APPLYING SATELLITE-OBTAINED OCEANOGRAPHIC DATA TO PREDICT RECRUITMENT BY LARVAL CLONING IN A KEYSTONE PREDATOR, THE SEA STAR *ASTERIAS FORBESI*

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Abstract

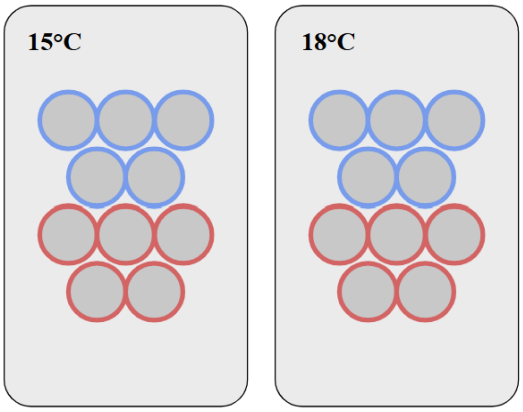
The sea star *Asterias forbesi* inhabits the rocky intertidal ecosystem along the western coast of the Atlantic. As a keystone predator, *A. forbesi* has an outsized effect on all trophic levels in its ecosystem (Bucci et al 2017). Absence of this species decreases shoreline biodiversity and damages nekton communities (Lubchenco & Menge, 1978). Because of this impact, knowledge about *A. forbesi* recruitment is critical to understanding coastal ecology, particularly as conditions fluctuate due to climate change.

A. forbesi can reproduce asexually as larvae by separating into its anterior and posterior components and regenerating to form two genetically identical clones. According to initial experimentation, *A. forbesi* demonstrates particularly high rates of cloning and regeneration compared to other sea star species. Additionally, increasing availability of the phytoplankton that *A. forbesi* consumes has a positive effect on cloning rate. Continued research over the summer of 2025 will further specify the cloning cue in *A. forbesi* and incorporate chlorophyll data from NASA's PACE satellite to predict locations where *A. forbesi* is likely to exhibit high recruitment by larval cloning. This will result in increased understanding of this keystone predator across environmental conditions and locations.

Experiment #1: The Effect of Phytoplankton Availability on Cloning Rate

Male and female *A. forbesi* adults were isolated in individual bowls of 32 ppt artificial seawater and spawned by injection of 100 µM of 1-Methyladenine (Kanatani 1969). Water containing eggs and sperm from one male-female pair were mixed and fertilized eggs were kept at 12°C in the cold room. After 4 days the larvae reached the bipinnaria stage and were sorted into 200mL beakers, 100 larvae per beaker. There were 20 beakers total, with four treatments. 10 beakers were placed in a water bath at 18°C, and 10 were placed in a water bath at 15°C. Beakers were cleaned and received a 50% water change three times per week, followed by feeding. Each beaker received cells of *Rhodomonas lens*, *Dunaliella tertiolecta*, and *Isochrysis galbana* according to its treatment.

Fig. 1: Experimental setup.



Each temperature-controlled water bath held 5 replicate beakers, each containing a population of 100 larvae. Beakers indicated in blue received 50,000 cells of each phytoplankton species per feeding; red beakers received 150,000.

Over the course of the semester, larvae that were observed to have split into "head" (anterior) or "body" (posterior) sections were isolated from the beakers in shotglasses and kept in a 15°C water bath, where they were monitored for regeneration. Larvae in shotglasses were fed the same concentrations of algae as the treatment group from which they were isolated. The 18°C

treatment was ended due to poor survivorship and our data on cloning focuses primarily on the food effect and the remaining two treatment groups in the 15°C water bath, hereafter referred to as “L15” (Low food) and “H15” (High food).

