***Discussion—***

1. **Something about the expected patterns of shell-building responses based on tolerance theory vs biological cycling.**
   1. Did the patterns match any of the potential scenarios described in the intro?
   2. What can we say about this?
   3. What are the limitations?
   4. Future directions? (measure across seasons to incorporate physiological differences and also look at how much food is enough food for other experiments
2. Processes that alter seawater TA operate at (when/how often) **different time scales**, as do events that alter TA through reductions to salinity.
   1. For example (reiteratie the low S thing above)
   2. Also through biological activity of photosynthetic SAV\*
   3. What about via weather agents that are slow release?
   4. Maybe also add more time windows to cover the ranges potential exposure types and also look at the potential feedback of organism responses to the chemical evolution of single-pulse events (vs multi-pulse episodes like ours).

For example, many studies have show the positive impact of seagrasses on oyster growth through uptaking CO2 during the day, corresponding to higher calcification rates. Recently, interest has risen for adding crushed shell, or commercial products that mimic shell, to areas swith growing oysters as a way to buffer low pH conditions (through increased TA as CaCO3 dissolves), though potential consequences for infection rise with growth rates that do not also have paired tissue growth (cite), and X consequences could happen from the product?

Juvenile *Crassostrea virginica* shell growth responded to changes in total alkalinity in two distinct patterns as a function of exposure window and was uninfluenced by lower salinity.

* The degree to which TA influenced surface area growth was not large enough to detect in relative shell growth, due to overall declines in shell growth rates in the later window, suggesting that the degree of shell-building activity of oysters likely mediated their interaction with surrounding seawater conditions.
* Neither TA nor salinity influenced the growth in shell area in the early window, nor did they influence relative shell growth overall, which might suggest oysters were able to overcome corrosive conditions in low TA, and yet, did not elevate growth in higher TA conditions.
* On the other hand, growth in shell area in the later window declined in lower TA conditions, which might suggest an inability to overcome abiotic tendencies to dissolve.
* However, we did not detect any trade-offs to sustained growth in the shell thickness, nor in the ability to increase tissue mass relative to shell (condition index).
* We did visually observe changes to the exterior coloration of the shells through time.
* Shells were noticeably bleached in the lower TA treatments in the later window, potentially indicating a disruption to the interaction between the surface of shells and epiphytes.

*\*****The first paragraph (as you note) should summarize/emphasize the findings. Sometimes it will take a second paragraph to talk over the implications, including potential broader applications to other taxa, other regions, or more general unknowns. We want to emphasize the relevance and value of the study before diving into nuances.***

Something about the expected patterns of shell-building responses?

Moreover, growth in shell area was higher in the early window and declined later on, regardless of treatment, demonstrating the unique trajectory that responses may have.

* The root cause of this pattern, however, was not tied to one of our controlled variables, which prevents us from more than speculation.
* One possibility is that oysters initially invested in building shell material until switching to tissue growth in the later window.
* Whether or not the initial shell building was done at a cost to tissue mass, however, remains known due limitations that prevented coinciding tissue mass sampling through time.
* The later window of exposure, therefore, could indicate natural cycling of growth patterns, a switch from shell to tissue growth to replenish a depleted condition index, or just general declines in performance due to extensive time in laboratory conditions (cite).
* Elevated food can off-set increased maintenance costs, which could have resulted in a heightened ability to cope with abrupt changes in seawater conditions. Future work disentangling how the temporal variability in food availability may interact with cyclical patterns of growth would be interesting.
* Does this go against our prediction of what was going to happen?
* Had oysters been unable to tolerate changes in conditions, we could have seen lower shell growth in the early window relative to later on, as oysters would have needed to invest more in other maintenance processes associated with osmoconformation to new seawater conditions.
* Had we seen declines in CI corresponding with TA condition it could have indicated a trade-off between building shell and building tissue mass.
* We did measure changes in CI through time, limiting our ability only to speculation.
* Future work that investigates the cyclical nature of shell and tissue growth in altered seawater conditions could also look at how food availability interacts with these cycles.
* we did not see trade-offs in other areas of shell growth, indicating an ability of well-fed oysters to quickly adapt to altered seawater TA.

