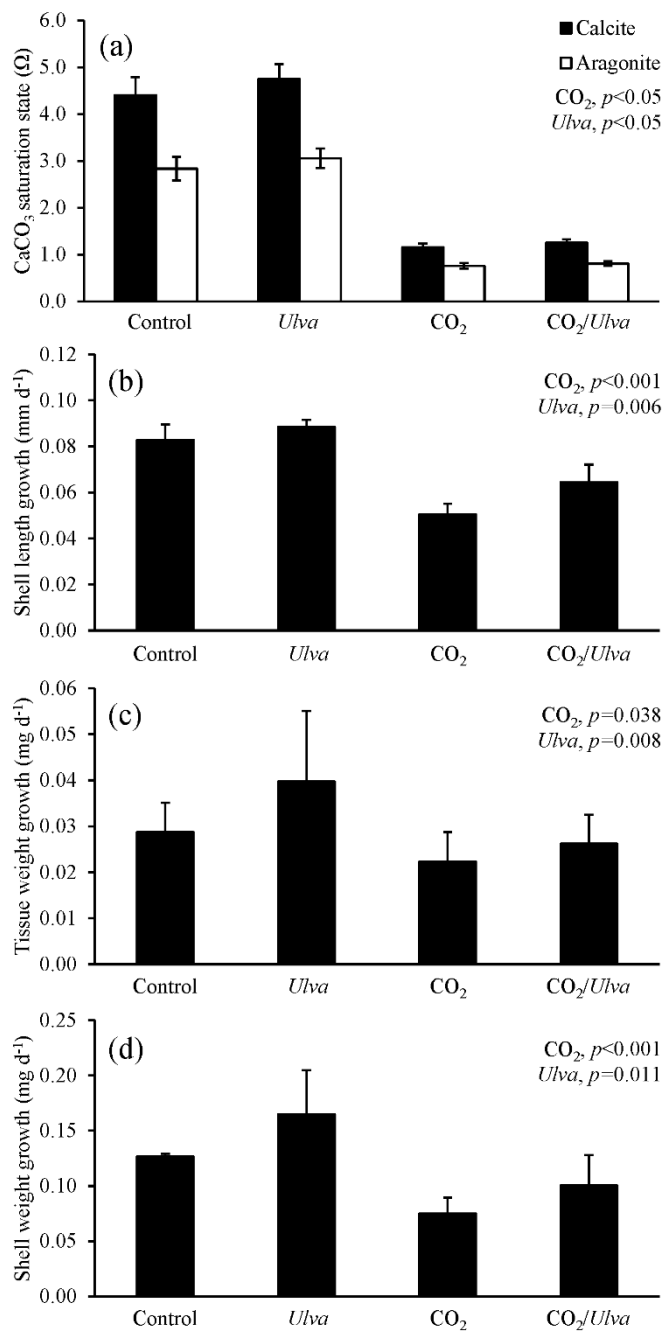
- Neori, A., Msuya, F. E., Shauli, L., Schuenhoff, A., Kopel, F., and Shpigel, M.: A novel three-stage seaweed (*Ulva lactuca*) biofilter design for integrated mariculture, *J. Appl. Phycol.*, 15, 543-553, doi: 10.1023/B:JAPH.0000004382.89142.2d, 2003.
- Neori, A.: Essential role of seaweed cultivation in integrated multi-trophic aquaculture farms for global expansion of mariculture: an analysis, *J. Appl. Phycol.*, 20, 567-570, doi: 10.1007/s10811-007-9206-3, 2008.
- 5 Newell, R. I. E.: Ecosystem influences of natural and cultivated populations of suspension-feeding bivalve molluscs: A review, *J. Shellfish Res.*, 23, 51-61, doi, 2004.
- Nobre, A. M., Robertson-Andersson, D., Neori, A., and Sankar, K.: Ecological-economic assessment of aquaculture options: Comparison between abalone monoculture and integrated multi-trophic aquaculture of abalone and seaweeds, *Aquaculture*, 306, 116-126, doi: 10.1016/j.aquaculture.2010.06.002, 2010.
- 10 Olischläger, M., Bartsch, I., Gutow, L., and Wiencke, C.: Effects of ocean acidification on growth and physiology of *Ulva lactuca* (Chlorophyta) in a rockpool-scenario, *Phycological Res.*, 61, 180-190, doi: 10.1111/pre.12006, 2013.
- Orth, R. J., Carruthers, T. J. B., Dennison, W. C., Duarte, C. M., Fourqurean, J. W., Heck, K. L., Hughes, A. R., Kendrick, G. A., Kenworthy, W. J., Olyarnik, S., Short, F. T., Waycott, M., and Williams, S. L.: A global crisis for seagrass ecosystems, *BioScience*, 56, 987-996, doi: 10.1641/0006-3568(2006)56[987:agcfse]2.0.co;2, 2006.
- 15 Palacios, S. L., and Zimmerman, R. C.: Response of eelgrass *Zostera marina* to CO₂ enrichment: possible impacts of climate change and potential for remediation of coastal habitats, *Mar. Ecol.-Prog. Ser.*, 344, 1-13, doi: 10.3354/meps07084, 2007.
- Pedersen, M. F., and Borum, J.: Nutrient control of estuarine macroalgae: growth strategy and the balance between nitrogen requirements and uptake, *Mar. Ecol.-Prog. Ser.*, 161, 155-163, doi: 10.3354/meps161155, 1997.
- Redfield, A. C., Ketchum, B. H., and Richards, F. A.: The influence of organisms on the composition of sea water, in: *The Sea*, edited by: Hill, M. N., Interscience, 1963.
- Salisbury, J., Green, M., Hunt, C., and Campbell, J.: Coastal acidification by rivers: a new threat to shellfish?, *Eos Trans. AGU*, 89, 513, doi: 10.1029/2008EO500001, 2008.
- Sfriso, A., Birkemeyer, T., and Ghetti, P. F.: Benthic macrofauna changes in areas of Venice lagoon populated by seagrasses or seaweeds, *Marine Environmental Research*, 52, 323-349, doi: 10.1016/S0141-1136(01)00089-7, 2001.
- 25 Short, F. T., Polidoro, B., Livingstone, S. R., Carpenter, K. E., Bandeira, S., Bujang, J. S., Calumpong, H. P., Carruthers, T. J. B., Coles, R. G., Dennison, W. C., Erfteimeijer, P. L. A., Fortes, M. D., Freeman, A. S., Jagtap, T. G., Kamal, A. H. M., Kendrick, G. A., Kenworthy, W. J., Nafie, Y. A. L., Nasution, I. M., Orth, R. J., Prathep, A., Sanciangco, J. C., van Tussenbroek, B., Vergara, S. G., Waycott, M., and Zieman, J. C.: Extinction risk assessment of the world's seagrass species, *Biol. Cons.*, 144, 1961-1971, doi: 10.1016/j.biocon.2011.04.010, 2011.
- 30 Shumway, S. E., and Parsons, G. J.: *Scallops: Biology, Ecology, and Aquaculture*, Elsevier, Boston, MA, 2006.
- Smetacek, V., and Zingone, A.: Green and golden seaweed tides on the rise, *Nature*, 504, 84-88, doi: 10.1038/nature12860, 2013.
- Sogard, S. M., and Able, K. W.: A comparison of eelgrass, sea lettuce macroalgae, and marsh creeks as habitats for epibenthic fishes and decapods, *Estuar. Coast. Shelf Sci.*, 33, 501-519, doi: 10.1016/0272-7714(91)90087-R, 1991.
- 35 Stenzel, H. B.: Oysters: Composition of the larval shell, *Science*, 304, 1005-1008, doi: 10.1126/science.145.3628.155, 1964.

