***Abstract—***

***Introduction—***

***Methods—***

**Study species—** The Eastern oyster, Crassostrea virginica, settle in dense aggregations forming subtidal coastal reefs along the eastern coast of North America (Gulf of St Lawrence to the Gulf of Mexico) (cite), along with the Chesapeake Bay and tributaries (as the only native oyster species) (cite). C. virginica prefer brackish or marine seawater in subtidal habitats, however, in warmer location they also form reefs intertidally (NOAA). Natural oyster reefs create important habitat for hundreds of species (cite), while commercial aquaculture growers favor C. virginica for X reasons (cite). We sourced oysters used in experiments from X brood location (settled X) that were grown to X size at a local shellfish farm (Hog Island Oyster Company, Tomales Bay, CA). Oysters were transported in chilled seawater from HIOC to the Bodega Marine Lab, where they were placed on flow-through seawater for 30 days and fed X, daily. 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.). We adhered oysters to X type plates using X marine silicone, which has negligible impact on mortality and growth rates (cite). We waited another 3 days following attachment to check for mortality and dislodgement.

**Seawater treatment conditions—** We exposed oysters to one of two salinity treatments and a unique [TA] in a pseudo-factorial design, where oysters (n = 49 per bin) were grown in tanks (ntotal = 12) of replicated treatments (n=2 per treatment). Within either ambient (S = 34) or low (S = 27) salinity conditions, oysters were exposed to one of three TA treatments. Oysters in ambient salinity treatments, were grown in elevated, X, or X TA conditions (X, X, X + SEs) whereas, oysters in low salinity treatments were exposed to one of three [TA]s (X, X, and X) likely to occur when seawater salinity is reduced by freshwater endmembers (High TA river [X], low TA river [X], hurricane/precipitation [X]) mixing with ambient seawater ([X] conditions).

Experimental setup— We quantified the relative influence of osmotic stress versus carbonate system stress on surface area shell growth in oysters by growing them in unique treatment conditions for 5 weeks and measuring shell and tissue growth responses in individuals. Exposure to treatment conditions was refreshed every three days (see X), at which time, measurements of seawater salinity, temperature, pH, and dissolved oxygen % saturation were measured with calibrated sensors (see X). Experiments occurred in a temperature-controlled room and largely in the dark, to prevent any influence of light changes to oyster gaping, thus feeding, behavior (cite). Oysters were exposed to light conditions similarly, once every three days when growth bin seawater was refreshed. We adhered individual oysters of similar size distributions (range: X – X, mean = X, SD = X) to individual plexiglass? plates (n = 49 per plate, n = X total) using marine epoxy (cite) and placed one plate in each growth bin, i.e., a unique seawater condition. Growth bin (13-L X brand) seawater was circulated continuously with aquarium pumps (Xgph) to ensure continuous access to food and oxygen. We fed oysters daily with X (concentration), following feeding guidelines from industry growers (personal comms.) to prevent food-limitations to growth.

**Estimating growth rate and biological condition—** We measured shell growth as the surface area of the X side of the oyster, using Image J software to analyze photos of individuals. Photos of oysters were captured prior to the experiment (day 1), following 2 weeks (day 15) and after 5 weeks of exposure (day 36) using a XMP camera to capture top-view photos of each plate with a scale bar included for size. We used measurements of oyster shell growth (surface area mm2) to compute net growth rate and shorter, incremental growth rates between 0-2 weeks, and 2-5 weeks. We sacrificed oysters after 5 weeks, and dried the shell separate from the tissue at 60°C for 48-hr. We measured dry tissue mass and dry shell mass on a microbalance (X = 0.0000mg) and used it to compute individual condition index (CI) values (Okumuş and Stirling 1998). We quantified individual condition index values as X, where CI values may indicate the relative availability for energetic effort (cite).

Figure: Schematic of Tank layout and photo of oysters adhered to plates

**Chemical manipulation of seawater—** We refreshed experimental conditions in the growth bins with chemically-adjusted seawater every three days for the duration of the experiment (n = 12 growth, n = 2 control bins). Prior to incubation adjustments, we reduced [TA] to undetectable amounts in filtered seawater (filter size) sumps (n = 4 sumps per water change date) by adding hydrochloric acid (HCl), to convert TA to CO2(gas), and then bubbling vigorously for 48-hrs, to off-gas CO2 and equilibrate seawater with atmospheric concentrations. In low salinity treatments, we diluted prepared seawater with deionized freshwater (milli-Q) to lower the salinity, and then adjusted the carbonate system with predetermined amounts of chemicals (NaHCO3 (sodium bicarbonate) + Na2CO3 (sodium carbonate) and HCl) to target specific [TA], while controlling seawater pH (Waldbusser et al. 2015, Ninokawa et al. In prep). Salinity minimally fluctuated over the duration of the experiment in both ambient and reduced treatments ( ) due to natural variations in lab flow through seawater. The reduced salinity treatments was roughly X% of the ambient treatment. In both salinity levels we were able to target three individual [TA]s, while keeping the refreshed pH above X, and average measured pH (between refreshed and day 3) above X in all treatments. Reported as the saturation state, treatments in low salinity, X, whereas treatments in ambient salinity X. Although temperature was largely controlled, temperatures were slightly cooler in refreshed seawater (X) than in day 3 seawater (X) conditions, however, these changes were observed in all of the growth bins. Oxygen concentrations remained high (mean = X SE) over the course of 3 days, ensuring oysters were free from stress that accrues in hypoxic conditions (cite).

