1. **INTRO:**
   1. Thanks for coming to hear my talk...
   2. Before I get started, I wanted to let everyone know that I have some sweet SEM stickers for a little shameless bribery – come grab them at the end!
2. **MEDIA:**
   1. When I thought about how to frame the story of my research, I thought a lot about how the media often translates science, specifically the headlines. I happen to be related to a journalist, and have had many conversations about this.
3. **HEADLINES**:
   1. I thought back to some of the headlines I’ve seen recently…these are just a selection from the Washington post….
4. **MY HEADLINE**:
   1. So I thought I’d try to make my own headline that could be the basis of my talk – to give you the take-away points.
   2. But the problem with this exercise is that it’s so complex…as many of us have seen, those headlines on the last page are heavily debated in the scientific community (except for the Coldplay one, I don’t know how much debate there is for that one).
   3. So, my headline has caveats…
5. **OUTLINE**:
   1. So, I’m still sticking with the common format, so you can follow along chronologically, I’m going to go through a bit of background information, my methods, results and discussion. I’ll jump between methods and results at times, but hopefully it will be clear.
6. **CHAOS:**
   1. So, to jump start- climate change. I don’t put this up here to overwhelm you, although maybe I kind of do, but not with the expectation that you’ll read it. I just want to showcase the complexities of climate change. There are processes that create stressors that lead to organismal responses and community responses. The point of this is that it’s chaotic. There are a lot of factors and variables. Unfortunately – I couldn’t answer every question on there on climate change, so my focus is on temperature increase and acidification, which alters the pH, and the response of skeletal development and growth.

1. **WARMING**
   1. So, to break those two factors down a little bit more.
   2. As we drive our cars and burn fossil fuels, this leads to an increase in CO2, a known greenhouse gas, in the atmosphere. This traps heat, which is then absorbed in the ocean, and increases the sea surface temperature. If we continue the path of emissions we are on today, indicated by the red line, there is an expected increase of SST of 2-4°C by 2100.
   3. This temperature change can lead to variable responses, such as reductions in body size, changes in the timing of life cycle events, range shifts toward to poles, and potential increases in invasive species.
2. **ACIDIFICATION:**
   1. In addition, the increased CO2 in the atmosphere absorbs directly into the ocean, altering the seawater carbonate chemistry. The average ocean pH is around 8.1 (give-or-take depending on location) and is predicted to drop 0.3 to 0.5 units by 2100 (indicated by the red line) if we do nothing – making it more acidic.
   2. This can lead to changes such as reduced calcification, increased dissolution, changes to reproductive fitness etc.
3. **INTERACTIVE EFFECTS:**
   1. Now it may be simple to explore these stressors singularly, but the reality is that they occur simultaneously and can lead to what we call interactive effects. So we have stressors on the x and the performance detriment, or whatever measure of stress effect is being explored, on the Y.
   2. So A could represent warming and B acidification. If those stressors happen simultaneously, they can add up to create an additive response or create a synergistic response, where the negative impact is even more amplified.
   3. Or, we can have an antagonistic effect, where one stressor might mitigate the effects of the other. For example, when temperature and acidification are explored simultaneously, sometimes warming can mitigate some of the negative impacts of acidification.
   4. This interactive component can often make interpretation of results very difficult, and that increases as you continue to add stressors – which do naturally occur simultaneously.
4. **INVERTS:**
   1. Further confounding the complexities of understanding climate change impacts is the sheer amount of organisms to study –
   2. Invertebrates, like corals, urchins, lobsters etc. make up 99% of all animals and many of those are marine.
   3. Many marine inverts are calcifiers – meaning that they build their own skeletons by utilizing different elements from the surrounding seawater.
   4. Play important roles: Food and other uses for humans! Filtration of water and sediments, Grazing to control algae overgrowth, Provide habitats etc.
5. **ECHINODERMS**:
   1. One such category of marine invertebrate, Echinodermata ( which literally translates to “Spiny skin”) encompasesses organisms such as sea-stars and sea urchins. echinoderms are hugely important for their natural ecosystems and often act ecological managers – maintaining algae growth that could otherwise overwhelm the ecosystem.
