

A Letter for Mary Gates Endowment Vu Le Nguyen

As the quarter comes to an end, I reflect on memories that happened when I was an apprentice of the Bio-Imaging course from Friday Harbor. I realized how blessed I was to receive such an opportunity to operate complex machines that few college students could get hands-on experience. Without the tremendous financial support from the Mary Gates Endowment, this would not have been possible. So, I sincerely thank the Mary Gates Endowment from the bottom of my heart for providing me with the opportunity to be in such an incredible project. In the last three weeks of the course, I was given a chance to showcase my mastering of different photography techniques with a final project.

With the fascination in immunology and the marine life in San Juan Island, I set to investigate *Pandalus platyceros*, a native shrimp from San Juan Island. By looking at its hemocytes, and the vascular system of *Pandalus platyceros* through scanning electron microscopic (SEM) photography, fix and stained microscopy, and micro CT scan.

For preparation for SEM photography, I extracted the hemolymph from the subject's ventral carapace. The hemolymph was mixed with buffered glutaraldehyde and phosphate-buffered saline to fix and keep the integrity of the hemocyte. Hemolymph was centrifuged and washed with distilled water to dry off a stub inside the fume hood for six hours. The stub would then sputter in gold atoms for SEM photography. This method allowed me to differentiate and classify different types of hemocytes in the highest definition. Thus, these images allow for further studying of hemocyte's exterior morphology.

Although, imaging of live hemocytes does not give the best quality to study their morphology. By staining hemocytes with eosin, I was able to look at their differentiation under the 40x oil lens. This method gave hemocytes to preserve in their best condition, thus allowing me to capture the hemocytes' lifetime and differentiation.

Lastly, I used micro CT to draw a 3D depiction of the *Pandalus platyceros* vascular system. This method created the most difficulty because the phosphotungstic acid (PTA) staining agent took extremely slow to penetrate through the chitin wall. To resolve this, I dissected the carapace then sonicate for six hours to allow PTA to penetrate at its fastest rate. The vascular system was segmented digitally with 3DSclicer and labeled. This method wraps everything up with how the hemolymph circulates and distributes inside the subject. Ultimately tie up the vascular and immunology relationship of *Pandalus platyceros*.

In the future, I want to research hydrothermotherapy through the relationship between hemocyte concentration and internal temperature, using the imaging techniques above. Hydrothermotherapy has been hypothesized to alleviate COVID-19 symptoms by increasing the body's internal temperature to create a fever-like symptom; This leads to activation of the immune system and antiviral activities related to the Sars-Covid 2.

Histology and Microscope: 1-2

1. "Scallop Gonad" oc: oocyte; gc: granulosa cell; n: nucleus
2. "Hemocyte Timeline" A: pro-hemocyte; B: pro-plasmatocyte; C: matured plasmatocyte; D: Agranularcyte.

SEM: 3-6

3. "Hemocyte classification" A, D: granulocyte; B, E: semi-granulocyte; C, F: plasmatocyte.
4. "Scallop Eye"
- 5-6. "Abstract SEM of Scallop Eyes"

