

# A Cyte to See: An Exploratory Investigation of Hemocytes and Cellular Biomineralization in *Crassostrea virginica*



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# Rationale



- Biogenically constructed calcium carbonate is one of the most utilized raw materials in the modern world.
- One common biomaterial is that of the mollusc shell, and our research focuses on how the Eastern oyster deposits shell.
- Previous research and the composition of hemolymph



# Materials and Methods



## Obtain Sample

Hemolymph was collected using 20 gauge needle.

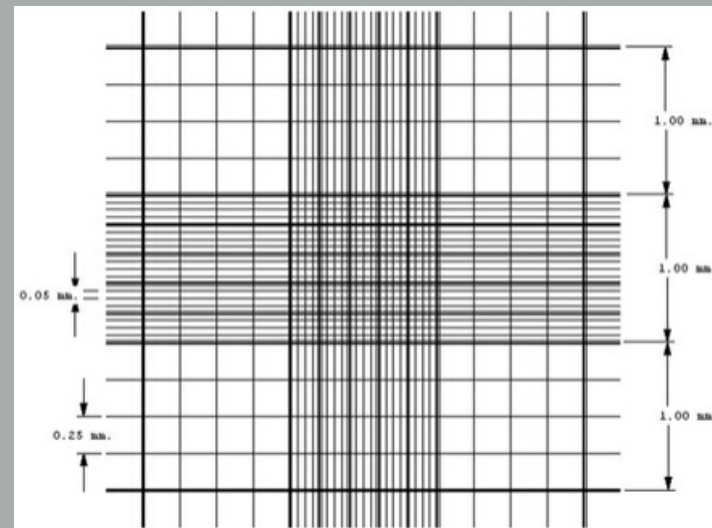
Experimental and Control groups

Samples collected every 24 hours for 7 days



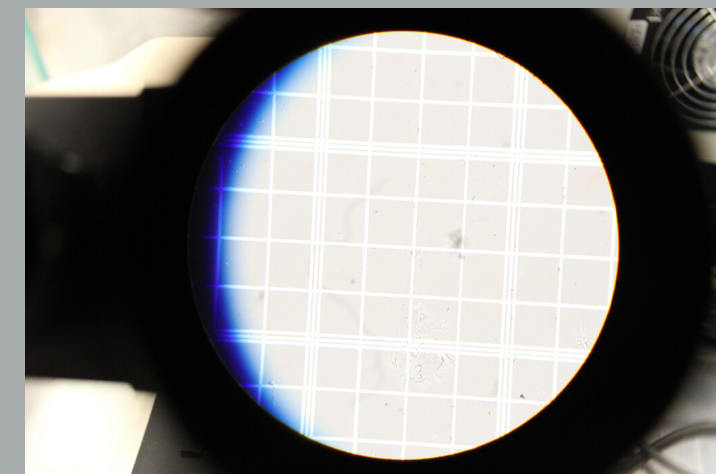
## Hemocytometer

A series of tiny squares



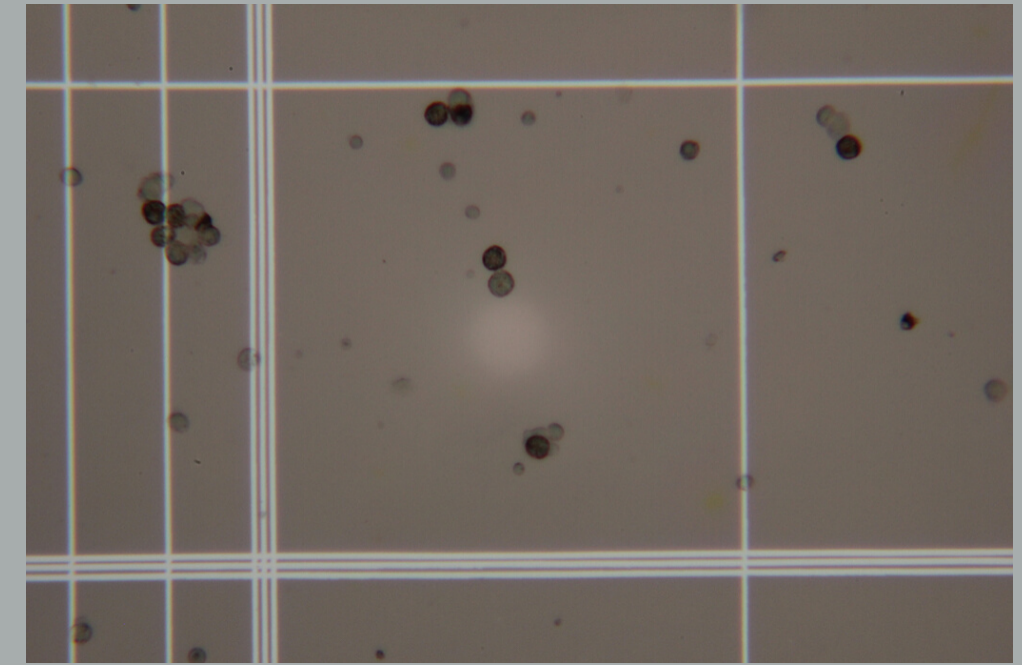
## Photograph

We used a microscope-mounted Canon eos rebel to systematically photograph the grid