Use omega to connect to OA paragraph?

which could have implications for other calcifiers with varying levels of external shell protection…

* Similar to the effect of increasing the concentration of CO2 in seawater to lower omega, the saturation state of calcium carbonate molecules (specifically for calcite mineral used in shell-building), changes in TA also changes omega.
* The two lowest TA treatments had average omega values that were < 1, indicating a tendency for CaCO3 to dissolve in undersaturated conditions. This likely means that in these conditions alone, shells experienced uncontrolled exterior dissolution, which would require an increase in biologically-controlled calcification.
* Oysters shells are more vulnerable to dissolution, as the calcite mineral form used for CaCO3 dissolves easier, relative to other marine calcifiers like mussels and X. However, when oysters are also able to rapidly cope with altered conditions (or close up and wait them out) through upregulated biological calc, it likely makes their perceived vulnerabililty to corrosive conditions more complex.
* Because overall shell growth is as a combination of many calcification and precipitation events, both driven by abiotic and biological processes, we may anticipate differences in net shell growth based the degree to which oysters face abiotic tendencies to dissolve. Through time stress factors can strengthen with low omega saturation, whether driven by elevated pCO2 or declines in TA.
* -this also could have had something to do with the temporal erosion of something on the exterior of the shell. we observed visible color deterioration in oysters from low omega treatments, suggesting that seawater interactions on the external face of the shell may have differed as a function of omega. We were unable to quantify the change in color, however, personal feedback from the oyster growers indicates that oysters in this region will fall into what they call ‘anemic condition’ in sunless tanks, but that introduction to light will encourage epiphyte growth. we may have been limiting natural epiphyte growth on the exterior of the shells in low omega conditions, though, we speculate.
* -Other growers have also mentioned that they see a similar bleached look in oysters seasonally. In the late winter / early spring we see the most noticeable bleaching in our pacific (gigas) oysters in areas will historically little food and very little tidal exchange. That coupled with the density of animals there has led to the assumption it's a nutrient availability effect. main parameters influencing that are assumed to be food availability and temperature. Since we did not alter the temperature directionally throughout the course of the experiment, it is possible that oysters somehow became food-limited in low omega treatments, however, we didn’t see the tradeoff decline in tissue.

P7: conclusion paragraph

In culture 2 the decline was sharp during the first 20 days. The condition of the unfed individuals in culture 3 was consistently lower than the condition of the fed individuals. In routine cultures 1 and 2 the gonad index increased slightly, whereas in culture 3 it declined, from 2.85 to 1.50 for unfed animals and from 2.85 to 1.70 for fed animals

a decline in the condition index during the winter and spring accompanied an increase in the gonad index

we therefore suggest that Mytilus edulis may continue normal development of the gametes whilst the body condition declines due to stresses in the environment, as long as the gametes themselves are not yet ripe.

**SUMMARIZE KEY FINDINGS**

1. **The influence of TA condition on surface area shell growth changed between initial and later periods of the exposure trajectory**
   1. **Surface area growth did not differ between oysters from different TA conditions initially**
   2. **Higher TA condition appeared to elevated surface area growth later on, but was only stat sig in the lower salinity**
2. **Growth rates were lower in the later part of exposure, talk about why this could be?**
   1. **Introduce limitation**
   2. **Does this go against our prediction of what was going to happen?**
3. **Net surface area growth rate patterns…talk about the change in omega across the TA levels in this paragraph?**
   1. **Implications for other calcifiers that are sensitive to changes in omega**
4. **No indication of physiological tradeoffs in shell thickness or tissue mass**
   1. **We did not see differences in shell thickness across the treatments**
   2. **We also didn’t see a difference in condition index, indicating an ability to maintain tissue mass to shell mass ratios, across very different growing patterns.**

**SHARE INTERPRETATIONS**

**DISCUSS THE IMPLICATIONS**

**ACKNOWLEDGE LIMITATIONS**

**RECOMMENDATIONS FOR FUTURE RESEARCH**

**Part 1: summarize your findings**

**Start this section by reiterating your research problem and concisely summarizing your major findings. To speed up the process you can use a summarizer to quickly get an overview of all important findings. Don’t just repeat all the data you have already reported—aim for a clear statement of the overall result that directly answers your main research question. This should be no more than one paragraph**