MicroCT Scan: 7-11

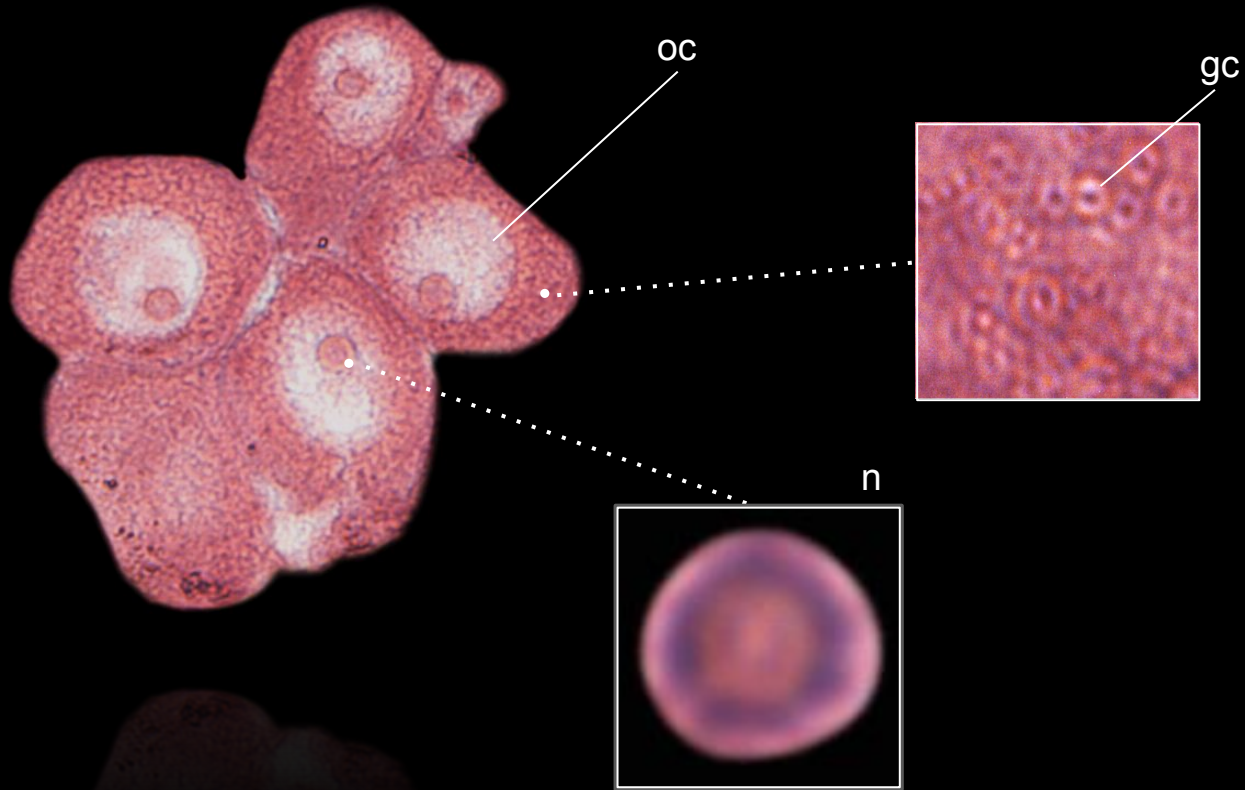
7. "Pandalus goniurus Vascular System" h:heart; pa: posterior aorta; pla1: first posterior lateral artery; pla2: second posterior lateral artery; pla3: third posterior lateral artery; pla4: fourth posterior lateral artery; pla5: fifth posterior lateral artery; da: descending artery; vv: ventral vessel.
8. "Pandalus goniurus Vascular System" P.2
9. "Sculpin Inside The Snailfish Gut"
10. "Sculpin Inside The Snailfish Gut" P2
11. "Face of The Snailfish"

