

# ***PISASTER* THE NEXT LARVAL REGENERATION**

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## **Introduction:**

Sea stars are common invertebrates that inhabit all oceans. These organisms belong to the phylum Echinodermata, which include sea cucumbers, sand dollars and sea urchins. The sea star *Pisaster ochraceus* is native to the Pacific Ocean, stretching from Northern Alaska to Northern California. They appear in different color morphs, ranging from purple to orange and even brown. Like other sea stars they have tube feet, which give them the ability to move and secure themselves on any surface, making it difficult for predators, including humans to obtain it. They normally thrive on common bivalves such as muscles, but also consume barnacles and chitons, however during their larval stage they mostly feed on algae. Using their tube feet they can latch on to different prey and bring them to their mouth to feast.

*Pisaster ochraceus* is known to have its mating Pisaster sea stars have separate sexes, which can be determined by making a slight incision at the base of the arm and locating the gonads, which will contain either sperm or eggs. . Many sea stars have the capacity to regenerate body parts lost during their lifetime, but only in their adult stages. However, I am more interested in learning about this mechanism during their larval stage. Not only does regeneration affect different organs in the specimen, but also different cells (Carnevali 2006). This paper will show the processes of obtaining the specimens, bisecting the larval sea stars, and finally displaying them in different colors, allowing viewers to not only see the common organs such as the mouth, anus, esophagus and stomach, but also different muscle tissues. In this study, we investigate the regenerative abilities of 200 larvae bisected in various morphological planes and fixed over a series of time points to observe the progress of each bisected culture.

## Materials and Methods

*Pisaster ochraceus* were gathered from Friday Harbor Laboratories, located in the Puget Sound, Washington USA. Adult males and females were kept at 10-12°C in a seawater tanks using an open water system. Spawning was induced by injection with methyl adenine and both sperm and eggs were collected. Growth and development through different stages were observed. Larvae were set up in five different cultures, each containing 20 anterior and posteriors, 20 left side and right sides, and 20 controls. Each culture was labeled according to the date they were cut .with the first culture having the longest time to regenerate and the final culture fixed and stained immediately. The first culture was made on July 6, 2012. All of the bisections were made on the day because if not it would make the culture invalid. The next culture was done on July 9, 2012. This, too, had bisections completed on the exact day. The next culture was completed July, 10 2012, however the day for fixation was missed and a new date was assigned to July 18, 2012. The next culture was done July, 11 2012. The final culture was cut July, 19 2012.

Some husbandry work performed was moving the larvae to new dishes filled with filtered seawater to prevent the possibility of having protozoans in the dish that could ultimately affect the test. The larvae were also given portions of *Dunalliella sp.* and *Isochysis sp.* (Dun,Iso) algae as a food source. When the larva reached the bipinnaria stage the larvae were bisected. A razor was used to cut each larva vertically and horizontally, separating the posterior from the anterior. Larvae that were pipetted but not bisected were used as a control. This was done on each assigned date. A sample of posterior and anterior larvae, as well as some controlled larvae was soaked in a solution of 4% paraformaldehyde in filtered seawater for two hours in order to fix them. The high concentration of paraformaldehyde was diluted by placing the entire larva in a

PBS wash. After washing, larvae were placed in 150  $\mu$ l of phalloidin 1:100 and 1ml of PBS, and then left to sit in the dark for two hours. After the two hours the washing process was repeated by soaking the larvae three times in PBS, each for five minutes. Cultures were then split so there was an even amount of each part including: posterior anterior (which was 20 at first but now is split evenly), left side, right side and control. The PBS solution was drained and 100  $\mu$ l of 1:100 concentration serotonin to it. The others went to the remaining two wells where they were drained of any PBS solution and acetylated tubulin was added. Both stains went on for around five to six hours. After the initial stain, larvae went through a Secondary Antibody Stain. The serotonin stained larvae got 100  $\mu$ l of Anti-Rabbit and the Acetylated Tubulin stained larvae were stained again by 100  $\mu$ l of Anti-Mouse. After an overnight stain, they were ready to be mounted using DAPI, and viewed under the microscope in order to observe the newly regenerating larva.