- Stevens, A. M., and Gobler, C. J.: Interactive effects of acidification, hypoxia, and thermal stress on growth, respiration, and survival of four North Atlantic bivalves, *Mar. Ecol.-Prog. Ser.*, doi: unavailable, in revision.
- Stoecker, D. K., Adolf, J. E., Place, A. R., Glibert, P. M., and Meritt, D. W.: Effects of the dinoflagellates *Karodinium veneficum* and *Prorocentrum minimum* on early life history stages of the eastern oyster (*Crassostrea virginica*), *Mar. Biol.*, 5 154, 81-90, doi: 10.1007/s00227-007-0901-z, 2008.
- Talmage, S. C., and Gobler, C. J.: The effects of elevated carbon dioxide concentrations on the metamorphosis, size, and survival of larval hard clams (*Mercenaria mercenaria*), bay scallops (*Argopecten irradians*), and Eastern oysters (*Crassostrea virginica*), *Limnol. Oceanogr.*, 54, 2072-2080, doi: 10.4319/lo.2009.54.6.2072, 2009.
- Talmage, S. C., and Gobler, C. J.: Effects of past, present, and future ocean carbon dioxide concentrations on the growth and survival of larval shellfish, *Proc. Natl. Acad. Sci. U.S.A.*, 107, 17246-17251, doi: 10.1073/pnas.0913804107, 2010.
- Talmage, S. C., and Gobler, C. J.: Effects of elevated temperature and carbon dioxide on the growth and survival of larvae and juveniles of three species of northwest Atlantic bivalves, *PLoS ONE*, 6, e26941, doi: 10.1371/journal.pone.0026941, 2011.
- Talmage, S. C., and Gobler, C. J.: Effects of CO₂ and the harmful alga *Aureococcus anophagefferens* on growth and survival of oyster and scallop larvae, *Mar. Ecol.-Prog. Ser.*, 464, 121-134, doi: 10.3354/meps09867, 2012.
- Tang, Y. Z., and Gobler, C. J.: *Cochlodinium polykrikoides* blooms and clonal isolates from the northwest Atlantic coast cause rapid mortality in larvae of multiple bivalve species, *Mar. Biol.*, 156, 2601-2611, doi: 10.1007/s00227-009-1285-z, 2009.
- Tang, Y. Z., and Gobler, C. J.: The green macroalga, *Ulva lactuca*, inhibits the growth of seven common harmful algal bloom species via allelopathy, *Harmful Algae*, 10, 480-488, doi: 10.1016/j.hal.2011.03.003, 2011.
- Tang, Y. Z., Kang, Y., Berry, D., and Gobler, C. J.: The ability of the red macroalga, *Porphyra purpurea* (Rhodophyceae) to inhibit the proliferation of seven common harmful microalgae, *J. Appl. Phycol.*, 27, 531-544, doi: 10.1007/s10811-014-0338-y, 2015.
- Thomsen, J., Casties, I., Pansch, C., Körtzinger, A., and Melzner, F.: Food availability outweighs ocean acidification effects in juvenile *Mytilus edulis*: laboratory and field experiments, *Global Change Biol.*, 19, 1017-1027, doi: 10.1111/gcb.12109, 2012.
- Troell, M., Joyce, A., Chopin, T., Neori, A., Buschmann, A. H., and Fang, J.-G.: Ecological engineering in aquaculture - Potential for integrated multi-trophic aquaculture (IMTA) in marine offshore systems, *Aquaculture*, 297, 1-9, doi: 10.1016/j.aquaculture.2009.09.010, 2009.
- 30 Tyler, R. M.: Effects of coverage by benthic seaweed mats on (northern quahog = hard clam) *Mercenaria mercenaria* in a eutrophic estuary, *J. Shellfish Res.*, 26, 1021-1028, doi: 10.2983/0730-8000(2007)26[1021:EOCBBS]2.0.CO;2, 2007.
- ~~Unsworth, R. K. F., Collier, C. J., Henderson, G. M., and McKenzie, L. J.: Tropical seagrass meadows modify seawater carbon chemistry: Implications for coral reefs impacted by ocean acidification, *Environ. Res. Lett.*, 7, 024026, doi: 10.1088/1748-9326/7/2/024026, 2012.~~
- 35 Valiela, I., Foreman, K., LaMontagne, M., Hersh, D., Costa, J., Peckol, P., DeMeo-Andreson, B., D'Avanzo, C., Babione, M., Sham, C.-H., Brawley, J., and Lajtha, K.: Couplings of watersheds and coastal waters: Sources and consequences of nutrient enrichment in Waquoit Bay, Massachusetts, *Estuaries*, 15, 443-457, doi: 10.2307/1352389, 1992.

- Valiela, I., McClelland, J., Hauxwell, J., Behr, P. J., Hersh, D., and Foreman, K.: Macroalgal blooms in shallow estuaries: Controls and ecophysiological and ecosystem consequences, *Limnol. Oceanogr.*, 42, 1105-1118, doi: 10.4319/lo.1997.42.5_part_2.1105, 1997.
- 5 Viaroli, P., Azzoni, R., Bartoli, M., Giordano, G., and Tajé, L.: Evolution of the Trophic Conditions and Dystrophic Outbreaks in the Sacca di Goro Lagoon (Northern Adriatic Sea), in: *Mediterranean Ecosystems*, edited by: Faranda, F. M., Guglielmo, L., Spezie, G., Springer, Milano, 467-475, 2001.
- Wahl, M., Schneider Covachã, S., Saderne, V., Hiebenthal, C., Müller, J. D., Pansch, C., and Sawall, Y.: Macroalgae may mitigate ocean acidification effects on mussel calcification by increasing pH and its fluctuations, *Limnol. Oceanogr.*, doi: 10.1002/lno.10608, 2017.
- 10 [Waldbusser, G. G., Bergschneider, H., and Green, M. A. 2010. Size-dependent pH effect on calcification in post-larval hard clam *Mercenaria* spp. *Mar. Ecol.-Prog. Ser.*, 417, 171-182, doi: 10.3354/meps08809.](#)
- Waldbusser, G., Hales, B., Langdon, C. J., Haley, B. A., Schrader, P., Brunner, E. L., Gray, M. W., Miller, C. A., and Gimenez, I.: Saturation-state sensitivity of marine bivalve larvae to ocean acidification, *Nat. Clim. Change*, 5, 273-280, doi: 10.1002/grl.50449, 2015a.
- 15 Waldbusser, G. G., Hales, B., Langdon, C., Haley, B. A., Schrader, P., Brunner, E. L., Gray, M. W., Miller, C. A., Gimenez, I., and Hutchins, D. A.: Ocean acidification has multiple modes of action on bivalve larvae, *PLoS ONE*, 10, e0128376, doi: 10.1371/journal.pone.0128376, 2015b.
- Wallace, R. B., Baumann, H., Grear, J. S., Aller, R. C., and Gobler, C. J.: Coastal ocean acidification: The other eutrophication problem, *Estuar. Coast. Shelf Sci.*, 148, 1-13, doi: 10.1016/j.ecss.2014.05.027, 2014.
- 20 Wallace, R. B., and Gobler, C. J.: Factors controlling blooms of microalgae and macroalgae (*Ulva rigida*) in a eutrophic, urban estuary: Jamaica Bay, NY, USA, *Estuar. Coast.*, 38, 519-533, doi: 10.1007/s12237-014-9818-1, 2015.
- Wang, C., Yu, R.-c., and Zhou, M.-J.: Acute toxicity of live and decomposing green alga *Ulva (Enteromorpha) prolifera* to abalone *Haliotis discus hannai*, *Chinese J. Oceanol. Limnol.*, 29, 541-546, doi: 10.1007/s00343-011-0126-3, 2011.
- 25 Waycott, M., Duarte, C. M., Caruthers, T. J. B., Orth, R. J., Dennison, W. C., Olyarnik, S., Calladine, A., Fourqurean, J. W., Heck, K. L., Hughes, A. R. A., Kendrick, G. A., Kenworthy, W. J., Short, F. T., and Williams, S. L.: Accelerating loss of seagrasses across the globe threatens coastal ecosystems, *Proc. Natl. Acad. Sci. U.S.A.*, 106, 12377-12381, doi: 10.1073/pnas.0905620106, 2009.
- Weiss, I. M., Tuross, N., Addadi, L., and Weiner, S.: Mollusc larval shell formation: Amorphous calcium carbonate is a precursor phase for aragonite, *J. Exp. Zool.*, 293, 478-491, doi: 10.1002/jez.90004, 2002.
- 30 Wilson, K. A., Able, K. W., and Heck, K. L., Jr.: Predation rates on juvenile blue crabs in estuarine nursery habitats: evidence for the importance of macroalgae (*Ulva lactuca*), *Mar. Ecol.-Prog. Ser.*, 58, 243-251, doi: 10.3354/meps058243, 1990.
- Young, C. S., and Gobler, C. J.: Ocean acidification accelerates the growth of two bloom-forming, estuarine macroalgae, *PLoS ONE*, 11, e0155152, doi: 10.1371/journal.pone.0155152, 2016.
- 35 Young, C. S., and Gobler, C. J.: The organizing effects of elevated CO₂ on competition among estuarine primary producers, *Sci. Rep.*, 7, 7667, doi: 10.1038/s41598-017-08178-5, 2017.