   2. They are of additional interest because of their high magnesium content calcite skeletons, which is a more soluble form of calcium carbonate, similar to aragonite. Making them thought to be more sensitive to changing ocean conditions from acidification.
6. **LIFE CYCLE**:
   1. Now lets focus on sea urchins – their life cycle consists of a benthic phase – where they remain on the sea floor, and a pelagic planktonic phase, where they are free swimming.
   2. Urchins are broadcast spawners – releasing their gametes directly into the water column where fertilization and early development takes place.
   3. Then the larval form begins to develop, and this is where the first calcified bits appear in the body rods and arms.
   4. Then the begin to metamorphose into the juvenile stage, where they settle on the bottom to grow into a full adult.
   5. The timing of this cycle may vary by species, but in the species I studied, urchins are usually settled in their post-metamorphic juvenile stage roughly around day 30 after spawning.
   6. Because of the dynamic life-cycles of these organisms, with calcification occurring at different stages in different environments, responses to warming and acidification are variable. I’m going to try to give a brief background of urchin response to climate change at each stage, although keep in mind that depending on the conditions, regions, etc. results have often been opposing…
7. **FERTILIZATION/ED**:
   1. For % fertilization success , there has been found to be no effect of temperature or pH.
   2. Early development on the other hand, is more influenced by temperature rather than pH – which makes sense because these early development stages don’t have any calcified parts yet.
   3. However, ED is still pretty resilient to climate change both warming and OA
8. **LARVAL**:
   1. Larval stages can get more variable, depending on the conditions set by the study. But often, as shown in this study, there is an interactive effect, with warming offsetting the negative influences of pH.
   2. Negative impacts may be a delay in development, abnormal growth, or reduced calcification.
   3. You can see, at three different pH levels (8.15 (ambient), 7.8, and 7.6), the formation of larvae declines at lower pH conditions. The two temperature treatments offset the effects of low pH.
9. **JUVENILE - ADULT**:
   1. Lastly, the adult stage is generally thought to be resilient to OA and warming, with sublethal effects. In this study, urchins grew less at both 22° and pH 7.6.
   2. They also measured the reproductive index, which had interactive effects – warming increased it and pH decreased it. However, there were virtually NO gonads at pH 7.6, regardless of temperature.
10. **CHAOS and LIFE CYCLE**:
    1. So, to circle back around, climate change is chaotic. With a plethora of responses possible. The varying life stages of urchins also add to the complexity. Generally, fertilization/ED and the adult life stages are pretty resilient to climate change with larval and juvenile stages being the most sensitive.
11. **TRIPNEUSTES GRATILLA**:
    1. Which brings me to my species of choice to study. I wanted to understand how this ecologically important species, native to Hawaii, could be sensitive to climate change from the juvenile to adult stages. Specifically, I wanted to look at relevant levels of warming and acidification to see how it would influence growth and skeletal development.
12. **INVASIVE ALGAE**:
    1. To give you a bit of background on this specific species, I’ll start with their particular ecological importance.
    2. Multiple species of invasive algae have been either intentionally or unintentionally introduced to the Hawai’ian islands.
    3. This can lead to phase shifts from coral dominated to algae dominated, monopolizing the reef habitat.
    4. It was found that manual removal – super sucker barges – followed by the outplanting of urchins as biocontrol, could maintain reasonable levels of macroalgae and prevent coral smothering.
    5. Since Tripneustes are relatively easy to grow in aquaculture, are generalist feeders that eat a lot, they were deemed an effective option for use in biocontrol.
13. **DAR HATCHERY:** 
    1. Because of this, the Division of Aquatic Resources established the sea urchin hatchery to create an army of urchins to defeat the algae.
    2. A peek inside the hatchery…
14. **SUCCESSFUL:** 
    1. This process has been found to be successful on reefs investigated in Kaneohe Bay, with percent cover of invasives decreasing without effecting native species.
15. **MAP**:
    1. These urchins are native to Hawaii, but are also found elsewhere. They experience a range of temperatures…
16. **OUTLINE:**
    1. Now, we’ve gone over….
    2. So lets get into the meat of my experiment.
    3. Experimental design and environmental condition results
17. **HATCHERY COLLECTION**
    1. Collected at the hatchery at around 7 mm in diameter. Big enough that they eat macroalgae, rather than biofilm in early stages, but small enough that they have a lot of room for growth.