## **Results**

### *Survival Rate*

There were many cultures that were set up in this experiments that had their own survival times. Roughly 80% was the total maximum success survival rate in all the cultures, however, the three day regenerated culture experienced 100% mortality, regardless of cut. The cause of mortality is unknown but still under investigation. Furthermore, the four day regeneration also experienced increased mortality. Currently the left side and the right side both have been decimated, but the posterior, anterior, and control are all still prevalent in their culture. There is a 66% regeneration rate that did occur for the day 11 culture.

### *Day 0 Control Staining Results*

Throughout the past weeks we were able to proceed with fixations and staining with acetylated tubulin and phalloidin. This resulted in a grand scale of staining that was observed under confocal. There was a mixture of staining of green and red with red representing the phalloidin stain (Figure 1) used as a marker for where the muscles were located. The main component of the muscles in the larvae is actin and that was stained. Green can also be seen representing acetylated tubulin in Figure 1. The staining was to be directed towards neurons to serve as a layout for the actual cuts that precede it; however the stain managed to stain the cilia which paint the outline of the controlled larvae.

As stated, staining was also done to the anterior and posterior of Day 0 bisections. The bisected larvae were stained with phalloidin, serotonin and acetylated tubulin. The result of the staining under the fluorescent microscope with the acetylated tubulin is shown in Figure 2. Cilia can be seen in the stomach allowing food to be moved around. With the phalloidin staining muscles such as the stomach as well as the esophagus are highlighted. Also due to the inclusion of the DAPI, we were able to see perfect staining of the nuclei in the larvae both anterior posterior and control.

### *Day 11 Staining Anterior results*

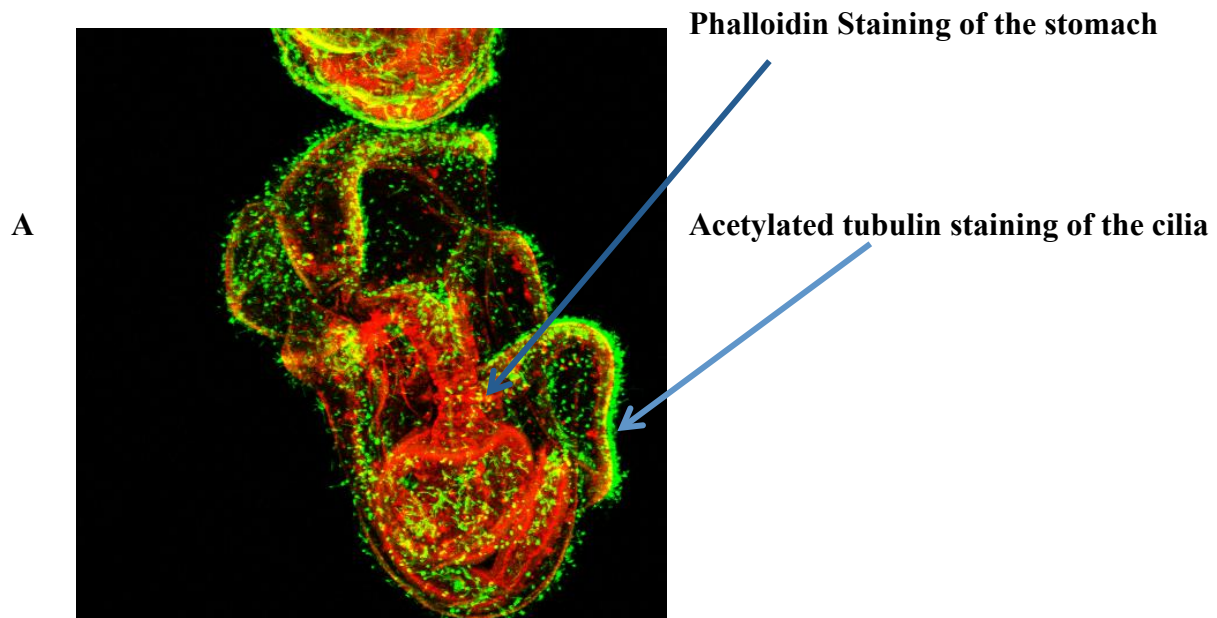
Based on the figures 4 and 5 regeneration did occur in the anterior bisections as well, and it occurred at a rate of 66%. With the regeneration completed we could see the other part of the esophagus as well as the stomach and anus being reformed.

