

# Integrated impacts of temperature increase and ocean acidification on larval shell development in the American Lobster, *Homarus americanus*

Christine San Antonio<sup>1</sup>, Michael Tlusty<sup>2</sup>, Robyn Hannigan<sup>1</sup>

<sup>1</sup> University of Massachusetts Boston, 100 Morrissey Blvd, Boston, MA 02125

<sup>2</sup> New England Aquarium, 1 Central Wharf, Boston, MA 02110

## Introduction

- The American lobster fishery is an important component of the coastal New England economy and community with a dockside value of over \$560 million in 2014.<sup>3</sup>
- Lobster shell is composed of layers of chitin fiber interspersed with CaCO<sub>3</sub> crystals.<sup>1, 7</sup>
- Previous research on the American lobster has focused on single stressor (pH) lab experiments to determine the effects on calcification, growth, and survival rates.<sup>2, 5</sup>



Figure 1. Stage II larval American lobster photographed under stereomicroscope.

- Larval American lobsters have four stages of molts before becoming a benthic juvenile; this is a naturally stressful time in their life cycle.<sup>6</sup>

## Objectives

We have three primary objectives:

1. Examine the synergistic effects of elevated temperatures and decreased pH on larval shell development in the American lobster, *Homarus americanus*.
2. Assess if and how the results from objective #1 correlate with susceptibility to epizootic shell disease.
3. Conduct an initial pilot study to test the integrity of our system, experimental conditions, and methods for analysis. The pilot was also intended to preliminarily test our first hypothesis.

## Hypotheses:

Under conditions of decreased pH and increased temperature, we expect to see:

1. Decreased shell growth and changes in CaCO<sub>3</sub> mineral composition and abundance.
2. Increased susceptibility to disease and effects on survival.
3. Downregulation of genes involved in shell biomineralization.

The preliminary results of a 10 day pilot study are presented here.

## Methods

- Larval lobsters reared at the New England Aquarium (Fig 2), transported by cooler to UMB.