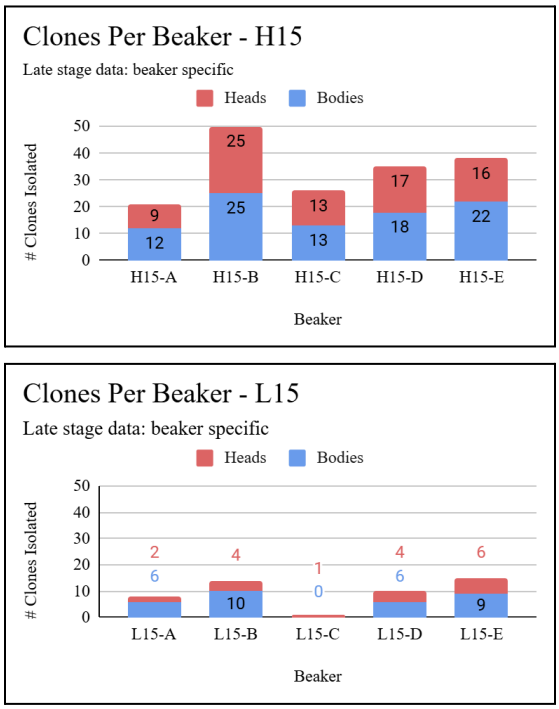
Over the course of the experiment, 41 clones were observed in the L15 and 114 clones in H15, which indicates a cloning rate of 8.2% in the low food treatment and 22.8% for the high food. Initially, data was assigned only to a treatment and not a particular beaker. Later data includes the beaker information (A through E).

Table 1: Clones isolated in each treatment.

Early Data	Bodies	Heads	Later Data	Bodies	Heads
L15 (All Beakers)	10	11	L15-Beaker A	6	2
H15 (All Beakers)	24	15	L15-Beaker B	10	4
			L15-Beaker C	0	1
			L15-Beaker D	6	4
			L15-Beaker E	9	6
			H15-Beaker A	12	9
			H15-Beaker B	25	25
			H15-Beaker C	13	13
			H15-Beaker D	18	17
			H15-Beaker E	22	16

For the later data, we can use the specific beakers to further analyze the effect of the food treatments. On average, there were 6.2 clones per beaker in L15 (stdv = 3.51) and 18 clones per beaker in the high food treatment (stdv = 5.61). A 2-tailed, type 2 T-test on this data determined the p-value to be < 0.05, indicating that high food causes an increase in larval cloning in A forbesi.

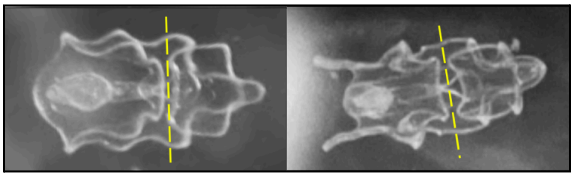
Fig. 2: Larvae in the high food treatment cloned more frequently.



Average clones per beaker: 6.2 for low food, 18 for high food.
Standard Deviation: 3.51 for low food, 5.61 for high food.
P value: 0.0044

The primary focus of the experiment was cloning rate, not regeneration. However the surviving larvae in each shotglasses at the end of the experiment were counted. Clone type was recorded, and the larvae were designated as “heads” (small, anterior portion clones consisting of the oral hood and its interior) and “bodies” (the trunk, gut, and posterior of the larvae lacking only the oral hood).

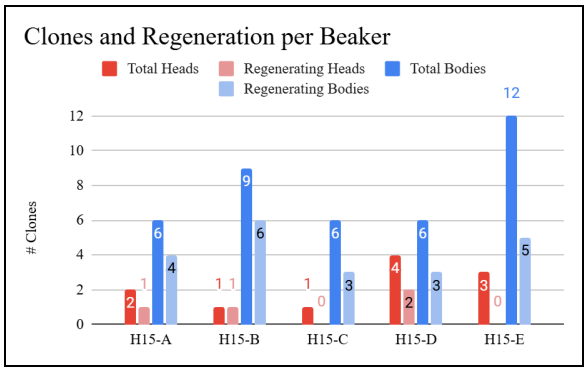
Fig. 3: Larvae most frequently clone by separating the oral hood or “head” portion from the posterior “body” which contains the gut.



Most larvae clone by separating along this approximate axis. Occasionally, some of the middle section is attached to the oral hood and the remaining posterior is smaller. This is less common.

Survival was low in L15 shotglasses. Body shotglasses for L15-B and L15-D each had one surviving body piece that showed no signs of regeneration. All other shotglasses in the L15 treatment were empty. By comparison, all H15 shotglasses had multiple surviving larvae. On average, 10 clones (heads or bodies) survived per beaker, as opposed to the average of 18 clones originally isolated, resulting in a 55% survival rate of clones in our shotglasses. Of the surviving clones, 36% of heads and 54% of bodies showed signs of regeneration.

Fig. 3: Survival and Regeneration in High Food Beakers.