## Hemocytometer Analysis

0.25mm -> 1.00mm -> total cell count



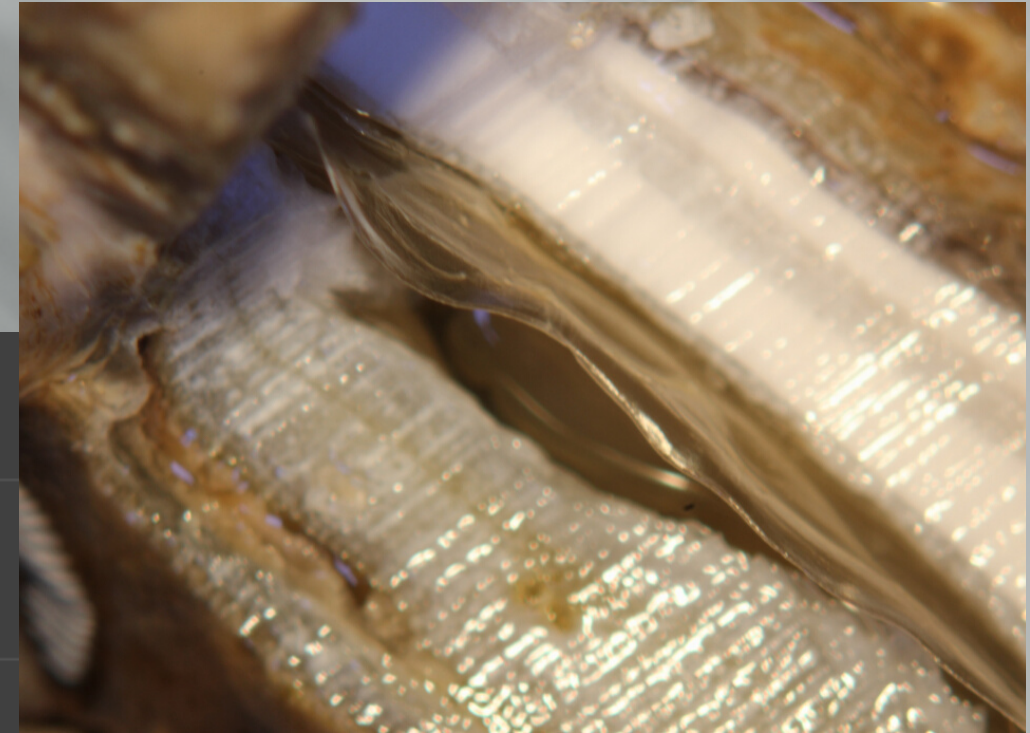