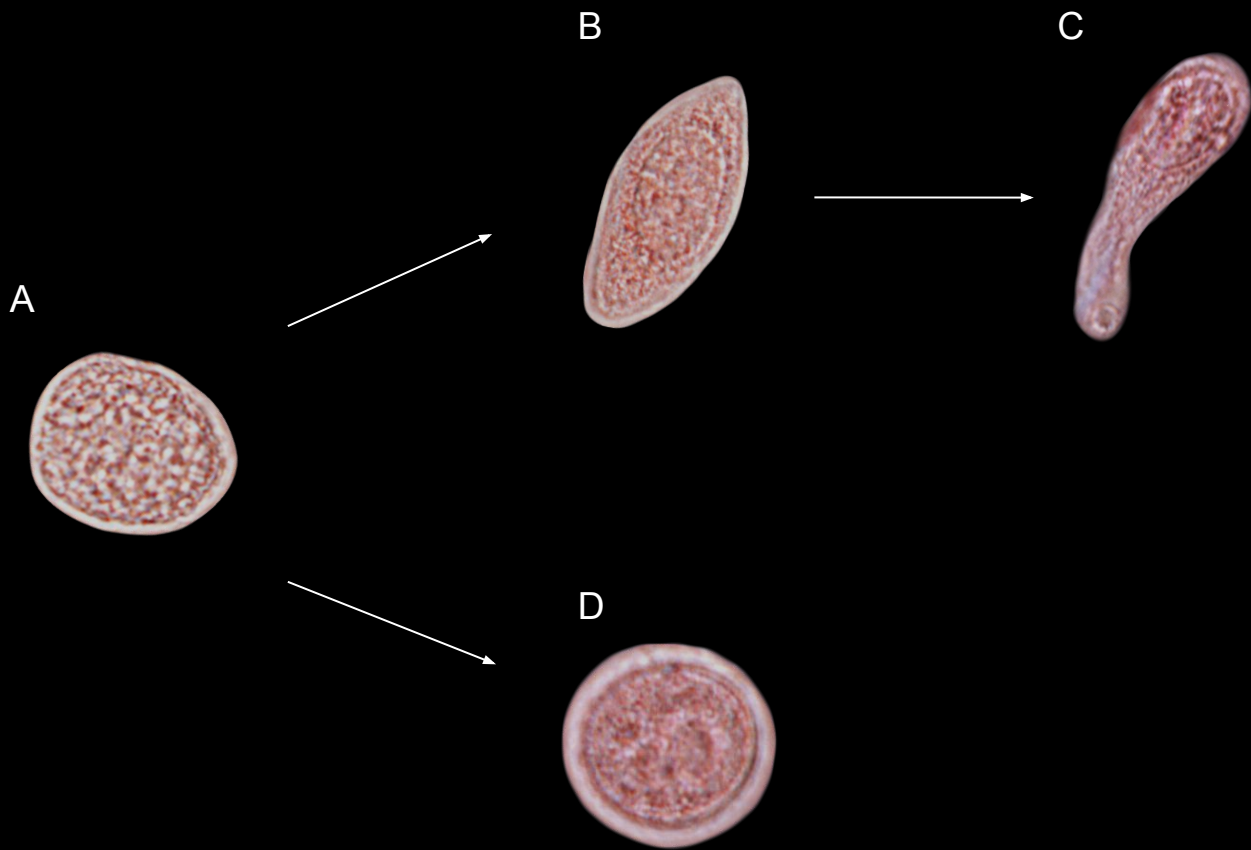
Drawing: 12

12. "Portrait of Liparis callyodon"

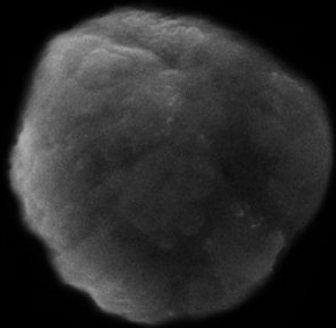
Photography: 13-14

13. "Macro Shot of Cleared & Stained Sculpin"
14. "Macro Shot of Cleared & Stained Sculpin"

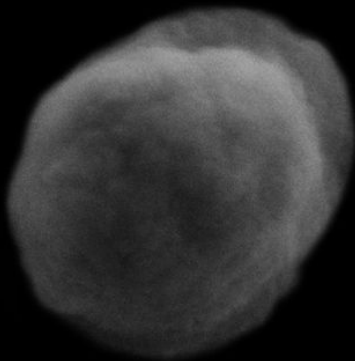




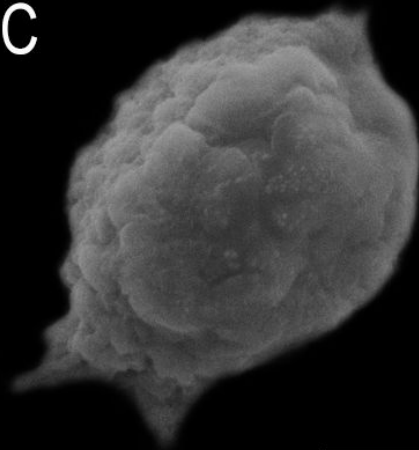
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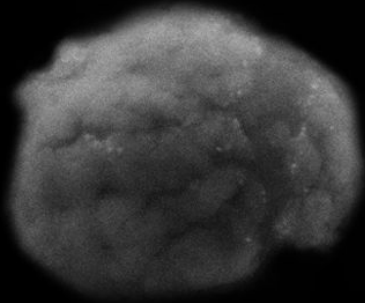
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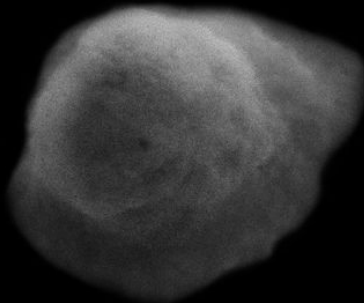
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D



E



F