Young, C. S., Peterson, B. J., and Gobler, C. J.: The bloom forming macroalgae, *Ulva*, outcompetes the seagrass, *Zostera marina*, under high CO₂ conditions, *Estuar. Coast.*, doi: unavailable, in revision.

Zimmerman, R. C., Kohrs, D. G., Steller, D. L., and Alberte, R. S.: Impacts of CO₂ enrichment on productivity and light requirements of eelgrass, *Plant Physiol.*, 115, 599-607, doi: 10.1104/pp.115.2.599, 1997.



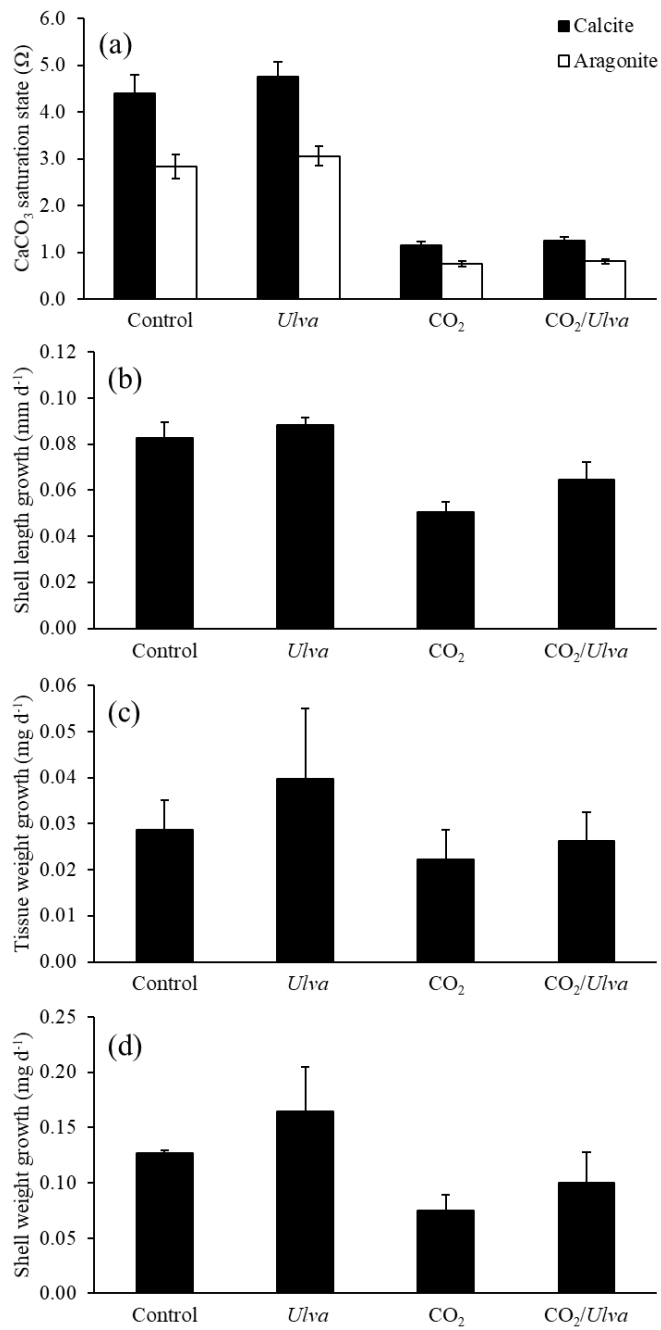
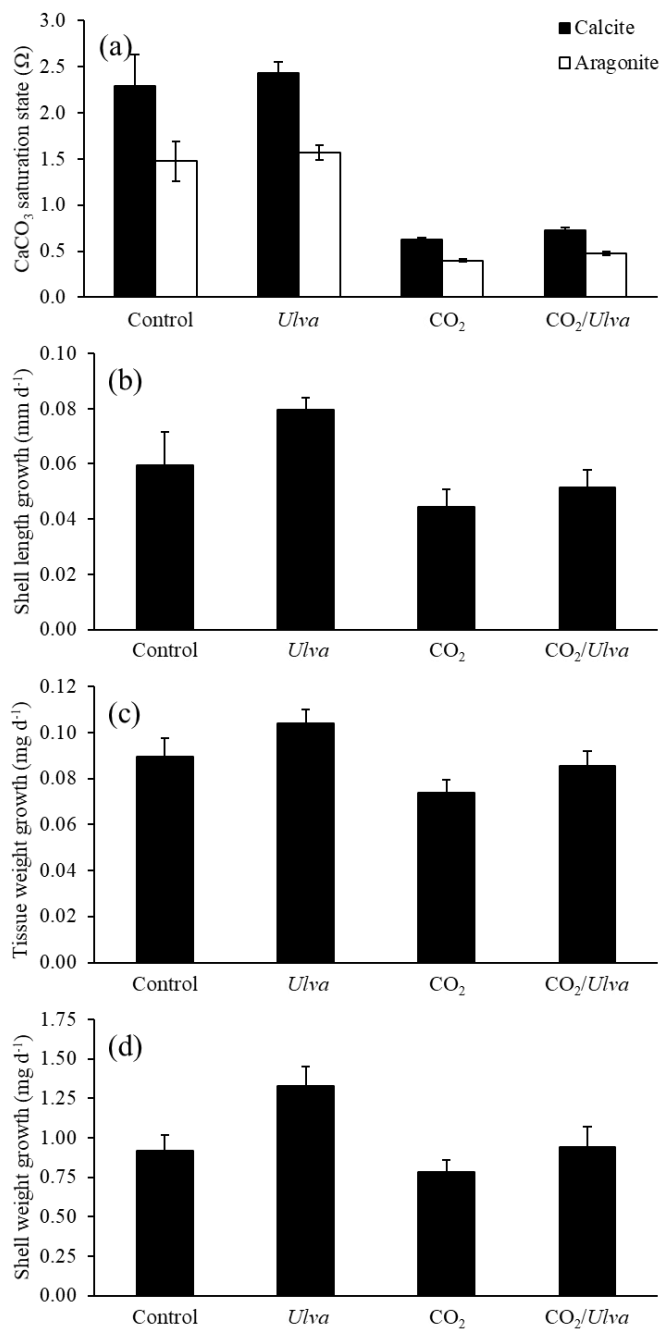


Figure 1. Experiment with small juvenile *Mercenaria mercenaria* exposed to ambient and elevated concentrations of CO₂ with and without the presence of *Ulva*.; (a) Ω_{calcite} and $\Omega_{\text{aragonite}}$; Growth was based on (b) shell length; (c) tissue weight; and (d) shell weight. Columns represent means \pm standard deviation. Significant main treatment effects (CO₂ and *Ulva*) appear on the top right of each figure.