### *Day 11 Staining Posterior results*

The day 11 culture was given 11 days to regenerate and then stained with serotonin, phalloidin, and DAPI. Staining with the phalloidin showed regeneration was a success in the posterior as both the muscle as well as the anus had regenerated. As expected, the regeneration that had occurred was smaller than the original larvae, as seen in figure 3 in which the phalloidin stains are clearly visible in the stomach region and the esophagus. Staining for day 11 was also a success based on the 66% rate almost every 2 out of 3 larvae had experienced regeneration. With that being said the posterior larvae were able to grow back their esophagus one on the account grew back two and their mouth.

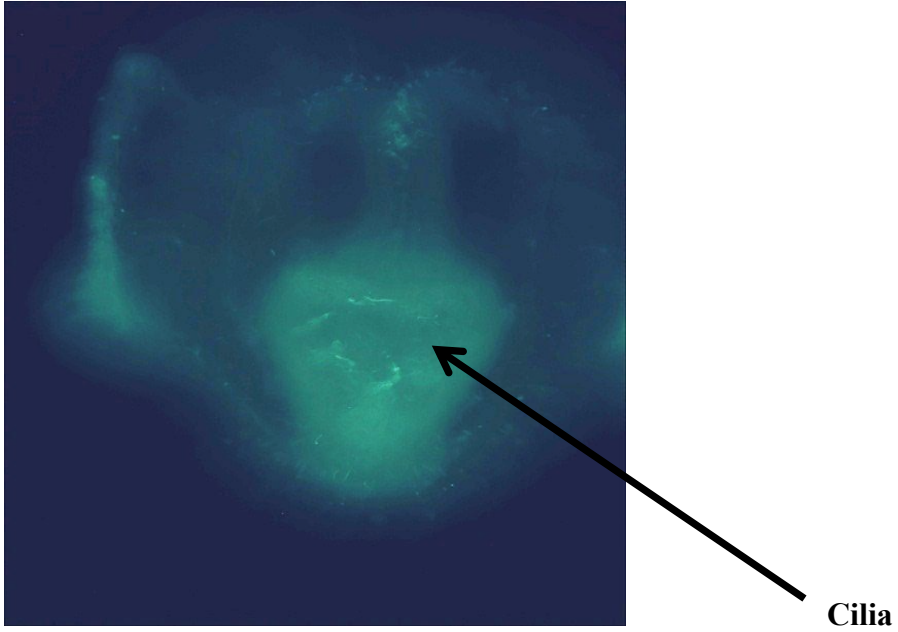
### **Discussion**

If more time were available to continue work with the *Pisaster Ochraceus* larvae, more time points for regeneration could be added. The majority of time spent at Friday Harbor Laboratories for the first couple of weeks, were spent learning how to conduct practice cutting of the larvae received. With more time allotted the experiments can be started immediately without the tutorials. The time frame for conducting experiments will be longer, allowing for more regeneration to occur and be observed. This means that when staining, I will be able to have better stains and much stronger results. More work would have to be done in order to figure out if the staining of the antibodies on the larvae would be nerve dependent.