**Part 2: give interpretation**

**Identifying correlations, patterns, and relationships among the data**

**Discussing whether the results met your expectations or supported your hypotheses**

**Contextualizing your findings within previous research and theory**

**Explaining unexpected results and evaluating their significance**

**Considering possible alternative explanations and making an argument for your position**

**WHAT COULD CHANGED RESPONSE TO TA STEM FROM? OUR SUGGESTIONS FOR FUTURE INVESTIGATIONS**

**Finding: shell growth responses to TA conditions in juvenile oysters changed through time.**

**-other studies have suggested that how responses change or don’t change, tells us something about the tolerance to conditions.**

**-or it could tell us something about the cyclical nature of growth processes, trading off between maximizing shell and tissue growth.**

**- something else.**

**Finding: Oyster shell growth was high in all oysters initially, but declined later in the response trajectory**

**-demonstrates an active downregulation of shell building activity through time. Could indicate an unknown stress introduced by laboratory conditions, or a switch from shell building to tissue growth at this time.**

-Limitation: Did not measure tissue mass subset to see whether there was a trade-off apparent in tissue mass through time

**other seawater parameters that may change in long residence times of exposure?**

-axes of pCO2, temperature, oxygen

-turbidity

**NEED TO ALSO CONSIDER THE RELATIVE ABIOTIC VERSUS BIOTIC SIGNALS IN TERMS OF SHELL GROWTH, EVEN THOUGH WE DIDN’T MEASURE THEM**

Findings: TA conditions experienced by the oysters were correlated with another parameter used to characterize the carbonate system, omega saturation state, which describes the tendency for calcium carbonate shell to precipitate or dissolve in seater, with known implications for shell building species.

-oysters overcame conditions that were undersaturated in respect to their CaCO3 shells, which means that although we didn’t directly capture it, we can assume those individuals were increasing their biological calcification to compensate for abiotic external shell loss. Later in the exposure trajectory, however, lower TA conditions corresponded to declines in average shell growth, indicating that oysters not only declined their shell growth overall, but did so more in low TA conditions where shell had a propensity to dissolve than those near ambient concentrations. Because overall shell growth is as a combination of many calcification and precipitation events, both driven by abiotic and biological processes, we may anticipate differences in net shell growth based the degree to which oysters face abiotic tendencies to dissolve. Through time stress factors can strengthen with low omega saturation, whether driven by elevated pCO2 or declines in TA.

-this also could have had something to do with the temporal erosion of something on the exterior of the shell. we observed visible color deterioration in oysters from low omega treatments, suggesting that seawater interactions on the external face of the shell may have differed as a function of omega. We were unable to quantify the change in color, however, personal feedback from the oyster growers indicates that oysters in this region will fall into what they call ‘anemic condition’ in sunless tanks, but that introduction to light will encourage epiphyte growth. we may have been limiting natural epiphyte growth on the exterior of the shells in low omega conditions, though, we cannot speculate.

-Other growers have also mentioned that they see a similar bleached look in oysters seasonally. In the late winter / early spring we see the most noticeable bleaching in our pacific (gigas) oysters in areas will historically little food and very little tidal exchange. That coupled with the density of animals there has led to the assumption it's a nutrient availability effect. main parameters influencing that are assumed to be food availability and temperature. Since we did not alter the temperature directionally throughout the course of the experiment, it is possible that oysters somehow became food-limited in low omega treatments, however, we didn’t see the tradeoff decline in tissue.

TA effects are likely manifest during times of downregulated biotic shell activity…what does this mean?

**OTHER PHYSIO CONSEQUENCES IN LONG RESIDENCE TIMES OF EXPOSURE TO ALTERED CONDITIONS?**

**NATURAL VARIATION IS COMPLICATED BOTH BY INCREASING FREQUENCY OF DISCRETE EXTREME EVENTS (LIKE HEATWAVES AND ATMOSPHERIC RIVER RAINSTORMS) AND GLOBAL CHANGES TO SEAWATER (CITE).**

**OTHER SPECIES HAVE DEMONSTRATED THAT CO-GROWING BIVALVES WITH PHOTOSYNTHESIZING SPECIES THAT MAY PROVIDE FOOD SOURCE DIRECTLY (AS EPIPHYTE ALGAE SLOUGHS OFF), AND MODERATES BOTH FOOD AND CARBONATE SYSTEM CONDITIONS COULD PRESENT. A similar study to understand early versus later responses in growth to naturally altered conditions would be a great next step. Would that be from living species though since not may algaes can tolerate the extremes that oysters do… or maybe even implementing shell hash material when food is readily available.**