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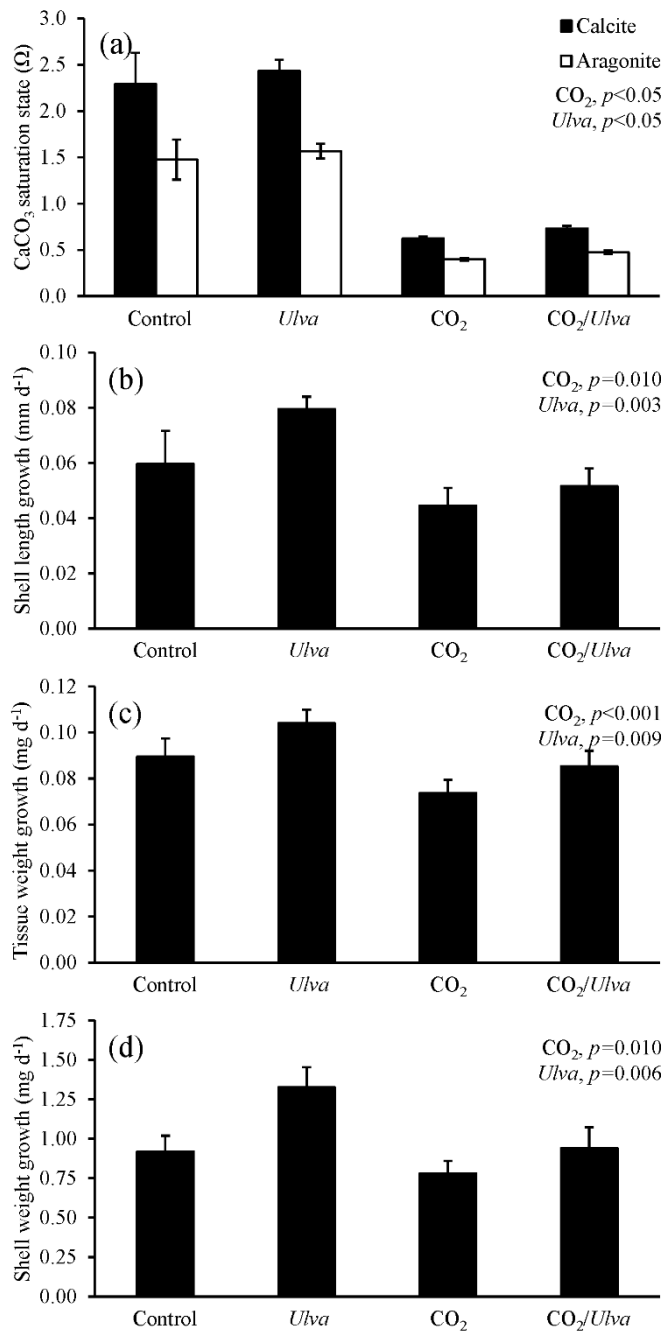
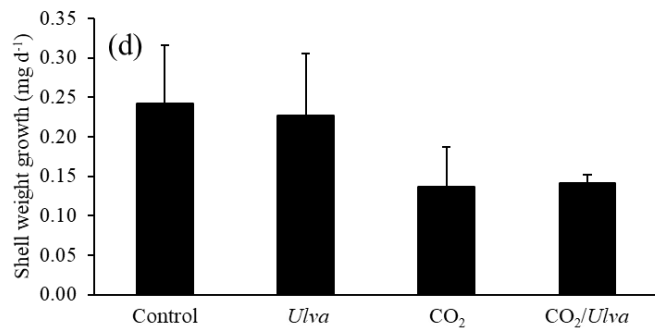
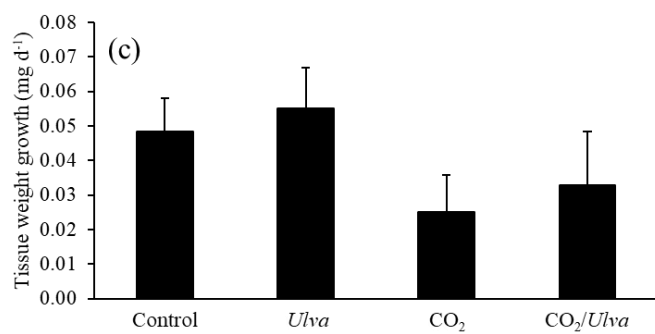
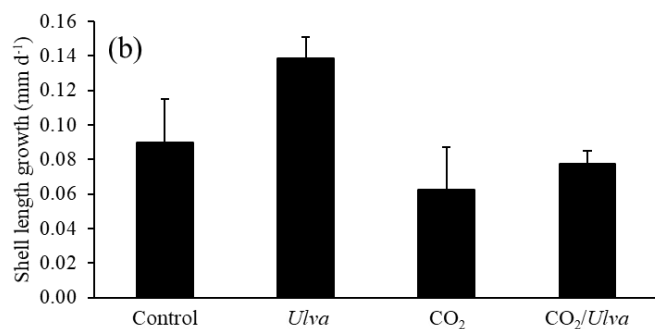
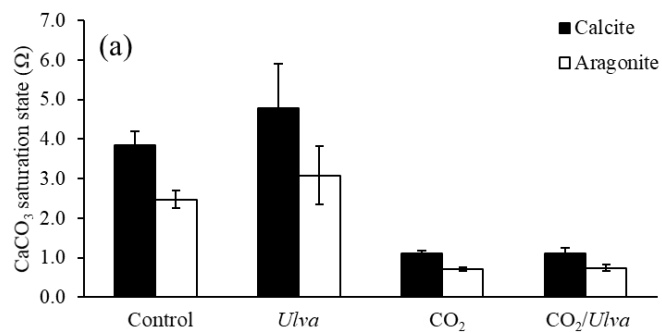


Figure 2. Experiment with large juvenile *Mercenaria mercenaria* exposed to ambient and elevated concentrations of CO₂ with and without the presence of *Ulva*. (a) Ω_{calcite} and $\Omega_{\text{aragonite}}$; Growth was based on (b) shell length; (c) tissue weight; and (d) shell weight. Columns represent means \pm standard deviation. Significant main treatment effects (CO₂ and *Ulva*) appear on the top right of each figure.