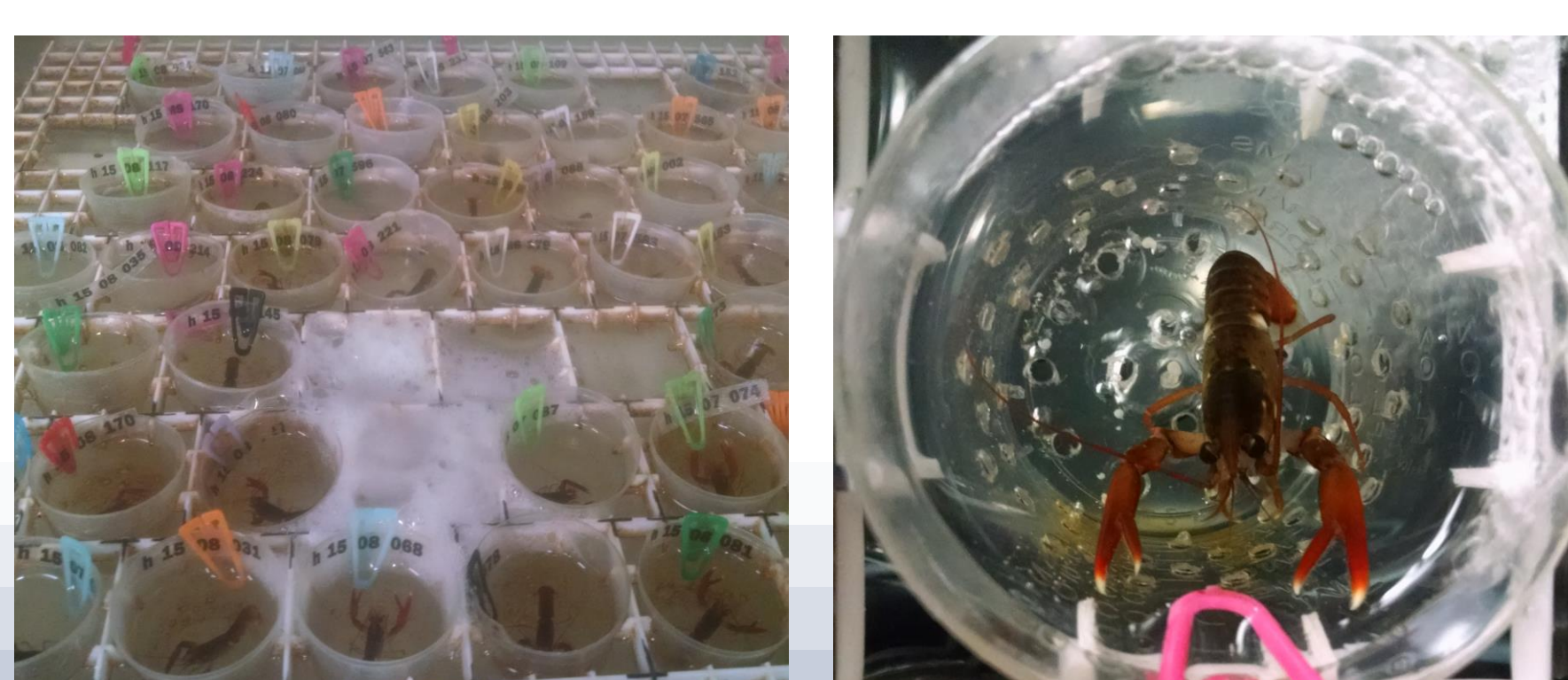


Figure 2. Left: Larval lobsters at the New England Aquarium; right: juvenile lobster in holding cup.

- Four pH/Temp pilot treatments of: 8.1/16C, 8.1/24C, 7.6/16C, 7.6/24C.
- Experiment run on a unique pH-stat CO<sub>2</sub> dosing system that continuously logs data and allows for true independent replicates (Fig 3).<sup>8</sup>

## Methods (cont'd)



Figure 3. Top: System electronics including solenoids (control pCO<sub>2</sub> dosing), SLI modules (log water quality data), temp probes, pH electrodes, and tubing for ambient air and CO<sub>2</sub>; bottom: individual tanks showing structural set up for holding larvae.

- Salinity, temperature and total alkalinity were measured daily; DIC, pCO<sub>2</sub> and Ω<sub>Ar</sub> were modeled using the CO<sub>2</sub>SYS excel macro.<sup>4</sup>
- Larvae that survived the 10 days were euthanized using tricaine mesylate (MS-222) (Fig 4).