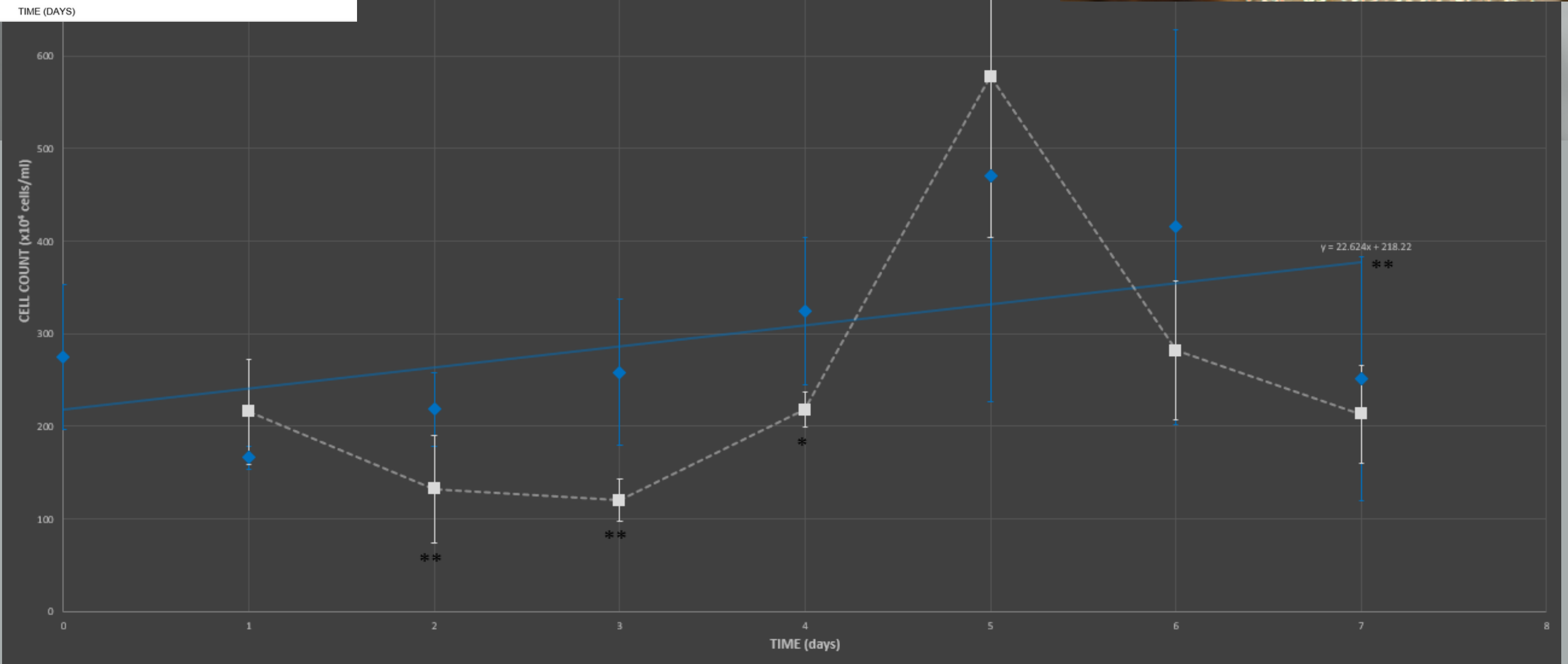
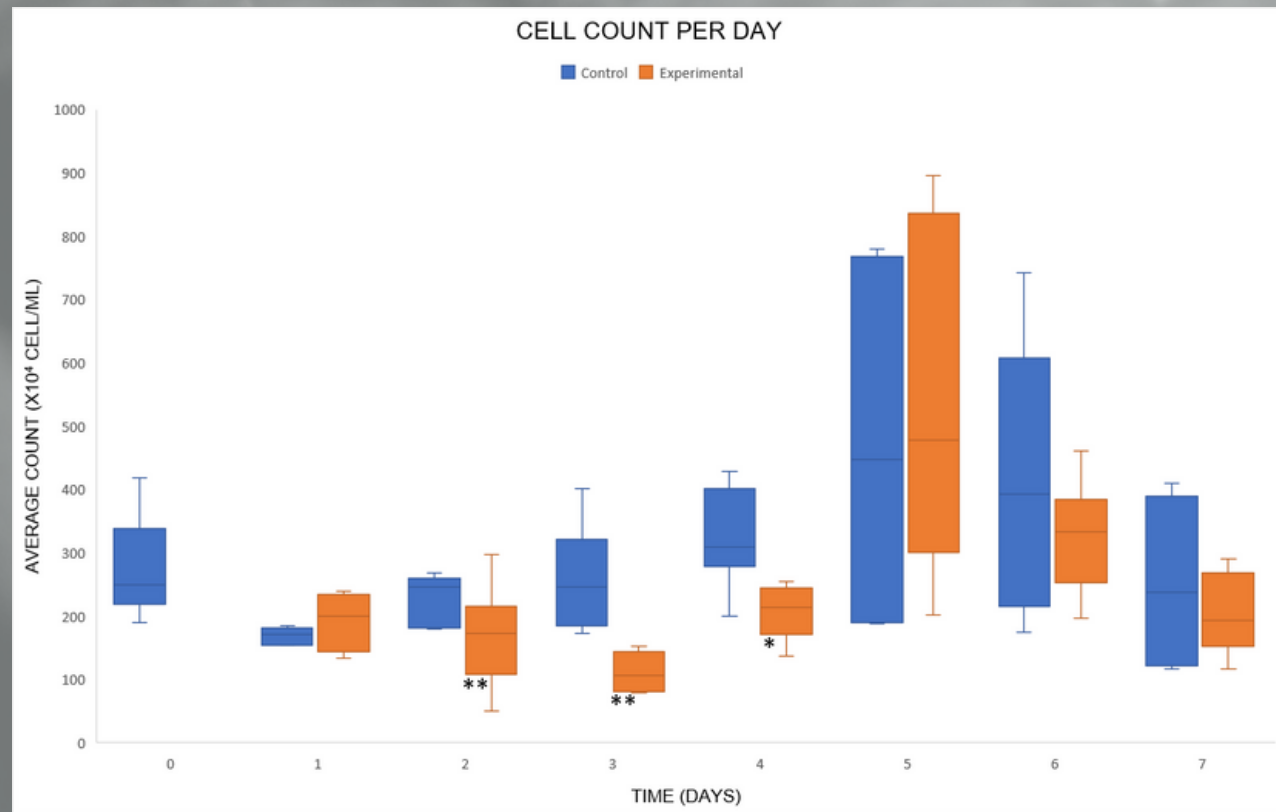
## Data Analysis