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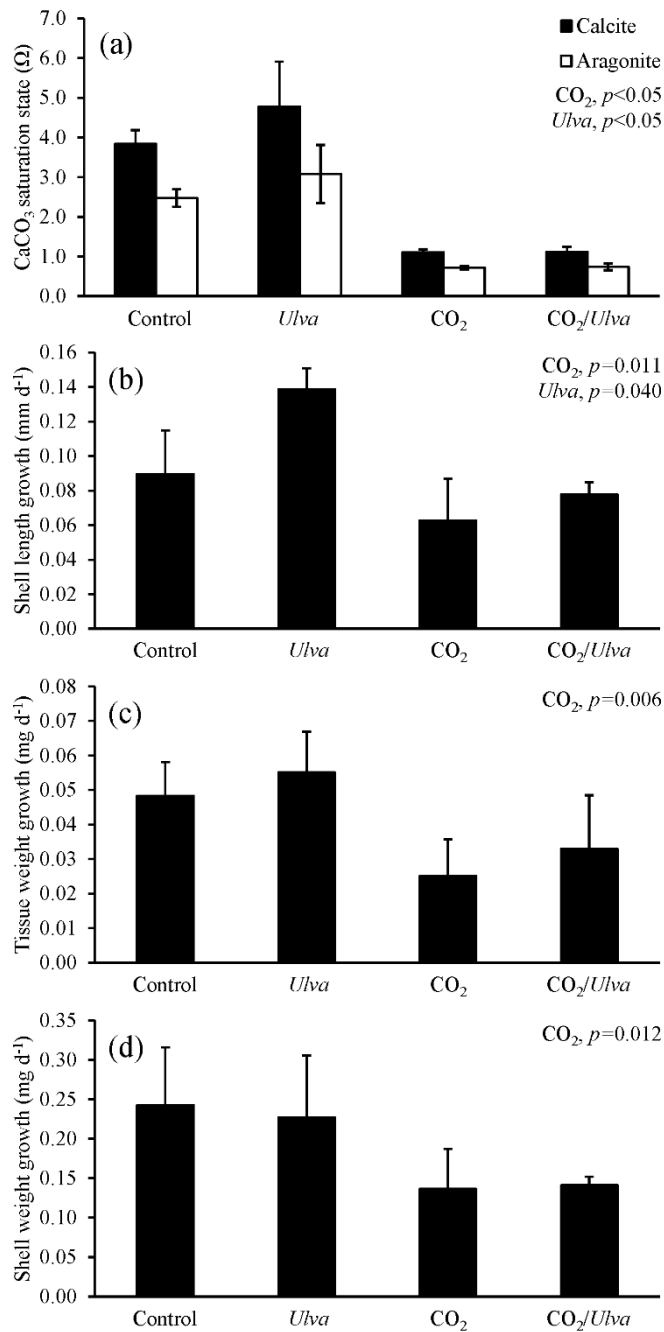
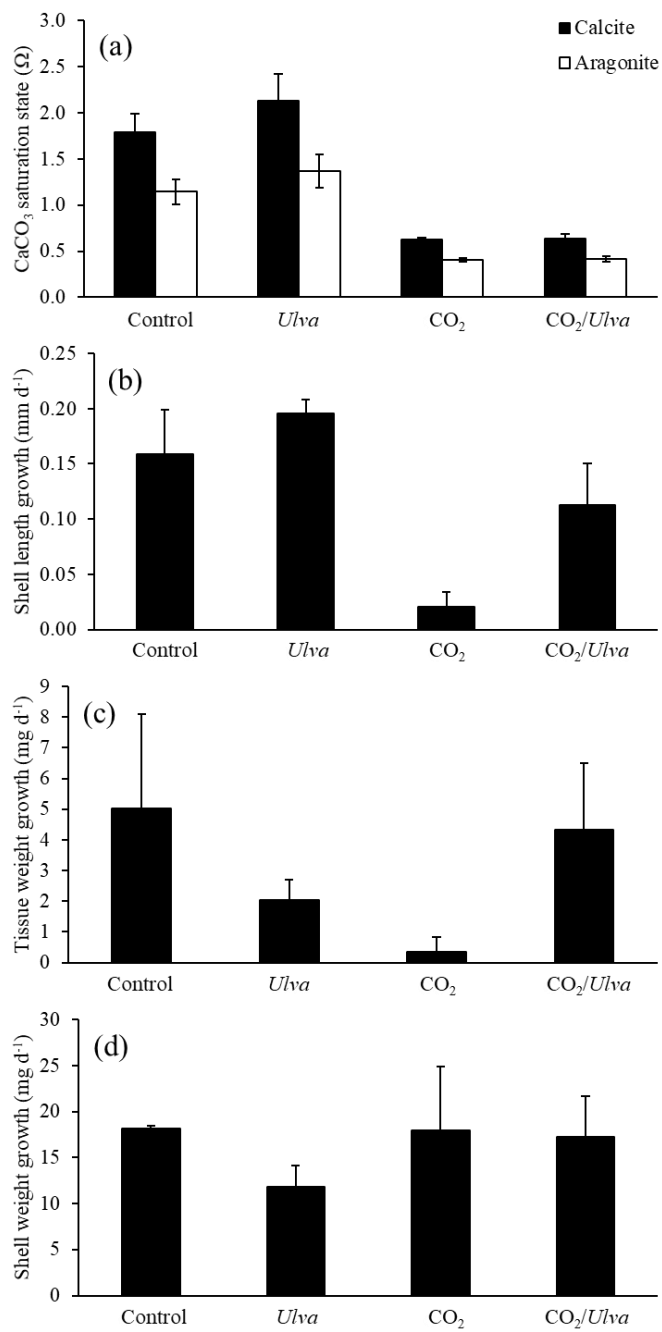


Figure 3. Experiment with small juvenile *Crassostrea virginica* exposed to ambient and elevated concentrations of CO₂ with and without the presence of *Ulva*. (a) Ω_{calcite} and $\Omega_{\text{aragonite}}$; Growth was based on (b) shell length; (c) tissue weight; and (d) shell weight. Columns represent means \pm standard deviation. Significant main treatment effects (CO₂ and *Ulva*) appear on the top right of each figure.