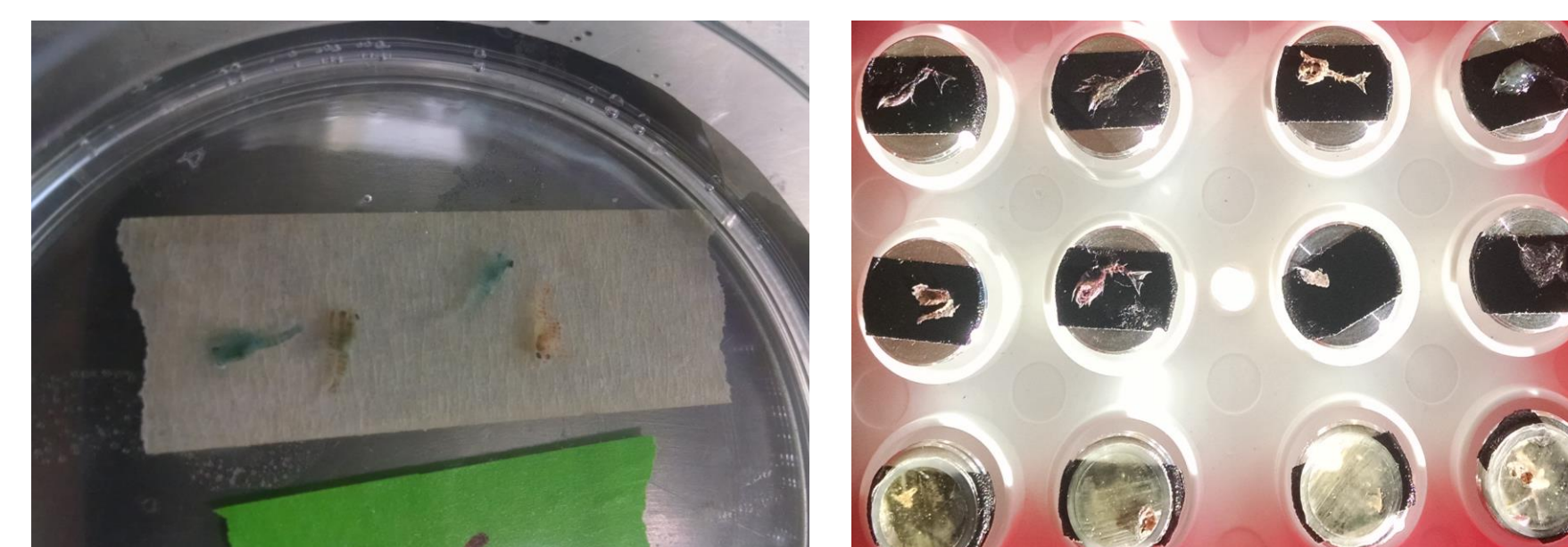


Figure 4. Left: Larval lobsters being euthanized in MS-222; right: selection of SEM stubs, bottom row are cross sections in Crystal Bond™ adhesive (Electron Microscopy Sciences).

- Using scanning electron microscopy, we examined the outer epicuticle of select larvae from treatments to determine if CaCO<sub>3</sub> crystals and bacteria could be detected.
- One larva from each pilot treatment was embedded in Crystal Bond™ adhesive and sectioned using an IsoMet® low speed saw to test the method for creating a quality cross section for SEM (Fig 4).

## Pilot Results

pH	Temp (C)	Alk (μmol/kg)	Sal (ppt)	DIC (μmol/kg)	pCO <sub>2</sub> (μatm)	Ω <sub>Ar</sub>
7.60 (0.05)	23.39 (0.37)	3055.00	34.83	2949.23	1675.33	1.83
7.80 (0.06)	15.87 (0.14)	2804.43	33.70	2669.40	911.90	1.89
7.85 (0.07)	23.46 (0.21)	2960.52	34.25	2737.86	862.08	2.92
7.60 (0.04)	15.14 (1.50)	2898.51	32.92	2849.48	1561.57	1.22

Table 1. Carbonate chemistry collected from each treatment continuously over the 10 period. Shows mean of variables (±SD where applicable) and CO<sub>2</sub>SYS data output.

- All larvae (but one) grown in the 24C treatments died prior to pilot completion.
- Procedure using IsoMet® low speed saw to get a cross section of did not produce clear images under SEM (Fig 5).