ANOVA, t-test, outliers



# Results

STATISTICALLY  
SIGNIFICANT  
DECREASE IN CELLS  
FROM DAYS 2-4





# Alyssa's Work

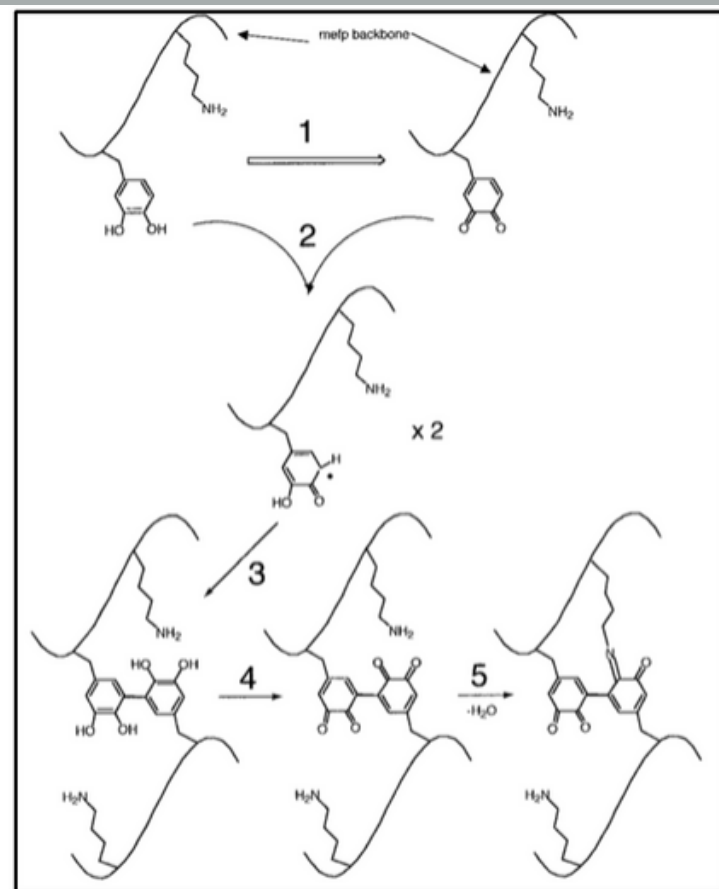


Figure 3: Diagram of L-DOPA Structure. Image adapted from Burzio and Waite 200. L-DOPA residues are attached to a protein backbone. When crosslinking occurs, bond between L-DOPA residues are created.

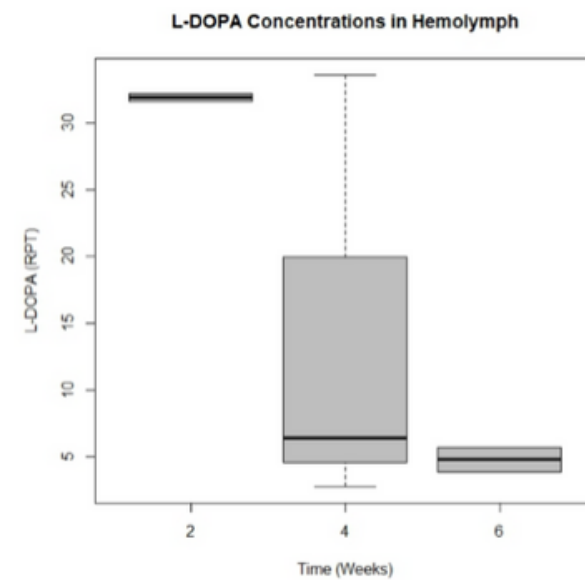


Figure 7: L-DOPA levels in Hemolymph from 6-week experiment  
L-DOPA concentration levels in hemolymph from 2 to 6 weeks, decreases through time.