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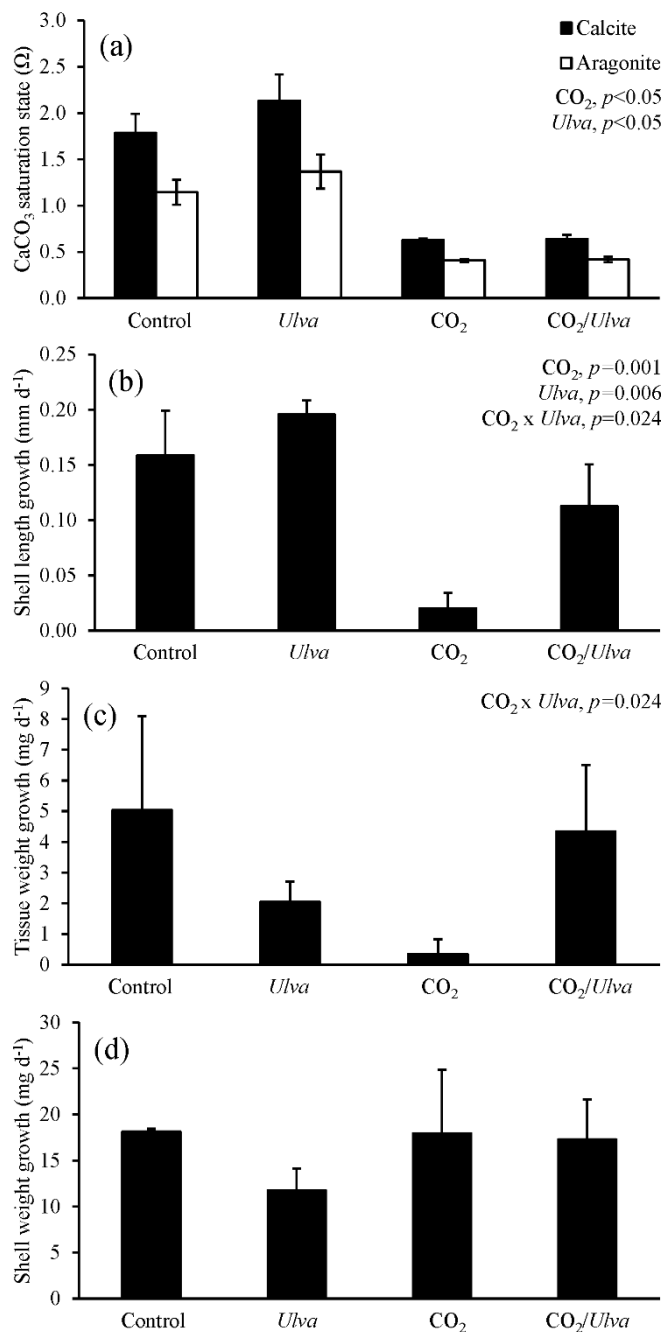
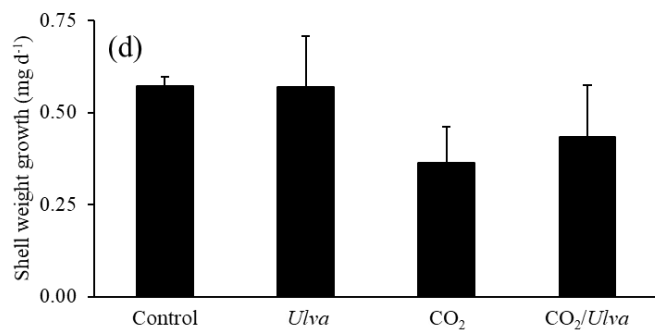
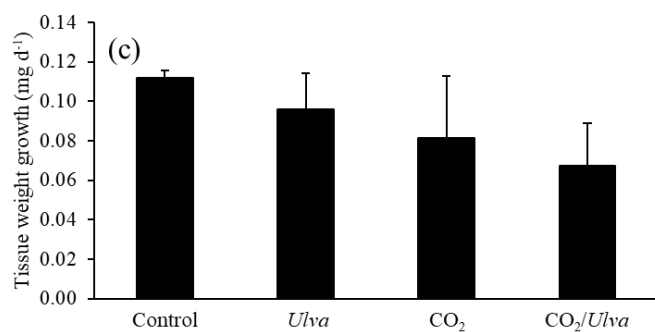
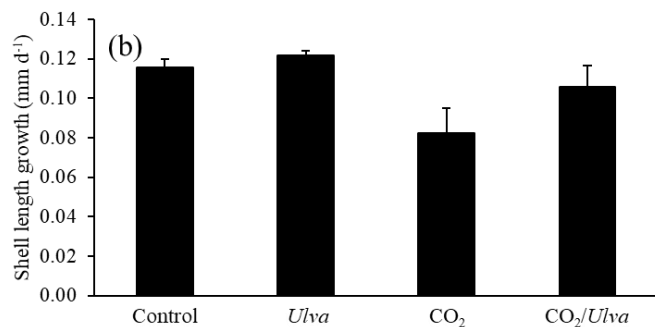
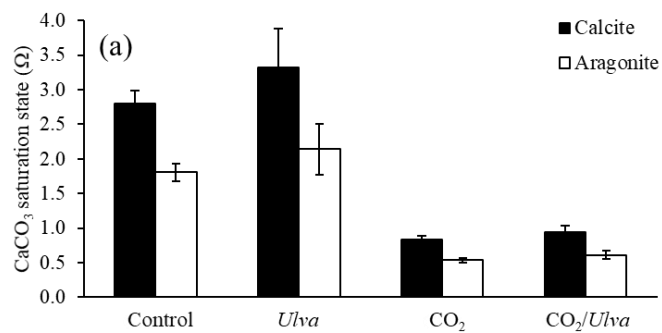


Figure 4. Experiment with large juvenile *Crassostrea virginica* exposed to ambient and elevated concentrations of CO₂ with and without the presence of *Ulva*. (a) Ω_{calcite} and $\Omega_{\text{aragonite}}$; Growth was based on (b) shell length; (c) tissue weight; and (d) shell weight. Columns represent means \pm standard deviation. Significant main treatment effects (CO₂ and *Ulva*) appear on the top right of each figure.

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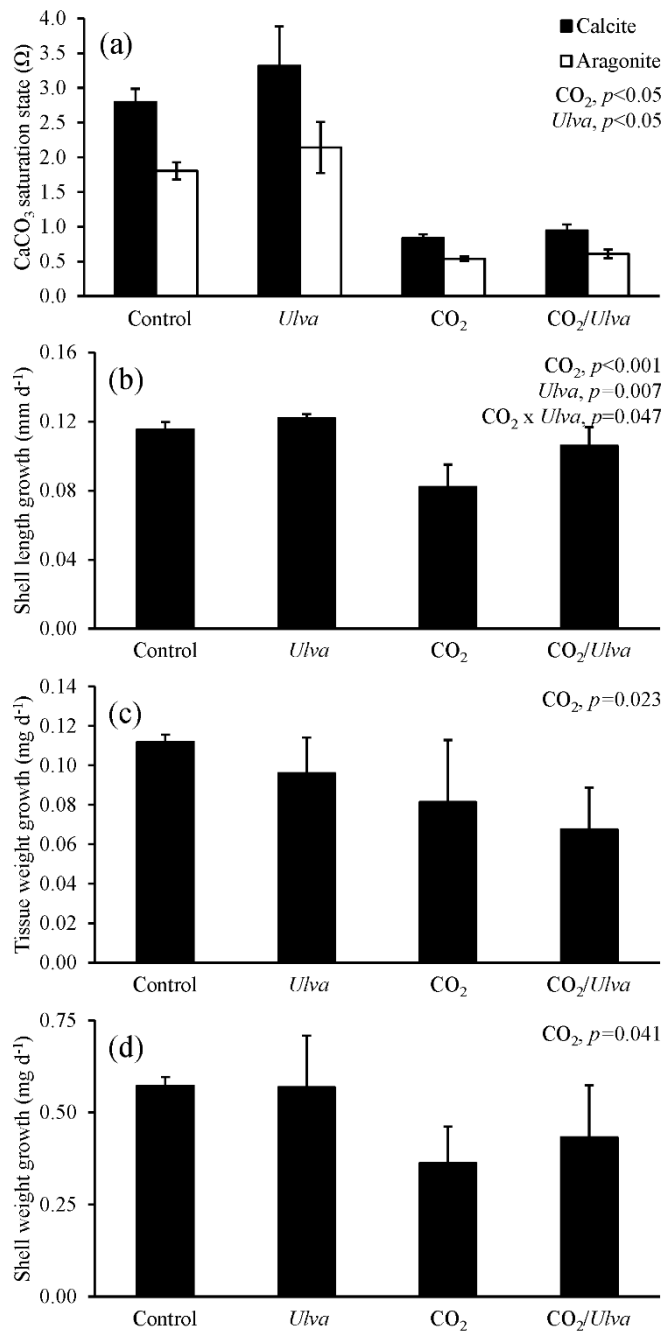
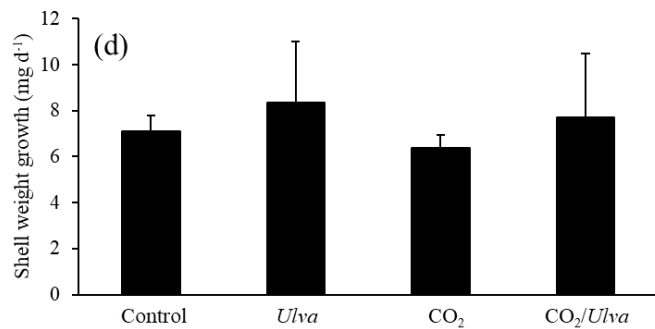
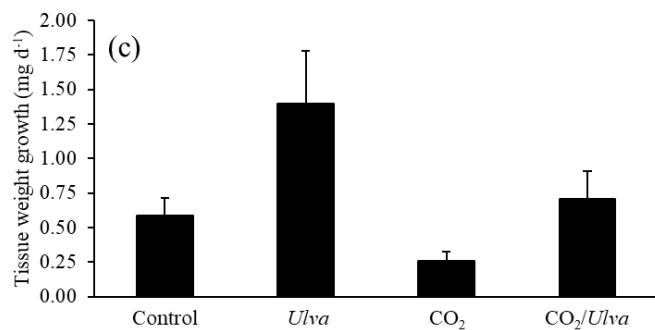
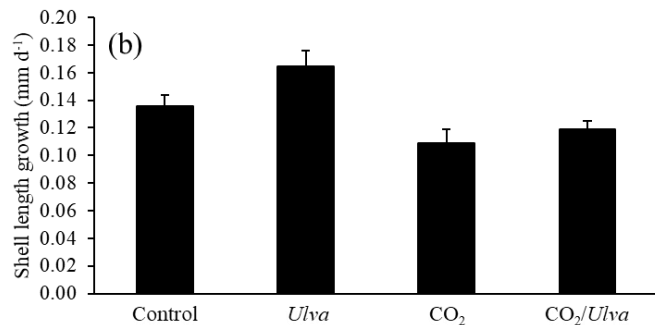
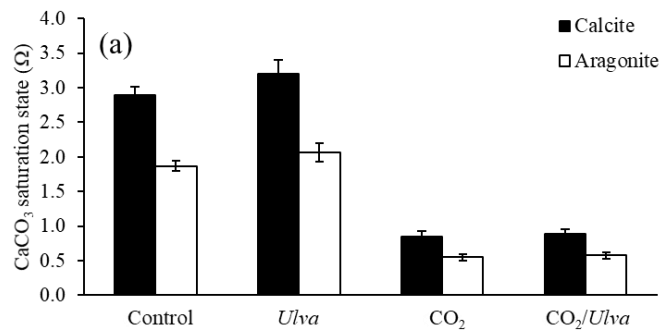


