***Methods—***

Study species:

* Oyster name, location found, role in habitat
* Preferred substrate type/seawater conditions
* Interesting adaptations/ relevance to aquaculture
* Acclimation to BML seawater from Tomales Bay, CA; original spawn date, etc prior to pick up?
* adhesion to plates (similar size distributions and ranges)
* Another 3 days acclimation

**Treatment layout—** Lalala

Schematic?

* Pseudo factorial design, where oysters (n = 49 per bin) were grown in tanks (ntotal = 12) of replicated treatments (n=2 per treatment) of either ambient (S = 34) or low (S = 27) salinity conditions.
* seawater was agitated and then added to bin. Within growth bins, pumps recirculated water so as to not limit growth based on pumping potential. We lidded the acclimation tanks to control conditions in the bit, however, due to a small amount of headspace, conditions drifted (similarly) over 3 days.
* Within each salinity level, oysters were grown in one of three [TA] treatments, pertinent to our question
  + In ambient salinity, we exposed osyters to X to simulate X
  + In low salinity we exposed oysters to X, to simulate X

**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) 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. 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 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).

**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], 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).

**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.

**Oyster growth rate—** We measured 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 quantified individual condition index values as X, where CI values may indicate the relative availability for energetic effort (cite).

Statistical analysis—

To test the effect of starting size (SA) and TA condition on the average net growth in juvenile oysters, we employed separate mixed effects linear models to net growth rate data in low salinity and high salinity treatments. We included starting SA (continuous) and TA conditions (factor) as fixed effects, and bin as a random intercept to control for non-independence between oysters from the same growth bin. We included a weighting X in our model equal to the X in order to account for heteroscedastic variability between TA conditions; initial attempts to transform the response parameter was unsuccessful meeting model assumptions.

We employed similar models to decouple the effect of lower salinity from TA condition by comparing net growth rates of oysters from similar, near ambient TA conditions (TA = ~ X +- SE), but varying salinity treatments.

Sampling independence was accounted for by including bin as a random effect.

Normal error distribution was visually verified with QQ-plots and histogram of the residuals following the addition of variance weighting.

In ambient salinity treatments we did not see an effect of TA condition on the average net growth rate and therefore did not conduct post-hoc analyses.

In low salinity treatments we tested for differences in mean growth rate using X, with a significance level a = 0.05)**.**

To test the effect of salinity and TA condition within different time increments following exposure to new ocnditions, we employed similar mixed-effects models to above, but also included increment as a fixed predictor (factor) that could have an interaction with TA condition, for both low salinity and ambient salinity treatments, separately.

Post hoc analysis was conducted using X, with an alpha significance threshold = 0.05.

We tested for a difference in tissue mass between treatment bins using a one way anova with treatment condition as a fixed effect, and bin as a random effect. We conducted a similar analysis for condition index.

we are allowing the variances to be different for each level of the TA.treat.x variable, but within each level, the variance is assumed to be constant (homoskedastic within each level). This can be a way to model heteroskedasticity within the context of a linear mixed-effects model, where you suspect that the variance of the response variable may vary across different levels of a categorical predictor (in this case, TA.treat.x)

*To understand whether abrasion from sand of differing coarseness might influence dissolution, we computed the difference between dissolution rates of mussel valves in abrasion treatments, and those of unsanded, control valves. We then used a Welch’s independent sample t-test to determine whether differences in average dissolution existed between the sanding treatments. Assumptions of normality and homogeneity of variances were assessed visually using qq-plots.*

*We used a two-way ANOVA to compare average periostracum cover in adult mussels collected from the field at two relative tidal heights and three levels of sun exposure. We dropped the interaction term between the treatments after confirming it failed to explain significant variability in the model. Using tidal height and sun exposure as categorical predictors to explain periostracum cover, we then ran a TukeyHSD test (95% confidence) to identify which microhabitat types differed from one another via pairwise comparisons.*

INCREMENTAL GROWTH MODEL FOR LOW SALINITY DID NOT NEED VARIANCE WEIGHTING IN MODEL; PASSED HETERSCEDASTICITY

**Tissue mass (treatment) = 5.92 mg +\_ 0.28**

**Tissue mass (control) = 3.67 mg +\_ 1.16**

**CI (treatment) = 0.02 +\_ 0.00**

**CI (control) = 0.01 mg +\_ 0.00**

**End shell area (treatment) = 143 +- 7 mm2**

**End shell area (control) = 83 +- 26 mm2**

**NEED TO MAKE A TABLE TO SEAWATER CARBONATE SYSTEM PARAMETERS?**

**LPB™ FROZEN SHELLFISH DIET®**

**whole-cell concentrate of Tetraselmis, Thalassiosira weissflogii, Thalassiosira pseudonana, and Schizochytrium**

**Physical Properties**

**Algal Cell Size……………….. 4.5–15 microns**

**Cell Density………………… ~1 Billion cells per ml**

**Algal Biomass……………. 150g Dry Biomass/L**

**Composition of Dry Algal Biomass (Typical)**

**Protein………………………. 52%**

**Lipids…………………………. 10.7%**

**DHA……………………... 8.0% (% of lipids)**

**EPA………………………. 9.5% (% of lipids)**

**Carbohydrate…………… 16.0%**

**Ash…………………………… 21.0 %**

**Feed 0.018 - 0.04ml LPB per animal per day**

**(assuming 1g meat weight); we fed as tanks in 20ml of a dilution mix that is 10% concentrate v/v, which equated to around 0.04mL per animal**