    2. Brought the HIMB where they would be acclimated to the bay water for two weeks, followed by a subsequent ramp up of conditions for two weeks before they reached conditions
18. **SCHEMATIC**
    1. Seawater pumped in from the bay so tracked natural cycle of temperature and pH. (take note of the colors)
19. **SET-UP**
    1. This is what the experiment actually looked like…describe
    2. So I had 24 urchins total, with 6 per treatment condition. I did have one mortality due to unknown conditions from an urchin in a heated condition on day 116.
20. **TEMP**
    1. The desired goal was to have a 2 degree difference between ambient and heated to replicate the predicted conditions of temperature change from climate change.
    2. The graph has time by day on the x-axis and the temperature in celcius on the y. Note that days below 0 indicate We achieved this.
    3. Note (?), I measured seawater temperatures weekly at midday.
21. **pH**
    1. The desired goal here was to manage a 0.3 difference in pH over the course of the experiment, similarly reflecting the expected changes.
    2. Same graph as before but looking at the pH.
    3. Note – I didn’t have any fancy controllors to maintain the pH, so had to do this manually, which led to the fluctuations and variability you see here. But the overall mean for the full 126 days was indeed -0.3.
22. **OUTLINE**
    1. Now we see what the experimental set-up looked like, lets move into what I measured.
    2. Growth Measurements and Growth Results
23. **GROWTH:**
    1. Measured two diameters once a week – they were often nonsymmetrical
    2. And…they grew!
24. **GROWTH PLOT:**
    1. To analyze growth, we used a linear mixed model with temperature and pH as fixed factor and the individual urchin as a random effect
    2. Growth was calculated as a % based on the initial size, so they could be standardized.
    3. This is consistent with other studies where growth often increases with associated temperatures, to a point.
    4. So, while this might seem like a positive response, this is not indicative of the temperature extremes experienced by this species that could become a stressor.
25. **OUTLINE:**
    1. So now we’ve seen what we did and the results of growth, lets move on to…
    2. SEM and Calcification Results
26. **SEM BACKGROUND**:
    1. Scanning electron microscopy has been used to describe skeletal formations previously. In this study that explored the structure of urchin spines to their function, deeper understanding of their life-style and habitats could be inferred.
    2. You can see the urchin as a whole photographed in the top left, the bare skeleton or test to the right, then the outside of the spine and internal structures
27. **BEMF:**
    1. This is the SEM we have here on campus
    2. Picture on left shows the whole machine, while the one on the right shows the ability to see into the machine directly to your sample.
28. **STUBS:**
    1. For my study, I wanted to explore the internal structure, not just the outside, so cut at the tip and the base to see both. I took three spines per individual to look at the base and tip cross sections.
    2. Preliminary testing showed that internal structure varied through the length of spine, so wanted to see both.
    3. Can control the stub to look at the samples on an x, y and z-plane to get a clear overhead viewpoint of the spines for comparisons.
29. **INITIAL RESULTS:**
    1. This is what we got!
    2. Take note of some of the basic patterns and structures of cross sections. Some are more organized and structured, while others are more disorganized.
30. **RESULTS OF TREATMENTS**
    1. There was a lot of variation that made my initial interpretation difficult, even from spines from the same urchin.
    2. Notice the colors again
31. **BASE BREAKDOWN**
    1. Upon looking more closely
32. **MACRO VIDEO**
    1. In order to quantify the calcification response, we used imagej and wrote a script for it to convert these images to black and white. This allowed us to create a ratio of structure-to-void to quantify the content of material.
    2. I just did the analysis on the inner portion of the spines, to account for the central structure rather than the edges.
    3. The higher the ratio, the more black-to-white area there was, and therefore more calcified material was present
33. **IMAGEJ BINARY**
    1. Resulting binary conversion pretttyyyy!
34. **CALC RATIO**
    1. Similar Linear mixed model
    2. On the x-axis we see the treatment conditions, note the colors still apply to the given treatment, and the y-axis is the calcification ratio. The right side plot is what we see at the tips, and the left is the bases.