Figure 5. Experiment with small juvenile *Argopecten irradians* exposed to ambient and elevated concentrations of CO₂ with and without the presence of *Ulva*. (a) Ω_{calcite} and $\Omega_{\text{aragonite}}$; Growth was based on (b) shell length; (c) tissue weight; and (d) shell weight. Columns represent means \pm standard deviation. Significant main treatment effects (CO₂ and *Ulva*) appear on the top right of each figure.

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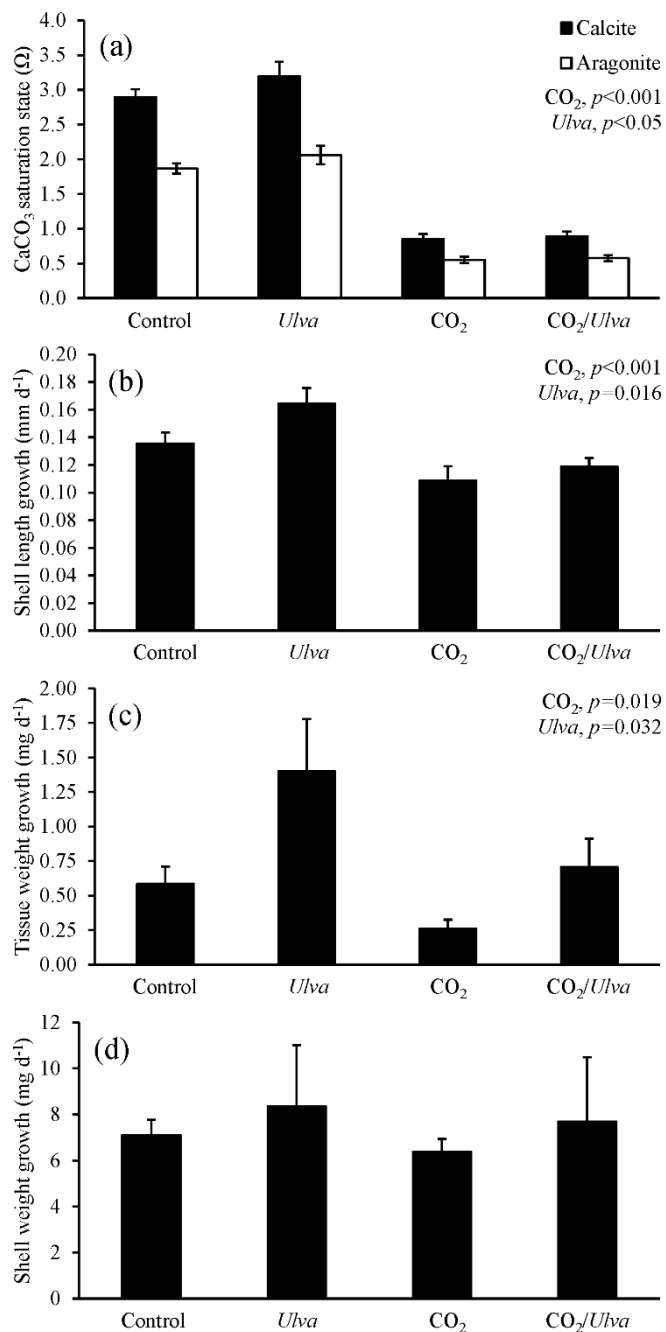
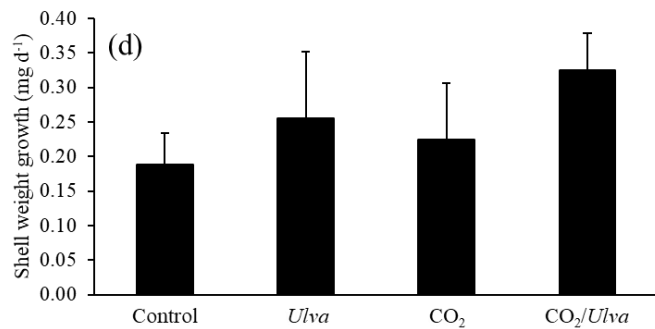
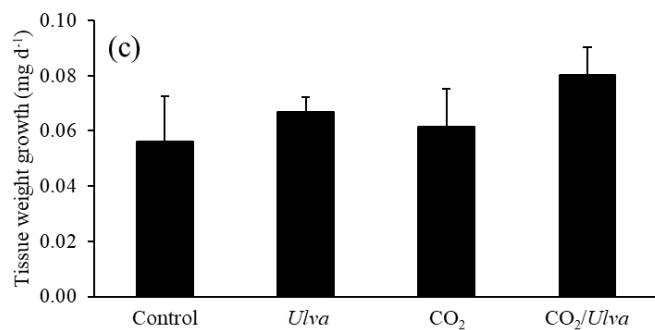
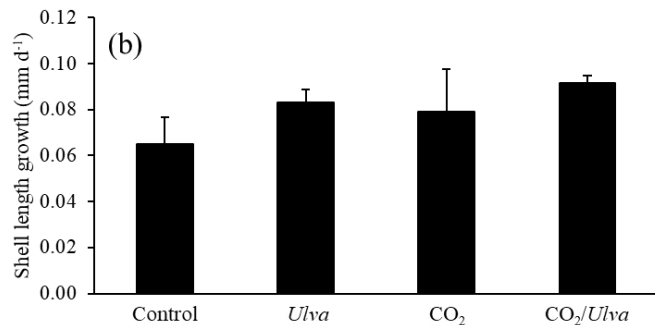
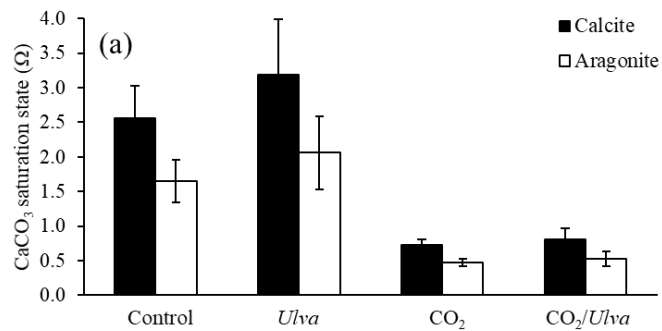