**Or looking at other species that have responded to changes in pH due to altered pCO2, to see if they respond similarly through time.**

***In line with the hypothesis…***

***Contrary to the hypothesized association…***

***The results contradict the claims of Smith (2022) that…***

***The results might suggest that x. However, based on the findings of similar studies, a more plausible explanation is y.***

**Part 3: Implications of the work?**

1. **What does this mean in the context of future ocean change? FW precip regime shifts, droughts, OA, warming, etc.**

***These results build on existing evidence of…***

***The results do not fit with the theory that…***

***The experiment provides a new insight into the relationship between…***

***These results should be taken into account when considering how to…***

***The data contribute a clearer understanding of…***

***While previous research has focused on x, these results demonstrate that y.***

1. **Relative to other studies/for the discussion?** 
   1. **across high and low salinity data combined, we did not see an effect of pH on net shell growth in oysters (even though some treatments had med omega calcite values less than one).**
   2. **We did not see an influence of Ta on mortality.**
   3. **Low salinity and low TA (di or low TA river) trended to have reduced survival, but it was not significant at the salinities we tested (was not the aim of our study).**
   4. **similar: we saw trends of decreased survival in low S and low TA conditions only (but not profound effects like them). Difference: We did not see effects of low S and low TA on energy metabolism (ie gut wt).**
   5. **Our shell area was also not impacted by varying TA (and omega), which correlate with CO2. We did NOT see an effect of low TA on shell mass (ie shells were not thinner). Both our treatments and their span a range of omega values above and below 1. Suggest that just area or length may not be sufficient without understanding of mass.**

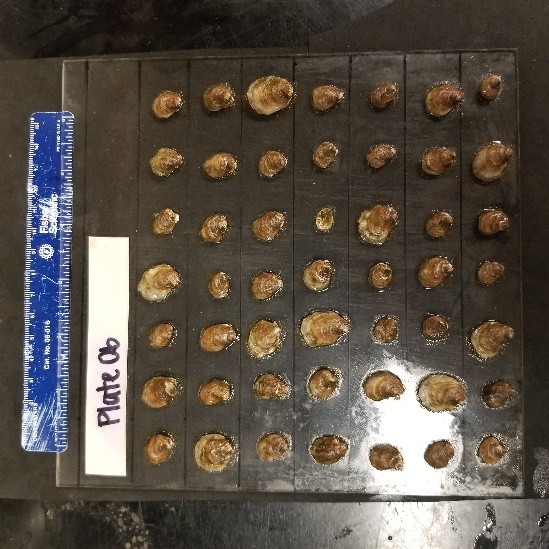
Plate 2:



**0 dps**



**18 dps**



**36 dps**

***Need to mention the notoriously high variability in the highest TA treatments,***

Prior to arrival at BML, conditions in Tomales Bay were marine-dominated due to upwelling (cite), with many oysters still characterized as ‘dormant’ to professional farmers (personal comm.).

Oysters repurpose periostracum material as ‘cement’ to adhere to firm bottom when settling naturally.-->changes in perio under OA or changed food/more maintenance costs.

coastal ‘living wall’ protection from sea level rise along X coast (cite). Shells have been posed as a potential mitigation tool for coastal acidification (cite) and are the preferred substrate for growers (cite), thus extending their importance after death.

Oyster growth was positively influence by [TA], in ambient and low salinity conditions

1. When changes in [TA] occurred in ambient S conditions X happened
   1. Which was largely driven by high incremental growth in the first two weeks, with incremental growth declining between week 2-5 in the ambient and high TA conditions…(Fig 5)
2. Oyster growth is maintained when alkalinity is severely reduced below ambient conditions (60% reduction) in ambient salinity(Fig 2)
   1. Which was largely driven by an increase in incremental growth **after** 2 weeks of exposure to low TA conditions (Fig 5)
3. We also saw X effect of changes to [TA] coinciding with abrupt acclimation to low S conditions.
   1. Oysters exposed to freshwater inputs that have elevated [TA], have higher growth than those exposed to rainfall/low TA rivers (Fig3)
      1. Which was largely driven by increased incremental growth in the first two weeks, with incremental growth declining between week 2-5…(Fig 5)
4. We do not observe an additional influence of salinity on top of the positive relationship with [TA], which may occur because oysters were fed often and not energetically limited.
   1. Often times we see a non-additive effect when stressors are combined. We would look at the first two week growth rate in the single vs multi stressor experiment to test this relationship. In fact, we see X in reduced [TA] with no change in S versus X when S was reduced with [TA].