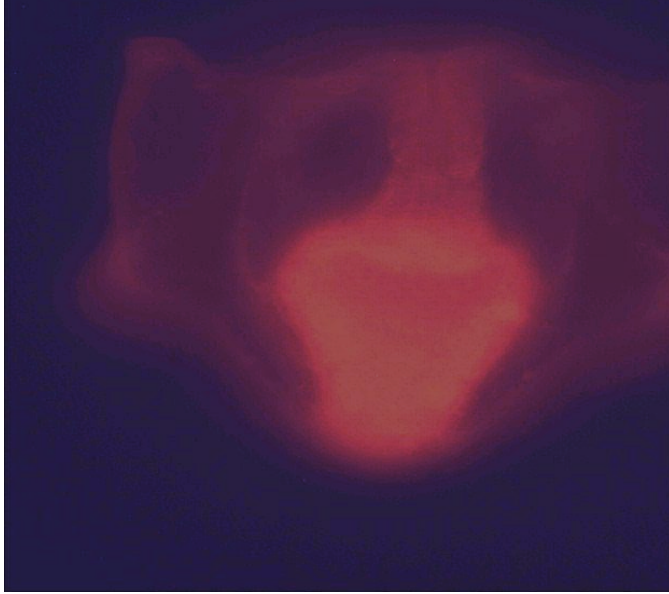
**Fig. 1. *Pisaster Ochraceus* Control Larvae**

**(A)** The red staining is the phalloidin in the controlled larvae. It allows the actin to be seen which is the primary component of the muscles which includes: esophagus, mouth and stomach. The green stain inside the larvae is the acetylated tubulin which did not show neuron located near the apical tuft, however it did stain the cilia around the larvae which grants it the ability to move



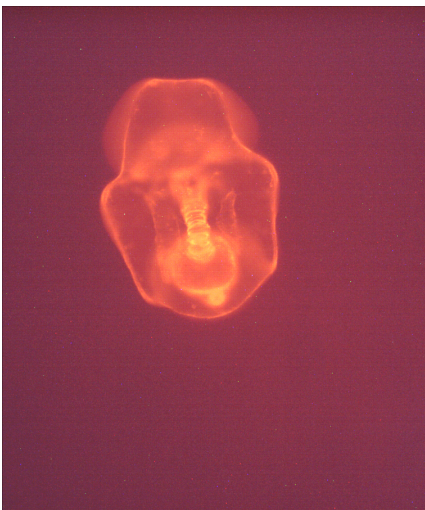
**Fig. 2. *Pisaster ochraceus* posterior larvae**

(A) Acetylated Tubulin is the primary stain that stains the posterior of the larvae showing the cilia located in the stomach.



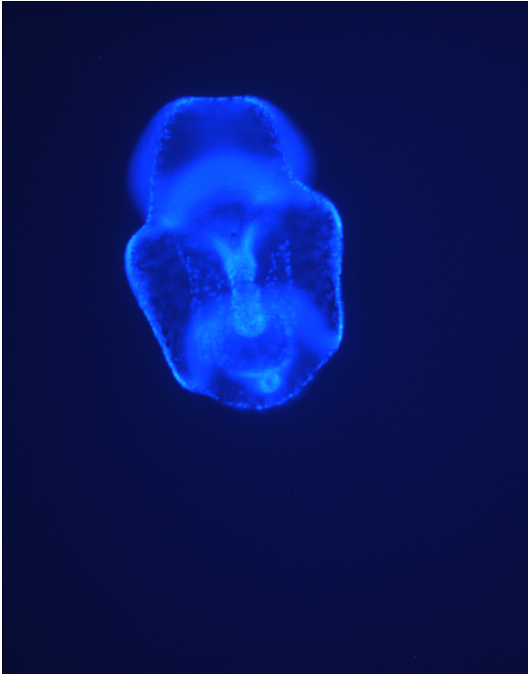
**Fig.3. *Pisaster ochraceus* posterior larvae**

(A) The posterior was given a phalloidin stain which allows us a perfect view of the muscles that are located inside the *Pisaster ochraceus*



