**~~Coastal estuaries have variable combinations of salinity and carbonate system conditions within small geographic spaces, with implications for sessile, organisms~~**~~.~~

~~We need to explore the nature in which responses to altered conditions persist through time to help understand how organisms may face future conditions?~~

~~s~~

~~n particular total alkalinity, as a result of biochemical processes (cite) and freshwater introducing its own alkalinity conditions. If estuarine mixing processes are slow, shifts in seawater conditions may happen abruptly and persist for long periods (> 1 month). This has significant import for calcifiers experiencing these shifts in conditions, due to their potential performance being heavily dictated by external seawater conditions.~~

**~~Paragraph 2~~**

**~~: Several studies have investigated disruptions to growth in calcifying organisms from abrupt exposure to altered carbonate system conditions over short or long-term exposures of time;~~** ~~It is hard to disentangle the nature in which altered species responses change as a function of exposure duration and most studies primarily have focused on net effects, both over short or longer-term exposures.~~

~~Have looked at: how~~ *~~when~~* ~~the exposure occurs may predict vulnrabililty later on in life (bottleneck) and also the~~ *~~nature~~* ~~in which exposure is constant or varies~~

~~As shifts in seawater conditions manifest as changes to shell growth, however, there is little understanding of how response patterns may change through time.~~

~~Patterns may be expected to change through time, due to the natural, necessary physiological shifts that increase the basal energetic demand in osmoconformers when confronted with altered seawater chemistry and salinity.~~

~~However, few studies have looked to see whether the effects they are seeing are similar between the days after an abrupt change in new conditions (0-18 day) and days longer after the exposure (18-36 days). This may have import for calcifiers in estuaries, as habitat conditions frequently shift, and persist, over different lengths of tme.~~

~~A lot of studies have demonstrated variable net shell growth responses of estuarine calcifiers from changes in the carbonate system, both in unchanged salinity and via low salinity dilutions that lower TA.~~

~~Conditions occur over various durations of time, suggesting that it is necessary to not only look at net responses to altered conditions over a certain duration, but also how those responses may change as a function of the length of exposure to altered conditions.~~

~~Changes in responses to conditions may show unique patterns in regards to whether conditions changed only TA, or changed TA in addition to salinity because co-occurring changes often demonstrate non-additive effects to performance.~~

**~~Paragraph 3: Oysters are present globally and have significant economic and ecological value to shoreline ecosystems. They also reside in habitats with some sort of routine changes in TA and s in their environment, where growth of the shell and tissue are both important to their coastal value. How do shells and tissue growth differ when considering them as potential trade-offs?~~**

~~often represented as the amount of tissue stores a bivalve has relative to the mass of the shell exterior.~~

~~Understanding environment effects on shell building requires investigating multiple components of net shell growth, both increases in shell surface area and increases in shell mass per area, in order to detect any shifts in shell thickness. Because energetic cost may limit growth when conditions increase basal metabolic demand, it is also necessary to investigate patterns in tissue growth as they relate to shell growth that happened over a similar period.~~

**Paragraph 4: Looking at the incremental growth responses in oysters to altered TA conditions represents a natural extension of prior work.**

We wanted to explore these questions in various combinations of perturbed salinity and alkalinity conditions, as they may be predicted to occur in estuarine systems (this is the incremental growth comparison).

Shifts in the carbonate system may occur from changes in TA and not from pCO2 directly, a concept that diverges from simulating ocean acidification effects. Specifically, total alkalinity in an estuary may vary for a given salinity, or could vary in response to low salinity water masses carrying their own TA conditions.

THIS IS WHAT PRIOR WORK HAS SHOWN in terms of next responses to disrupted carbonate system conditions in short term and long term (overall, general pattern).