Effect of TA in low salinity… how was this similar or different to mussel work?

Comparison:

Stevens and Gobler: CV

* low pH lowered growth rate
* low pH took away the negative effect of low DO when coupled (ie only see pH effect)
* *low DO coupled with warmer temp decreased survival*
* *higher temp led to lower tissue wt*
* *low pH, low DO and high temp led to lower tissue weight*
* **across high and low salinity data combined, we did not see an effect of pH on net shell growth in oysters (even though some treatments had med omega calcite values less than one). This suggests that they were well fed and robust. However, we do see a change in net growth when TA is elevated in contribution to TA. In our case, low S interacting with TA produced a higher influence than either change alone.**

Parter: Saccostrea glomerata

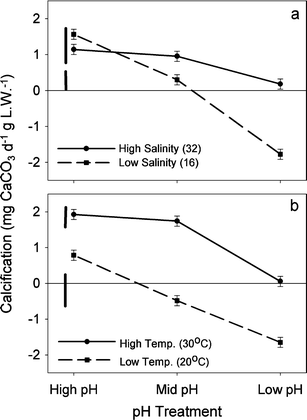
* high co2 acclimation led to significant metabolic depression
* acute exposure to elevated temperature and reduced S (and low TA) with the higher CO2 treatment led to extracellular acidosis

Dickinson 2012: CV

* A graph of different types of dry mass

  Description automatically generated with medium confidenceexposure to elevated pCO2 and or low salinity (and low TA) led to significant increase in mortality, reduction of tissue energy stores and negative soft tissue growth
* responses to high CO2 and or low salinity (and low TA) indicate
* oysters respond to these stressors by becoming energy deficient, or, juvenile oysters maintain their cellular energy status at the expense of energy stores
* Under the conditions of our experiment, low salinity (and low TA) is a greater single stressor than high PCO2, whereas the combination of these two factors produces greater changes in the physiology and shell properties of these mollusks than each of the factors alone
* **We did not see an influence of Ta on mortality. Low salinity and low TA (di or low TA river) trended to have reduced survival, but it was not significant at the salinities we tested (was not the aim of our study). We saw no impact of S or TA on energy stores, tissue growth, even though others have seen impacts of pco2 on these components. We did not see oysters becoming energy deficient (maintained similar gut tissue, higher than controls), but we did see activity decline. They found that their low S (and low TA) treatment was stronger than any of the high pCO2 treatments. When coupled with high pco2, low S (and low TA) had a greater impact on physio and shell properties than each factor alone. (impetus for researching?)**

Waldbusser 2011: CV

* comparing the effects of salinity (and TA) along pH treatments and temp treatments.
* High pH high the highest calcification (low S slightly higher and amb S), this also occurred at the highest TA
* The effect of high pH at high S is less than when coupled with decreasing S (and TA)
* *High Temp elevated calcification regardless of pH, and there was an independent decline in calcification from pH (similar to sal curve)*.
* The effect of pH at high S was minimal (black solid line)
* The effect of pH between S (and low TA) treatments were strong (dashed line)
* **We saw highest growth rates in the low salinity coupled with high alkalinity treatment, suggesting that oysters were able to optimize on their preferred condition quickly (effect was apparent by two weeks), with elevated TA becoming more important following the two week period, marginally.**
* **They didn’t see a strong effect of pH at ambient salinity; we didn’t see a strong effect of TA (and subsequently omega) at ambient salinity. The effect of diluting TA with freshwater (rain/hurricane) will also decrease the pH, and they showed it decreased calcification. We see that there is elevated growth when TA is elevated in low S, suggesting that the effect of low pH may not be as strong.**