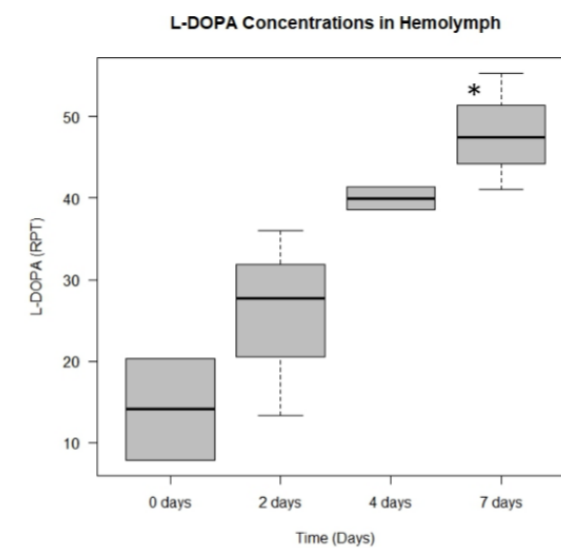


Figure 6: L-DOPA levels in Hemolymph from 1-week experiment  
L-DOPA concentration levels in hemolymph start out low then continue to increase out to the 1-week mark, with an average of 45 RPT. \*Indicates significance to baseline  $p < 0.05$ .

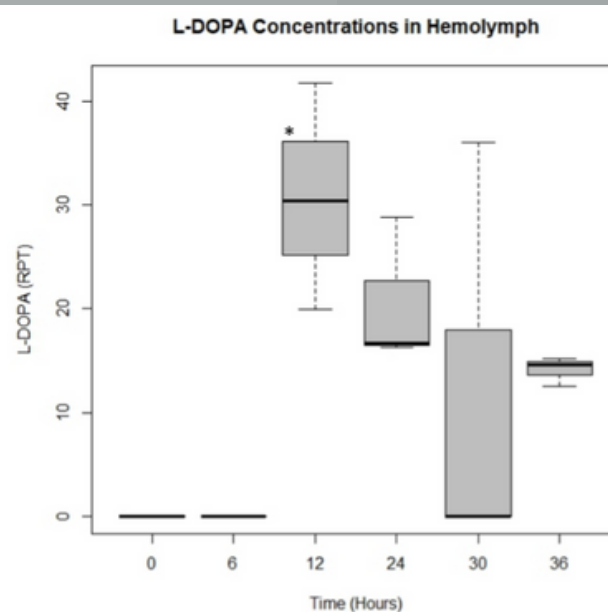


Figure 5: L-DOPA Levels in Hemolymph from 36-hour experiment  
L-DOPA concentration levels in hemolymph are shown across a 36-hour time period. Levels start out at 0 then increase to an average of 30 RPT at 12 hours. Levels decline over the course of the experiment. \* Indicates significance to baseline,  $p < 0.05$ .