As mentioned above, reducing salinity in laboratory experiments is often done with distilled or deionized freshwater, which not only dilutes salinity, but also lowers total alkalinity Although this is what we may anticipate with extreme precipitation events that introduce low TA freshwater, influence by streams may result in low salinities coupled with elevated total alkalinities. …this is what results have shown…

Low pH lowered growth rate but took away the negative effect of low DO when coupled in CV juveniles (changed by altering CO2) Stevens and Gobler

High CO2 leads to significant metabolic depression and when coupled with low S and diluted TA at elevated temperature, can lead to extracellular acidosis (def) in other oyster species Saccostrea glomerata (Parter)

Extreme declines in salinity (30 v 15) that also lowered TA increased mortality, reduced tissue energy stores and led to negative soft tissue growth rates in CV (Dickinson 2012)

Effects of diluting salinity and TA across pH showed that highest CV calcification occurred in high pH environments, regardless of salinity. The effect of pH on TA/Salinity dilutions showed individuals in ambient salinity (and TA) to have almost maintained growth, whereas those in low salinity and low TA have severely impacted growth (negative). (Waldbusser 2011)

Similar reductions in shell growth, following medium-term exposures (>2 weeks) to strong pH decrease ([-0.5 pH unit), have been reported by Beniash et al. (2010) and Talmage and Gobler (2011) for juvenile Eastern oysters (Crassostrea virginica). Interestingly, in the study of Beniash et al. (2010), in contrast to shell mass, the average shell area was not affected by hypercapnic conditions, suggesting that juvenile oysters were depositing thinner shells.

**Here we build on this knowledge base with C virginica oysters and found X.**

***####################################***

A lot of studies have demonstrated variable net growth responses of estuarine calcifiers from changes in the carbonate system, both in unchanged salinity and via low salinity dilutions that also lower TA (like DI). However, few studies have looked to see whether the effects they are seeing are similar between the days after an abrupt change in new conditions (0-18 day) and days longer after the exposure (18-36 days). This may have import for calcifiers in estuaries, as habitat conditions naturally shift, and persist, over different lengths. Comparing incremental growth through time may suggest (in)tolerance patterns if patterns are consistent, but stronger in the second increment (positive effect = tolerance, negative effect = increased vulnerability 18-36 days). We wanted to explore these questions in perturbed salinity and alkalinity conditions (this is the incremental growth comparison), as these two parameters decouple in these systems often and both have implications for calcifier performance. As mentioned above, reducing salinity in laboratory experiments is often done with distilled or deionized freshwater, which not only dilutes salinity, but also lowers total alkalinity. Although this is what we may anticipate with extreme precipitation events that introduce low TA freshwater, influence by streams may result in low salinities coupled with elevated total alkalinities. As such, investigations into how exposure to lower salinity conditions combined with different alkalinities influence calcifier growth (this is the net effect of TA across lower salinity treatment) would shed light on the potential import of freshwater systems to support calcification by elevating alkalinity, thus providing buffering capacity to coastal estuaries. Biogeochemical processing in estuarine waters with a higher residence-times, may lead to total alkalinity decoupling without corresponding changes in salinity. To this end, investigations that explore the relationship between oysters growth and alkalinity at an ambient salinity (this is the net effect of TA across ambient salinity treatment), would be valuable. Understanding environment effects on shell building requires investigating multiple components of net shell growth, both increases in shell surface area and increases in shell mass per area, in order to detect any shifts in shell thickness. Because energetic cost may limit growth when conditions increase basal metabolic demand, it is also necessary to investigate patterns in tissue growth as they relate to shell growth that happened over a similar period.

Influence of TA as a predictor of shell growth mechanistically

Separate influence of osmotic stress under maintained salinity conditions

Why this matters? Estuarine influence by freshwater is dynamic

Rain events causing mass die offs

Oyster performance elevated in estuaries, however, at risk to climate change shifts in precip regimes, nutrient loading (hypox), disease, etc

Focus not only on shell growth and aesthetics, but also on gut tissue

Focus on juveniles, more sensitive than adults and may be a bottleneck to extreme freshwater conditions

Food availability not limiting\* may or may not occur simultaneously with changing seawater conditions

***Methods—***

Experimental overview:

Species: Natural History

Chemical manipulation of seawater

Organismal performance quantification:

Shell growth

Net growth

Incremental growth

Energetic allocation

Condition index

% Organic carbon in shells

***Results—***

Seawater conditions

***Discussion—***

aslgnsdf