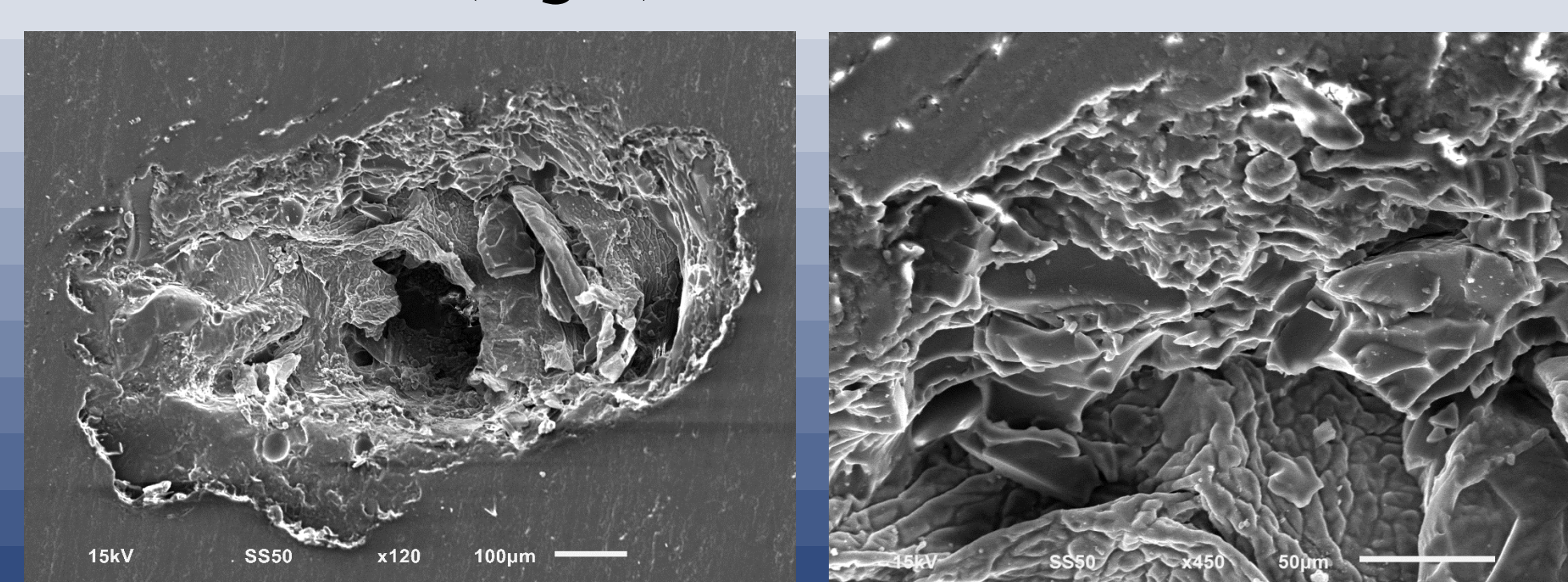


Figure 5. Cross section at different magnifications under SEM.

## Pilot Results (cont'd)

- CaCO<sub>3</sub> crystals of sufficient size and abundance were not consistently seen on larval epicuticle under SEM (Fig 6).
- Outer epicuticle of the larva grown under 7.6/24C was more textured than the larva from control (8.1/16C) treatment (Fig 7).