## HEMOLYMPH VERSUS HEMOCYTES

L-DOPA and Hemolymph

A cellular comparison

## THREE EXPERIMENTS

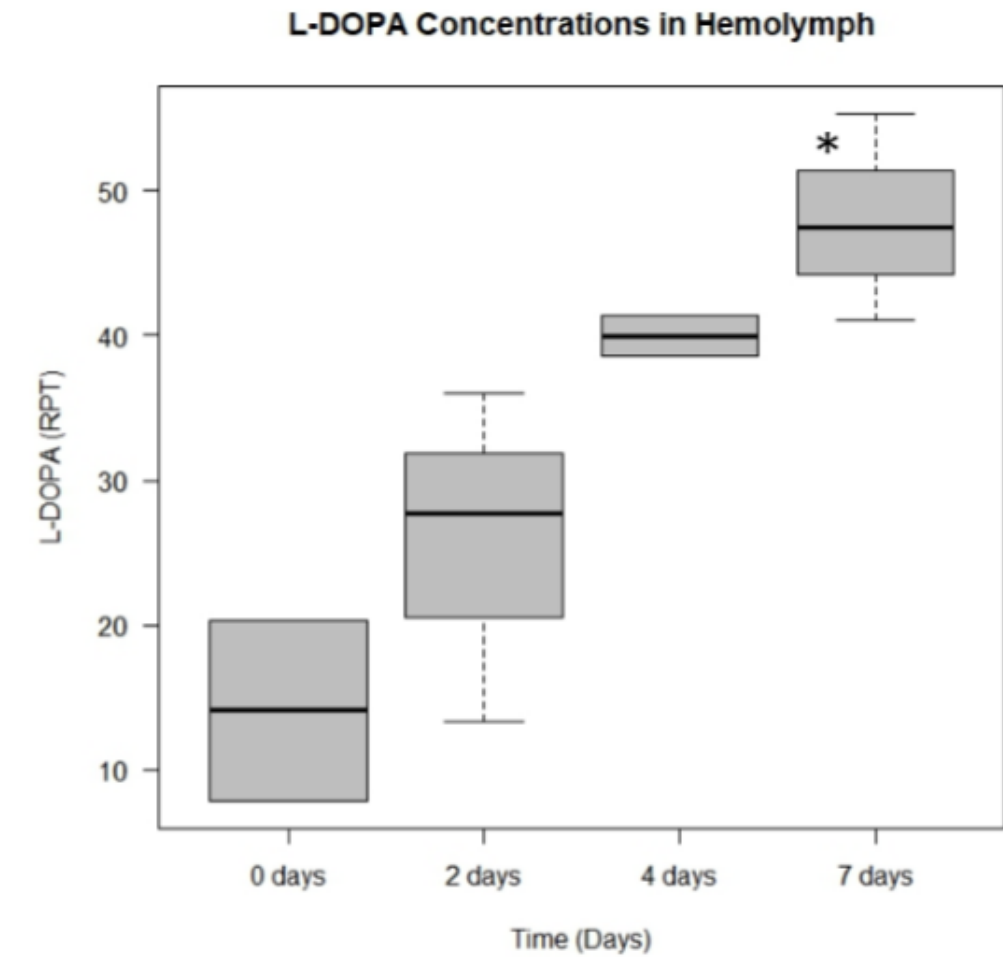
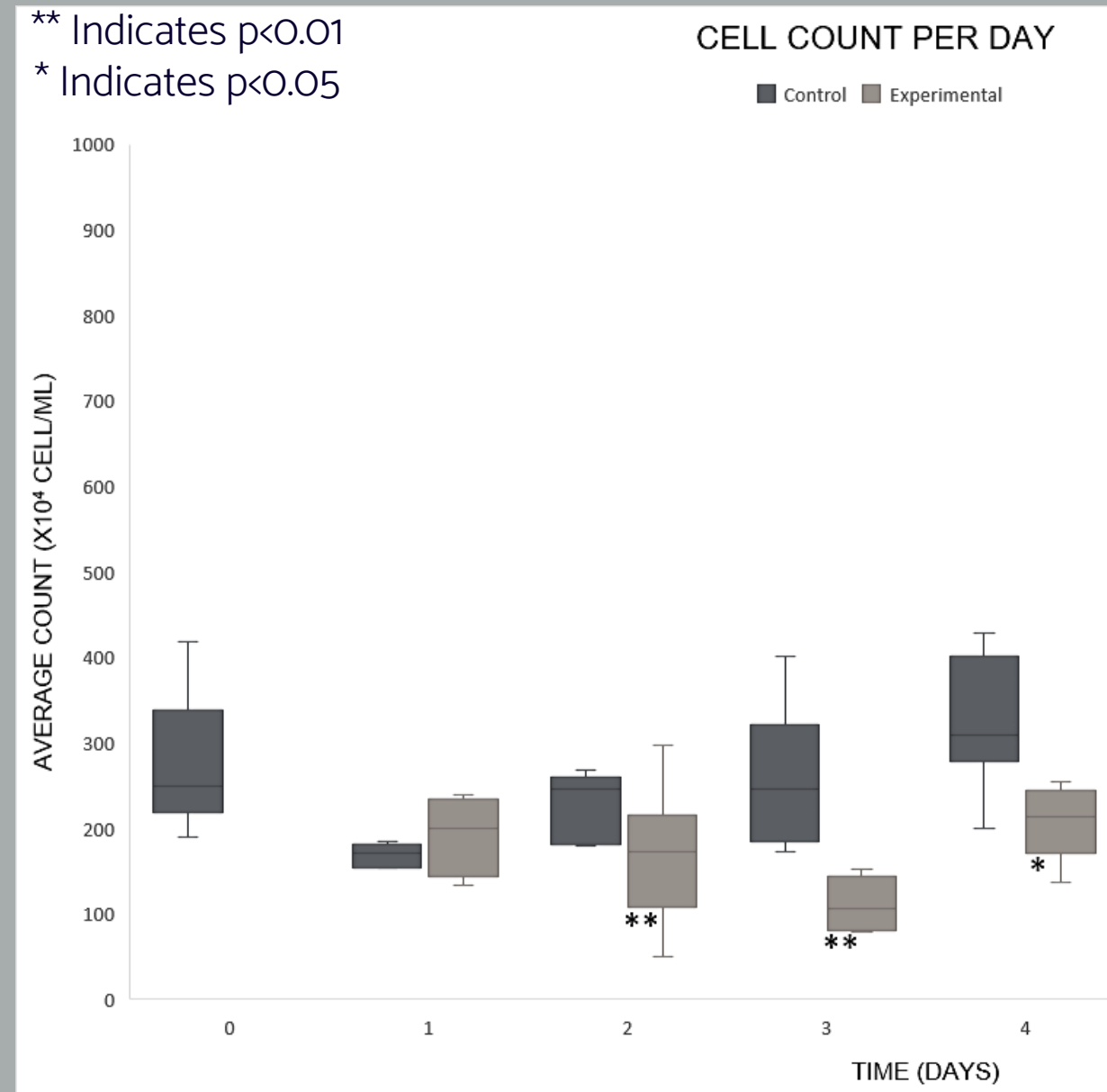
36 hours, 7 days, and 8 weeks