Figure: Multipanel; salinity conditions in tanks, TA conditions in tanks, T conditions in tanks, pH conditions in tanks?

**Characterizing experimental seawater conditions—** Prior to and immediately after refreshing individual bin seawater, we measured seawater temperature, salinity, and dissolved oxygen concentration with calibrated sensors (YSI blah blah blah) and pH spectrophotometrically and with the handheld multiparameter probe (YSI: pH blah blah). To report pH in total scale, we measured absorbance, calibrated daily with m-cresol dye standards (Easley and Byrne 2015). We measured pH absorbance in half of our sampling events at the same time as electrode charge measurements and applied the relationship to convert voltage measurements to pH. Concurrently, we collected and froze 250 mL of seawater for later total alkalinity concentration determination. We analyzed [TA] on a Metrohm 855 Titrosampler, correcting titration acid concentration daily using daily certified reference materials from the laboratory of Dr. Andrew Dickson (Scripps Institute of Oceanography). With two known parameters of the seawater carbonate system, in addition to salinity and temperature, we estimated the remaining carbonate system parameters (DIC, Omegacalcite, pCO2) using seacarb in RStudio with X coefficients (Table X, supplementary materials).

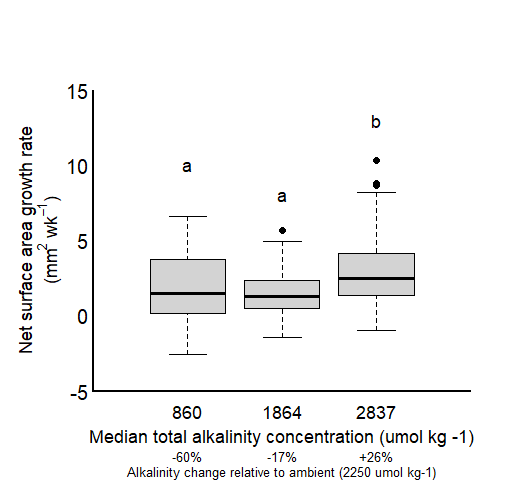
**Statistical analysis—**

***Results—***

**Independent role of [TA] on net growth—**

on average, when oysters only experience variability in [TA] but little salinity change (single stressor), to what extent does lowering and elevating [TA] (at ambient salinity) influence net oyster growth (~ X umol kg-1) . Oysters exposed to elevated alkalinity had higher net growth than those in ambient and reduced alkalinity conditions.

Figure X



**Influence of salinity and [TA] on net growth—**

Our **net growth data appear to have a log relationship with [TA],** common in growth data (cite); in order to test with a linear model, we log transformed the response variable (net growth) before running the model. When including salinity, TA as fixed predictors alongside bin number as a random effect (grouping) we found that when comparing a wide range of [TA] and salinity, there is an effect of [TA] on net growth, but the effect of TA does not differ between high and low salinity exposure.

We can also see that over the duration of the experiment, there **is no significant influence of salinity** across the range of [TA]. No impact on CI

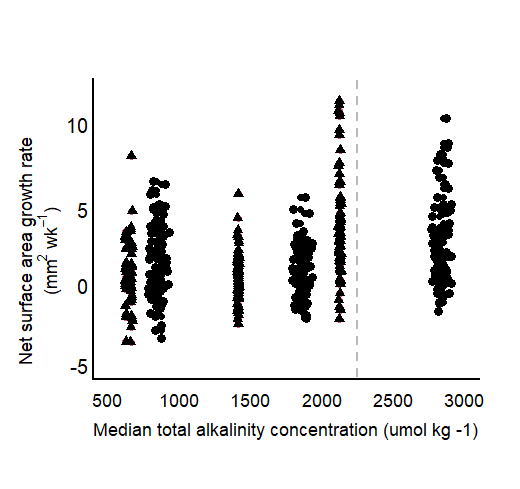


Figure X

**FIG of CI vs S and TA to show no trend?**

**Effects of [TA] on net growth in reduced salinity conditions—**

Now considering alkalinity change from a frequent source that also changes salinity (multiple stressor); for example, DI (hurricane/rainwater), diluted [TA], maintained [TA]; **to what extent does [TA] influence net growth during acclimation to low S conditions**

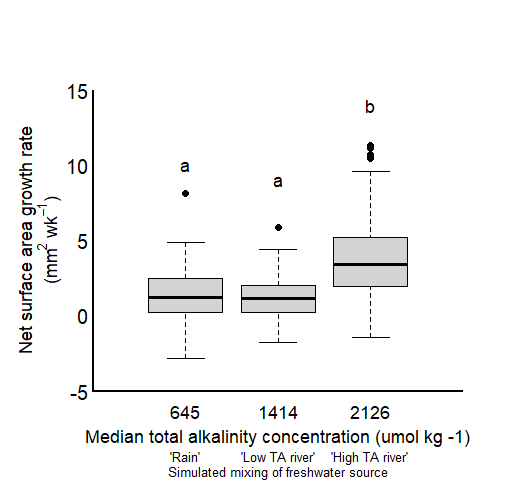


Figure X

**Incremental growth versus net growth rates—**

What does incremental growth say about how oysters are responding to environmental conditions?

* Figure 4 and 5: comparison of incremental growth rates comparing the first two weeks of growth and last two weeks of growth

Are there differences between growth seen in first two weeks versus that overall?

* Show a figure with incremental growth over first two weeks, and net growth overall (which includes the incremental) …may be a better way to show fig 4 and 5

***Discussion—***

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].