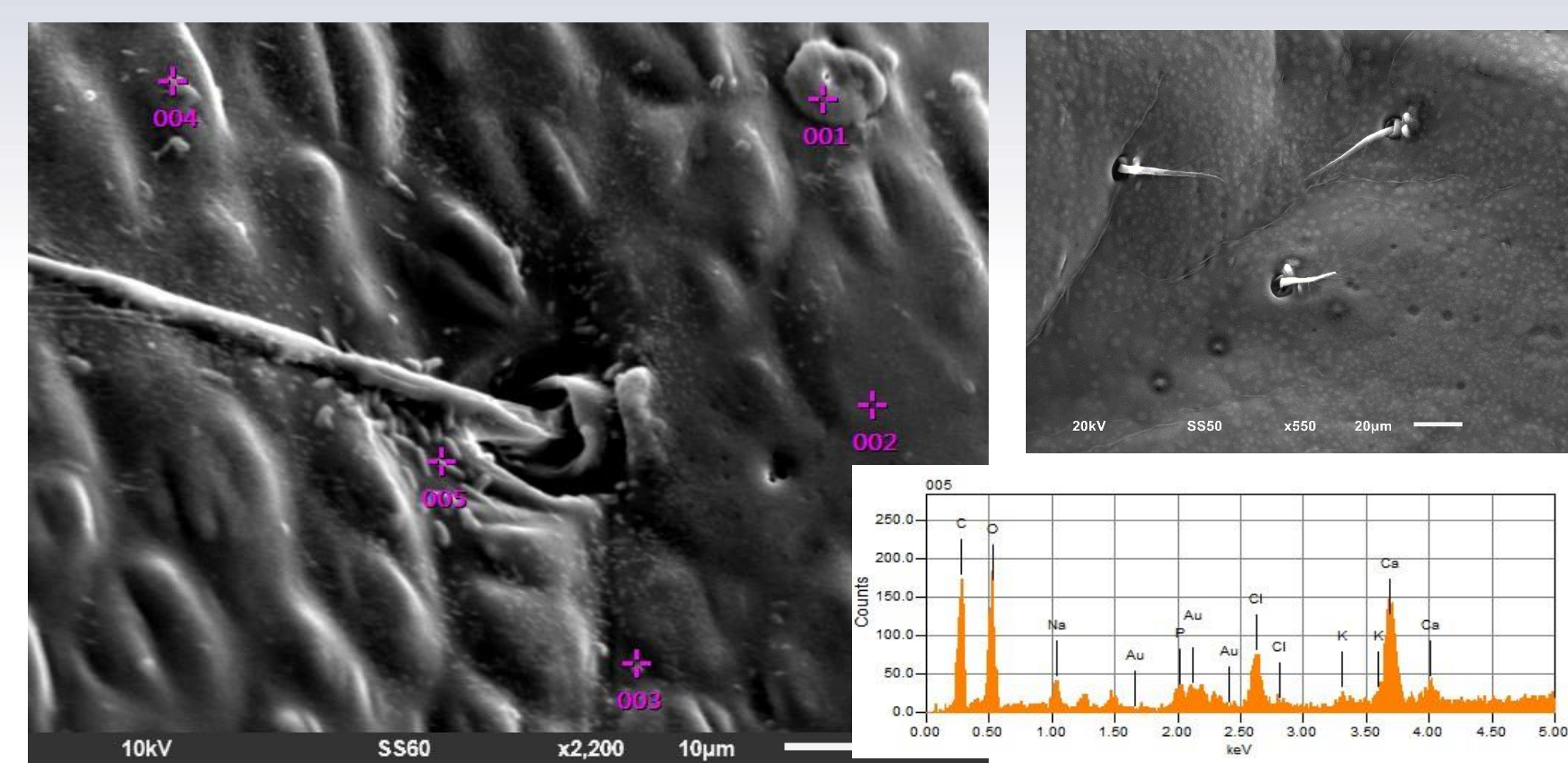


Figure 6. EDS analysis near a setal pit with a sample elemental composition output; top right: setal pits on control larva, EDS results were not consistent across larvae.