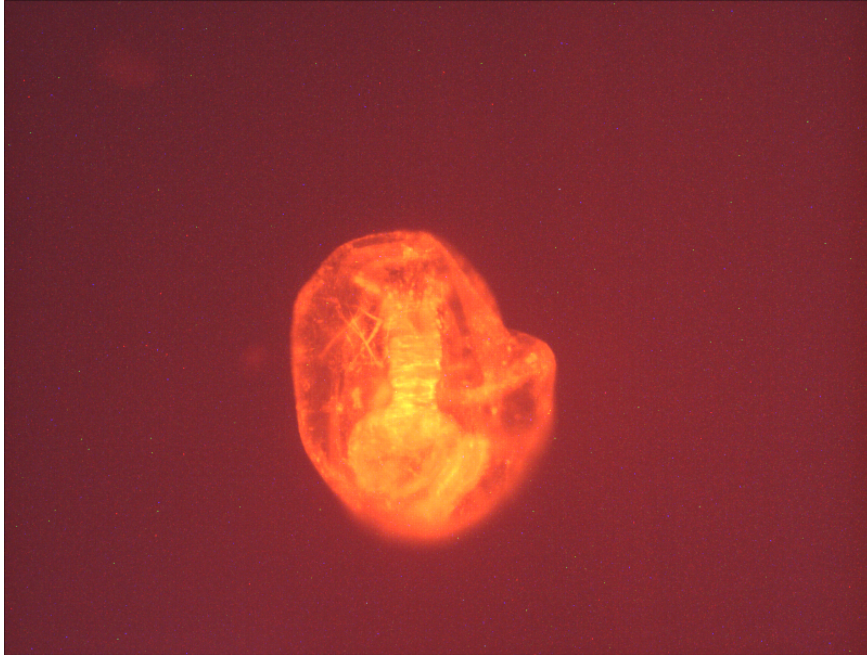
**Fig.4. *Pisaster ochraceus* anterior larvae**

(A) The phalloidin was able to stain part of the esophagus the stomach and the anus.  
Both muscle parts were able to regenerate after given amount of time.



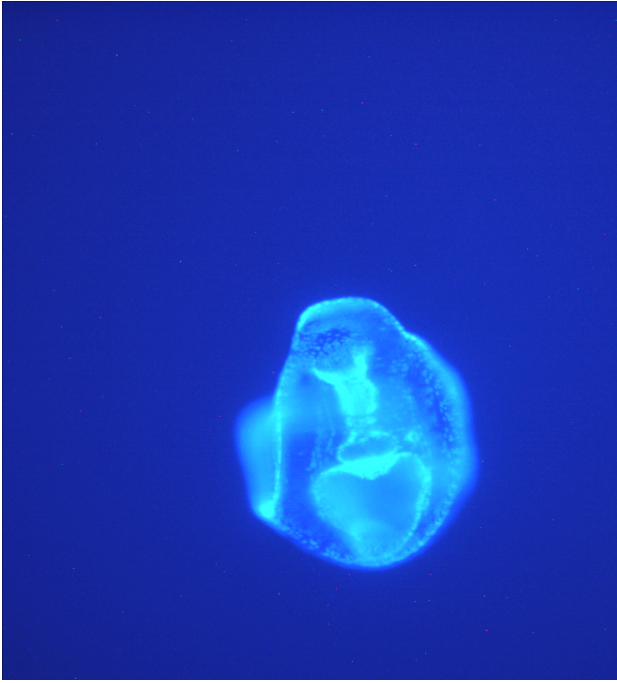
**Fig.5. *Pisaster ochraceus* anterior larvae**

(A) In this larva the DAPI was able to be stained the larvae allowing us to see the the nuclei in the larva.



**Fig.6. Pisaster ochraceus posterior larvae**

(A) This posterior larva was able to regenerate after 11 days. Anterior parts such as two esophagus were able to regenerate including the mouth.



**Fig.7. *Pisaster ochraceus* posterior larvae**

(A) The DAPI staining allowed for the larvae to show its nuclei which is seen in the brighter regions such as the stomach, esophagus, and the sides.

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