Figure 6. Experiment with large *Argopecten irradians* exposed to ambient and elevated concentrations of CO₂ with and without the presence of *Ulva*. (a) Ω_{calcite} and $\Omega_{\text{aragonite}}$; Growth was based on (b) shell length; (c) tissue weight; and (d) shell weight. Columns represent means \pm standard deviation. Significant main treatment effects (CO₂ and *Ulva*) appear on the top right of each figure.

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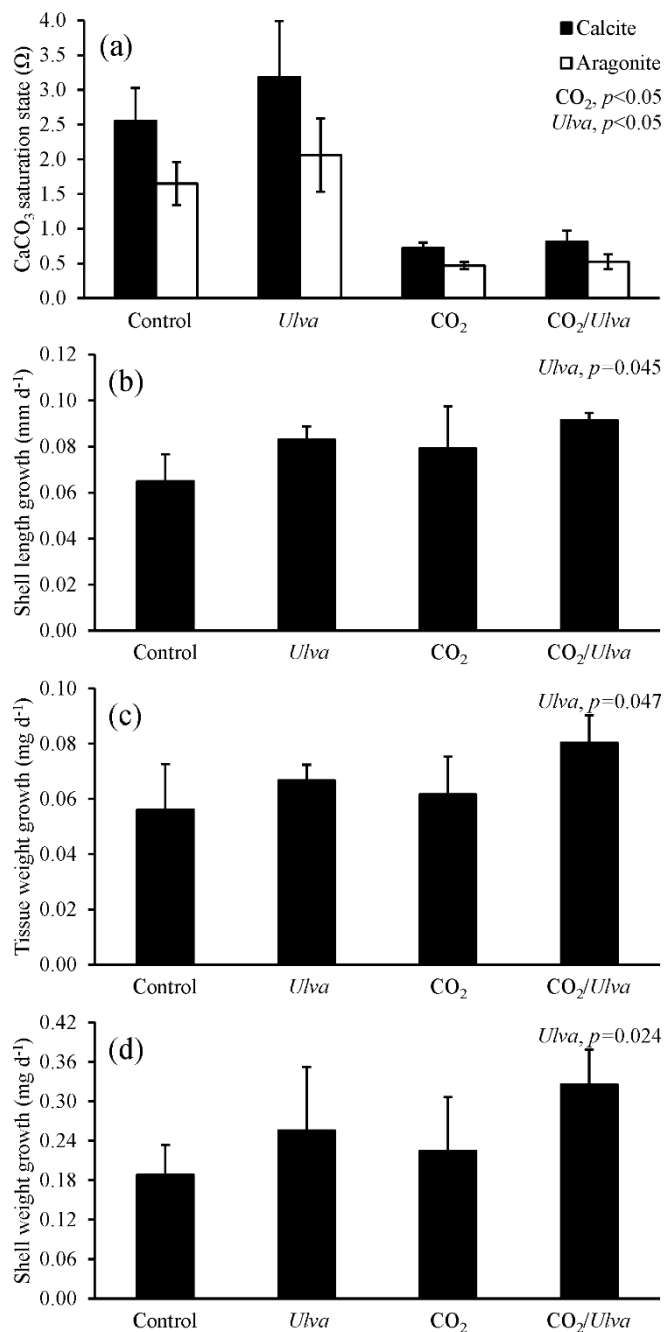


Figure 7. Experiment with *Mytilus edulis* exposed to ambient and elevated concentrations of CO₂ with and without the presence of *Ulva*. (a) Ω_{calcite} and $\Omega_{\text{aragonite}}$; Growth was based on (b) shell length; (c) tissue weight; and (d) shell weight. Columns represent means \pm standard deviation. Significant main treatment effects (CO₂ and *Ulva*) appear on the top right of each figure.

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5 **Table 1.** Values of mean pH (total scale), temperature (°C), dissolved oxygen (DO; mg L⁻¹), and salinity (g kg⁻¹), and final pCO₂ (µatm), total alkalinity (µmol kgSW⁻¹), total DIC (µmol kgSW⁻¹), HCO₃⁻ (µmol kgSW⁻¹), CO₃²⁻ (µmol kgSW⁻¹), OH⁻ (µmol kgSW⁻¹), Ω_{calcite}, ~~and~~ Ω_{aragonite}, and final microalgal cell counts of *Isochrysis galbana* and *Chaetoceros muelleri* (cells mL⁻¹) for June through November experiments (n=4 for all treatments). Values represent means ± standard error. Asterisks indicate parameters that were directly measured, and not calculated. Data from individual experiments appear within Tables S1.

Parameter	Control	<i>Ulva</i>	CO ₂	CO ₂ / <i>Ulva</i>
pH*	7.98±0.01	8.03±0.01	7.37±0.01	7.39±0.01
Temperature*	21.3±0.1	21.2±0.1	21.3±0.1	21.3±0.1
<u>Dissolved oxygen</u> Θ*	9.06±0.01	9.00±0.01	9.17±0.01	9.10±0.01
Salinity*	30.0±0.1	30.1±0.1	30.0±0.1	30.0±0.1
pCO ₂	373±8	335±9	1763±27	1721±27
Total alkalinity	1740±26	1759±26	1792±25	1803±21
Total DIC*	1561±19	1557±21	1782±22	1797±19
HCO ₃ ⁻	1428±16	1413±18	1690±21	1706±19
CO ₃ ²⁻	119±4	134±5	35±1	37±1
OH ⁻	3.84±0.12	4.51±0.18	0.95±0.02	1.01±0.02
Ω _{calcite}	2.97±0.11	3.36±0.13	0.86±0.03	0.90±0.03
Ω _{aragonite}	1.91±0.07	2.16±0.09	0.56±0.02	0.59±0.02
<u>Microalgae cells*</u>	<u>97273±5230</u>	<u>97727±4696</u>	<u>90455±4388</u>	<u>95000±5294</u>

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~~Dickson, A. G.: The measurement of sea water pH, Mar. Chem., 44, 131-142, doi: 10.1016/0304-4203(93)90198-W, 1993.~~

~~Dickson, A. G., Sabine, C. L., and Christian, J. R.: Guide to best practices for ocean CO₂ measurements, PICES Special Publication, 3, 191 pp., doi, 2007.~~

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