## RESULTS

Noteworthy fluctuation and many statistically significant findings



# How they compare



*Figure 6: L-DOPA levels in Hemolymph from 1-week experiment*  
*L-DOPA concentration levels in hemolymph start out low then continue to increase out to the 1-week mark, with an average of 45 RPT. \*Indicates significance to baseline  $p < 0.05$ .*

SO WHAT DOES THIS MEAN IS HAPPENING?



# Additional Observations

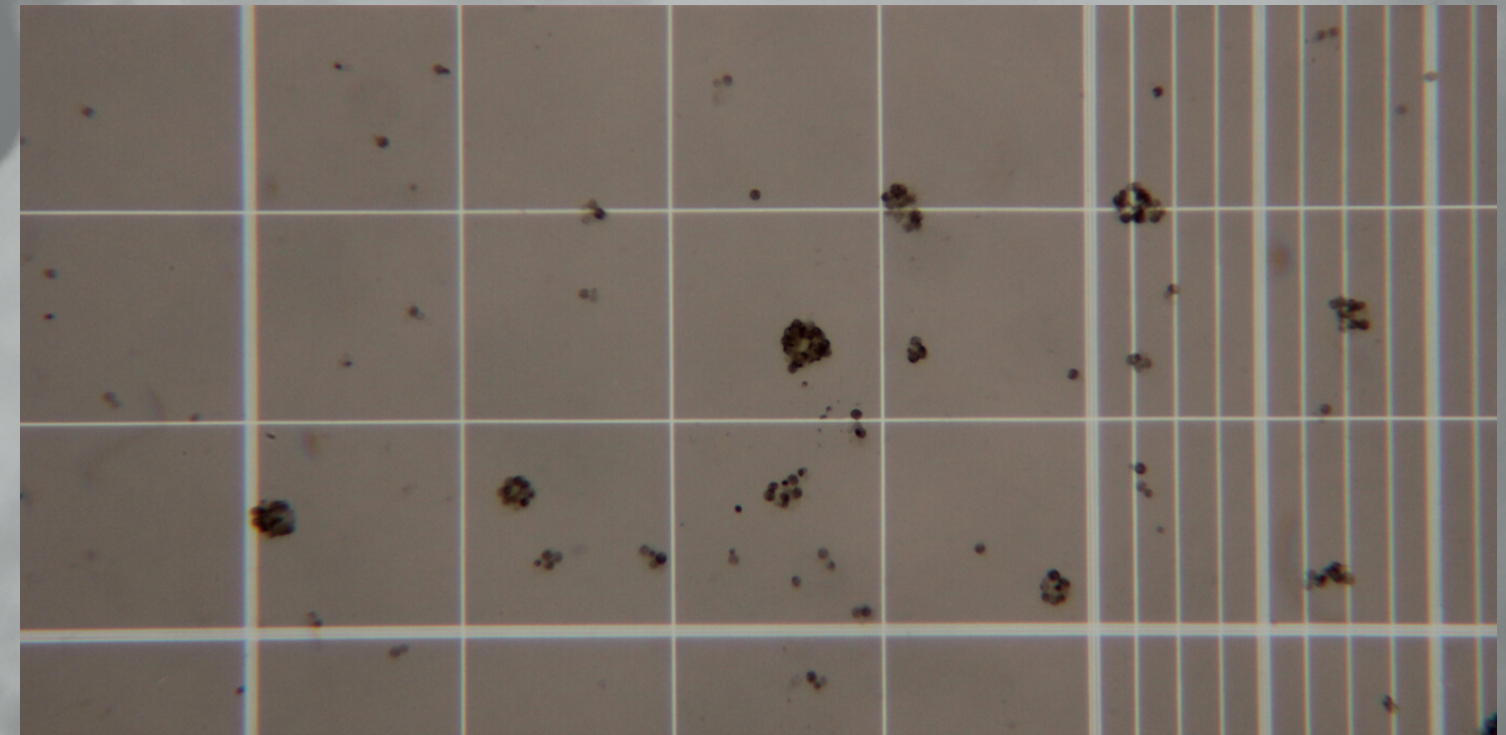
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Cells appear to be grouping together into organized shapes



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The number of these groupings are very abundant on day 1, occasionally present on day 2 and virtually non-existent on day 3





## PERHAPS...

Cells are actively working to deposit shell more immediately than proteins in the hemolymph.