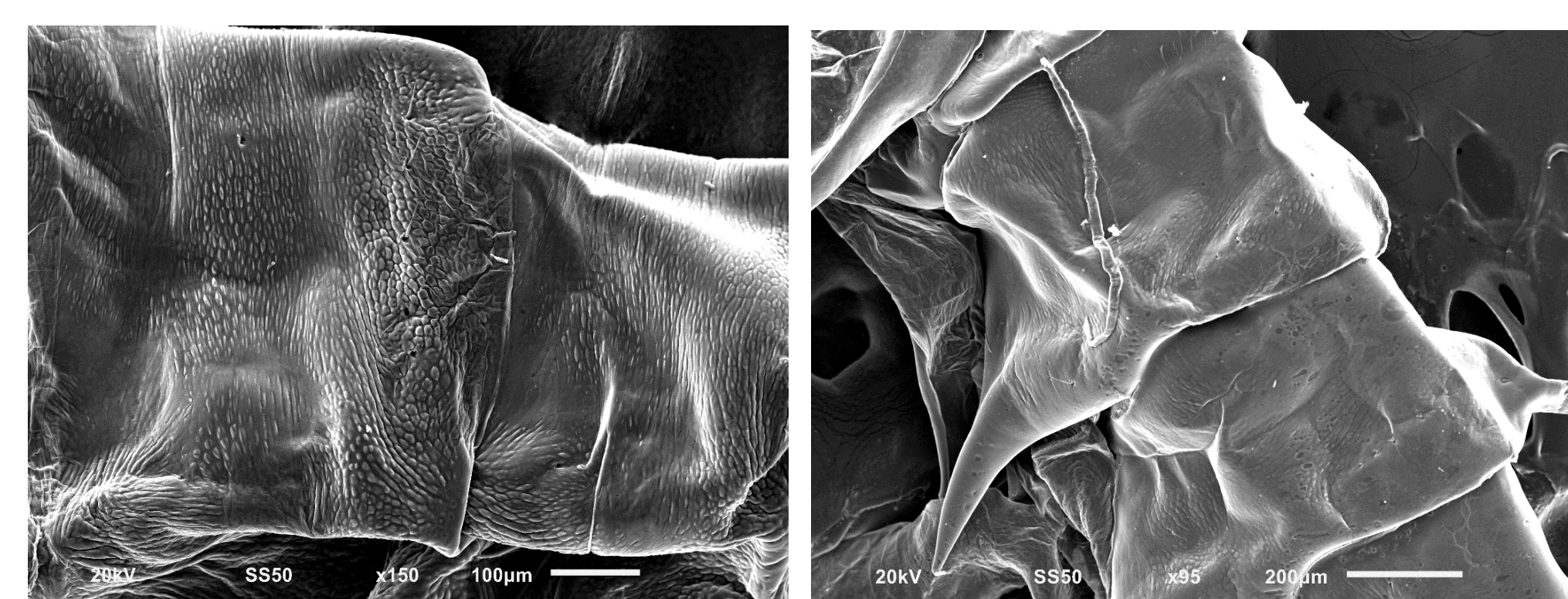


Figure 7. Epicuticle of the abdomen showing texture; left is larva from 7.6/24C, right is control larva 8.1/16C.

## Discussion

- Results of the pilot study were not sufficient for doing statistical analyses or to quantifiably show support for our first hypothesis.

## Pilot Study Lessons Learned and Next Steps:

- Adjust highest temperature for treatments.
- Develop alternative method for taking a cross section of fragile larval shell – resin?
- Cross sections are likely key to seeing and quantifying CaCO<sub>3</sub> crystals.
- Determine if differences in epicuticle texture translates to low pH and high temperature.
- Complete full experiment to test for connection between OA/temp and susceptibility to epizootic shell disease.

## Acknowledgements



Robert Holmberg  
MIT Sea Grant Program  
The New England Aquarium  
UMB Environmental Analytical  
Facility (EAF)  
Hannigan lab group

## References

- [1] Davies, C. E. et al. (2014). A comparison of the structure of American (*Homarus americanus*) and European (*Homarus gammarus*) lobster cuticle with particular reference to shell disease susceptibility. *Journal of Invertebrate Pathology*, 117, 33-41.
- [2] Keppel, E.A. et al. (2012). Ocean acidification decreases growth and development in American lobster (*Homarus americanus*) larvae. *J. Northw. Atl. Fish. Sci.*, 44: 61-66.
- [3] National Marine Fisheries Service. (2015). Fisheries of the United States, 2014. U.S. Department of Commerce, NOAA Current Fishery Statistics No. 2014.
- [4] Pierrot, D., Lewis, E., and Wallace, D. (2006). Program developed for CO<sub>2</sub> system calculations: Carbon Dioxide Information Analysis Center, Oak Ridge National Laboratory, U.S. Department of Energy, Oak Ridge, Tennessee.
- [5] Ries, J. B., Cohen, A. L., & McCorkle, D. C. (2009). Marine calcifiers exhibit mixed responses to CO<sub>2</sub>-induced ocean acidification. *Geology*, 37(12), 1131-1134.
- [6] Scarratt, D.J. (1973). Abundance, survival and vertical diurnal distribution of lobster larvae in Northumberland Strait, 1962-63, and their relationships with commercial stocks. *J. of Fisheries Research Board of Canada*. 30.12: 1819-1824.
- [7] Stirn, A. (2012). The formula for lobster shell. *Max-Planck Research: Materials and Technology Bionanocomposites*, 77.
- [8] Wilcox Freeburg, E. D. (2014). Exploring the link between otolith growth and function along the biological continuum in the context of ocean acidification.