Dickinson 2013: Hard shell clams

* Low S (and low TA) had profound effects on survival, energy metabolism and biomineralization of hard-shell clams
* Low S (and low TA) modulated clam response to high CO2
* Negative effects of low salinity (and low TA) were mostly due to the strongly elevated basal energy demand, indicating energy deficiency, that led to reduced growth, elevated mortality and impaired shell maintenance (evidenced by the extensive damage to the periostracum).
* Moderate hypercapnia (similar to 800 mu atm P-CO2) increased shell and tissue growth and reduced mortality high salinity exposures
* these effects were abolished under the low salinity (and low TA) conditions or at high P-CO2 (similar to 1500 mu atm)
* **similar: we saw trends of decreased survival in low S and low TA conditions only (but not profound effects like them). Difference: We did not see effects of low S and low TA on energy metabolism (ie gut wt). New material: we did see an elevated shell growth response to low S conditions coupled with high TA (they didn’t test). If low S doesn’t necessarily mean low TA, then estuaries may not experience the harmful consequences of high CO2. Moderate elevations in CO2 raised shell and tissue growth rates (maybe some sort of physio priming), like we see in low S and high TA?**

Gazeau 2013: Review

* in Benaish 2010 shell area was not impacted by high CO2 but shell mass lowered. This suggests that shells were thinner under high co2 conditions. As a note, their treatments spanned below omega calcite threshold of 1.
* (B) They suggest that shell area or length may not be sufficiently accurate as indicators of the effects of OA without measuring mass as well.
* **Our shell area was also not impacted by varying TA (and omega), which correlate with CO2. We did NOT see an effect of low TA on shell mass (ie shells were not thinner). Both our treatments and their span a range of omega values above and below 1. Suggest that just area or length may not be sufficient without understanding of mass.**

Hollarsmith 2019): Ostrea lurida and Crassostrea gigas

* the influence of carbonate system parameters, temperature, salinity, dissolved oxygen (DO) gradients is contingent upon the location in the estuary as well as seasonal timing (Hollarsmith 2019).
* During upwelling events (dry season), temperature, carbonate chemistry, and DO had the greatest impact on oyster performance. (Hollarsmith 2019)
* During runoff events (wet season), gradients in salinity, nutrient concentrations, and total alkalinity driven by river discharge were comparatively more important. (Hollarsmith 2019)
* the spatial importance *of carbonate chemistry and temperature* are seasonally variable and are two of several other factors that determine oyster performance. (Hollarsmith 2019)
* **Context for performance patterns overall: Performance was highest in upwelling season and declines following. We were between upwelling and river events and so there is some sort of transition in physio that is occurring such that, salinity, nutrients and TA are becoming more important. Future work should consider interval effects at different seasons, or consider outplants like these for CV in TX. Maybe because we were in a transition point, we saw depleted growth rates with time (coming down from upwelling?)**

A lot of studies have looked at the effects of altered conditions on net performances, however, this method doesn’t allow us to observe whether the effect is occurring in a similar nature throughout the course of the experiment. Plus, variability in the environment may be flashy, where conditions change abruptly, but persist over extended periods. It may be valuable to understand the degree to which responses to altered conditions change through time, as an organism may become more adjusted (especially in osmoconforming species like oysters).

Oysters are shell building species and respond to changes in carbonate system components. Others have looked to see how OA may impact oysters, but this occurred through varying pCO2. We were instead interested in seeing whether changes to TA conditions, either reducing or elevating, that occurs in estuaries without a corresponding salinity change, have a significant impact on shell growth.

Oysters preferentially live in low salinity estuaries that are impacted by freshwater. Especially within native ranges of this species, there is a high potential for rivers to elevate or maintain TA above ambient seawater. Most studies have looked at the effects of salinity on oysters by diluting with DI water, which simultaneously dilutes TA. These oysters may be responding to both salinity and carbonate system stress (cite), and therefore will likely have a different response to those experiencing low salinity and maintained TA.

We tested the influence of TA condition under lower, preferred salinity on net shell growth in oysters. We wanted to understand how the relationship between TA and shell growth may be mediated under low salinity by type of fresh water.

We were especially interested in comparing the responses of oyster growth to TA (in ambient and low salinity) through time, and so we compared growth patterns between two increments, the first spanning the first 2.5 weeks of exposure and the second, spanning 2.5 weeks after the first.

When oysters are really stressed they all do the same things

When they have ish stress, the variability in response increases

What is probably happening is that we are capturing changes in oyster growth phases where oysters are growing shell vs growing tissue, however, we do not have tissue samples from the midpoint to confirm.

Discussion : Low S (and low TA) had profound effects on survival, energy metabolism and biomineralization of hard-shell clams, Low S (and low TA) modulated clam response to high CO2 in Hard shell clams (Dickinson 2013)