    3. We see the tips had less material than the bases, but didn’t vary between treatments.
    4. The bases on the other hand, did vary, with acidified conditions regardless of temperature, having significantly less material present. So, even though the urchins grew more in warmer conditions, that growth spurt didn’t help them to calcify more, as there was no interactive effect.
35. **DROPPED SPINES**
    1. Something else we saw, urchins in acidified conditions had more loose spines at the base of tank.
    2. I want to note that although I noticed this throughout the experiment, this was only quantified at the end, when it became clear that something was going on in the structural abilities of the spines.
    3. The spines were also more fragile, often breaking off in my hand upon handling.
36. **OUTLINE**
    1. Alright, so far we’ve covered some basic background info, the methods of the experimental set-up, a
    2. does it all mean and why do we care?
37. **WHAT DOES IT MEAN: GROWTH**
    1. Recap of what we found
38. **WHAT DOES IT MEAN: CALCIFICATION**
    1. Graphic to showcase
39. **WHATS GOING ON AT THE TIPS**
    1. Wound healing phase where dermis grows over top
    2. Sclerocytes present in the dermis – outer organic layer
    3. Rate – in one species, full regeneration after 40-60 days.
40. **WHATS GOING ON AT THE BASES**
    1. Longitudinal growth followed by lateral thickening
41. **REGENERATIVE CAPACITY:**
    1. So this makes sense for what we found in the differences between the bases and the tips.
    2. And indeed, another study found that OA impacts the spine integrity – so the strength and oomph – but not the regenerative capacity.
    3. So they can grow, but they have a harder time in that secondary thickening stage.
42. **HOW DO THEY CALCIFY:**
    1. So let’s look more closely at how they actually calcify. I will say this is very much a black box of mystery of the cellular mechanisms going on here, but simplistically…
    2. We’ve already gone over that the pH of the ocean is decreasing from ocean acidification due to climate change.
    3. This really just translates to an increase in the proton concentration of the surrounding seawater.
    4. In order to calcify, urchins must actually increase the pH at the site of calcification thereby must expel the excess protons to promote the formation of carbonate from bicarbonate to build their skeletons.
    5. The energy required to do then increases when the external seawater concentration is high.
    6. So, urchins must increase the pH at the site of calcification, but if the surrounding seawater pH is lowered from acidification, this becomes difficult.
43. **DROPPED SPINES**:
    1. So, coming back to the increase dropped spines, we saw that they could grow well but not quite thicken enough, which either made them intentionally drop their spines to try and rebuild, or they just broke from being weaker. Either way, it’s a lot of energy to do this!
44. **SO, WHAT’S UP WITH SPINE CALCIFICATION?**
    1. Can grow in length but not thicken, likely due to being in a continuous state of regeneration
    2. This takes a lot of energy to calcify, potentially sacrificing other process (like reproduction).
       1. Remember I mentioned a previous study finding virtually NO gonads at pH 7.6, the level we explored here…so that’s bad for future populations.
    3. And they’re dropping their spines, thereby reducing defensive effectiveness.
45. **SPINE TIPS**
    1. Additionally, many studies use SEM images of the tips of spines to explore the effect of acidification. Which makes sense, seeing that they do build at the tip.
    2. But our study indicates that this actually isn’t a good proxy at all, as much more is at play, and more may be happening in other parts of the spines.
46. **WHY DO WE CARE?**
    1. These urchins are ecologically important, if climate change compromises aspects of their development and they become less effective as biocontrollors, that would not be good.
    2. If energy required to keep up with these challenges prevents them from keeping up with reproduction, future generations could be compromised.
    3. This is the first study to my knowledge to explore the calcification impacts at the base of spines and indicates that other studies that found no effect at the tip may be misled.
    4. Science and urchins are cool – duh!
47. **RECAP**
    1. What we did:
    2. What we found:
    3. What it means:
48. **RECAP**
    1. This is what we call a recall! We got there…
49. **NEXT STEPS**
50. **THANK YOU FUNDING**
51. **THANK YOU COMMITTEE**
52. **THANK YOU MISC**
53. **THANK YOU FAMILY AND FRIENDS**
54. **QUESTIONS**