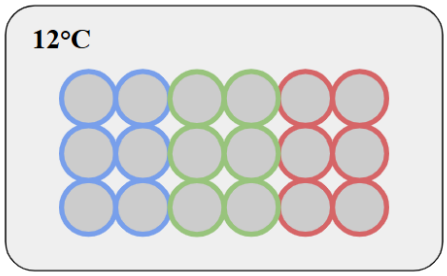


Average regeneration: 36% for heads, 54% for bodies
Average survival of clones: 55%

Experiment #2: The Effect of a Food Pulse on Cloning Rate

Male and female *A. forbesi* were spawned via the same methods as above and the resulting bipinnaria larvae sorted into shotglasses of 32 ppt artificial seawater and kept in a water bath at 12°C. Each shotglass contained 40 mL of seawater and 20 larvae. There were 18 beakers total, and 3 feeding treatments: High, Low, and Pulse. The Pulse treatment consisted of feeding the larvae "Low" amounts for the first 10 days, followed by "High" amounts for the rest of the experiment.

Fig. 4: Experimental Setup.



The temperature-controlled water bath held 6 replicate shotglasses per treatment, each containing a population of 20 larvae. Shotglasses in the high food treatment (red) received 400,000 cells of *I. galbana* and 200,000 cells of *D. tertiolecta* each, while low food replicates (blue) received 10,000 cells each of both algae species (McDonald and Vaughn 2010). Pulse treatment shotglasses received the high food amount followed by the low food amount. The shotglasses were monitored three times a week using a dissecting scope to search for clones and to count the surviving larvae. The experiment lasted for three weeks.

Over the course of this experiment, no clones were produced in any of the shotglasses. The time period and treatments were chosen to replicate a past pulse experiment in sand dollars (McDonald and Vaughn), but the time period proved too short to produce cloning results in *A. forbesi*. Data was collected on the survival of the populations in each shotglass and average survival can be compared across treatments. On average, survival by day 21 was 85% for the low food treatment, 82% for high food, and 92% for pulse. The p-value determined by one-way ANOVA for survival data is greater than 0.05, so the null hypothesis is accepted.

Table 3: The phytoplankton concentrations used did not significantly affect survival during the experiment.

Treatment	Mean Surviving (per shotglass, day 21)
Low	17
High	16.4
Pulse	18.4

P value: 0.3922

Current conclusions

- High food induces cloning in *A. forbesi* larvae.
- To improve survival, *A. forbesi* larvae should be cultured at temperatures less than 18°C.
- Cloning likely occurs late in the larval stage prior to metamorphosis.
    - Further experimentation will adapt the timing of the "pulse" experimental methods used for other echinoderm species (McDonald and Vaughn) to better suit A. forbesi's cloning
- The pulse food treatments used had no significant effect on larval survival over a 3 week period.

Future Directions

Spring 2025: Additional Pulse Experiments

The next aspect of this project is to perform the pulse experiment at least one additional time. Feeding treatments will remain the same since there was high larvae survival in the previous experiment. The experiment will be extended over a longer period of time. The pulse will be administered at approximately day 15, and the experiment will last until at least some larvae reach metamorphosis. Due to availability of adults for spawning, this experiment will be done with *Asterias rubens*, not *Asterias forbesi*. This northern population has a similar ecological niche and hybridizes frequently with *A. forbesi* (Wares, 2001). This pulse experiment will take place this semester.

Summer 2025: Natural Seawater and PACE Data

A significant portion of this project will take place over the summer (June-August, 2025) at the Bowdoin College Schiller Coastal Studies Center in Harpswell, Maine. First, the feeding experiments will be repeated using flowing natural seawater, to determine its effect compared to artificial seawater on cloning frequency and larvae survival.

Results from all the cloning experiments will be combined with data obtained from NASA's PACE satellite regarding environmental conditions throughout the geographic range of *A. forbesi*. This data will be imported into Esri's ArcGIS software. Data for environmental conditions in *A. forbesi's* range shown to have an effect on larval cloning will be imported as raster layers and reclassified to reflect their impact on cloning frequency. A higher value indicates that the condition is associated with increased cloning, and a lower value conversely indicates reduced cloning. If multiple conditions are shown to affect cloning, these conditions will be weighted according to the strength of their effect and the total value for each raster cell will be calculated based on all reclassified layers. This will produce a map showing suitable areas for cloning, where *A. forbesi* populations are likely to be able to recover from population loss more quickly, as well as areas that may be more affected by global climate change, or other natural or anthropogenic disturbances.

Works Cited

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