## WHAT'S NEXT?

Identify the action of cells as a potential reservoir of calcium carbonate through amino acid analysis and staining.

## HOW COULD THIS ALL BE HELPFUL?

Biomaterials and possibly even bioarmors.

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# Discussion





# *Acknowledgements*

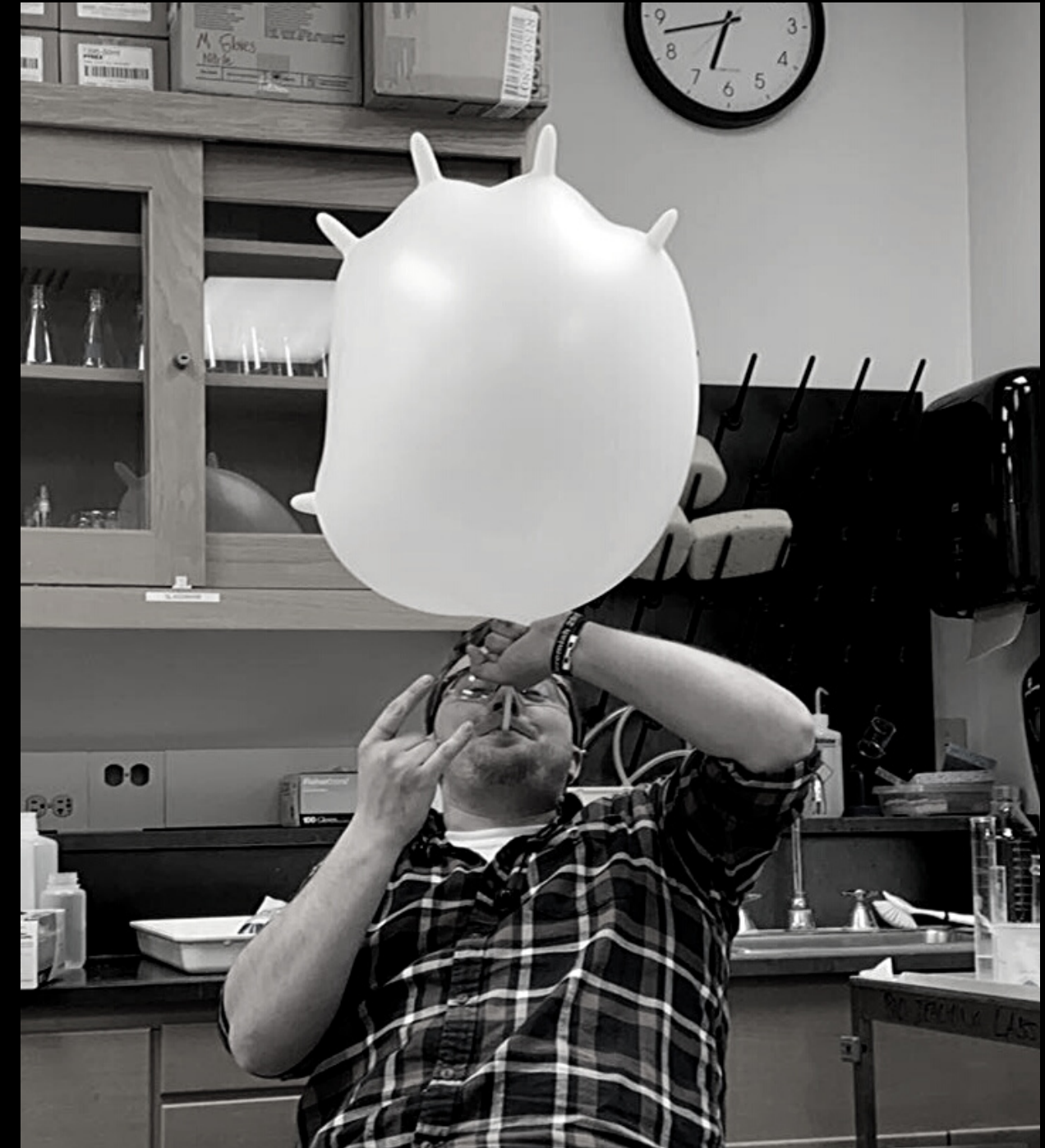
- Karolyn M. Hansen, Ph.D.
- Alyssa Outhwaite, M.S.
- Doug Hansen, Ph.D.
- All those friends and family members that encouraged our scientific curiosity





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# Questions?



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