Name: Rehmat, Sijia, Jaizi, Amirah, Chiara

Date: 7/2/19

Goals: 1. Make YM media plates with kanamycin

2. Transform the pCB302 plasmid from papers 1 & 2 into Agrobacterium tumefaciens

3. Make overnight cultures from pcb302 in E. Coli from papers 1 & 2 transformations

4. Verify algal life

5. Transform codon optimized RFP into E. coli

6. Colony PCR on pcb302 in E.Coli from papers 1 & 2

Name: Rehmat Babar

Date: 7/12/19

Goal:

1. Make YM media plates with kanamycin

Materials

Yeast extract
Mannitol
NaCl
MgSO4ï 7H2O
K2HPO4

Protocol

YM Media Recipe
Dissolve in 900 mL of diH2O
0.4 g Yeast extract
10 g Mannitol
0.1 g NaCl
0.2 g MgSO4ï 7H2O
0.38 g K2HPO4
15 g Bacto Agar

Autoclaved for 45 minutes and brought to a pH of 7
1 mL kanamycin was added and the plates were poured

Name: Rehmat Babar

Date: 7/12/19

Goal:

1. Transform the pCB302 plasmid into Agrobacterium tumefaciens

Protocol:

Electroporation of Agrobacterium tumefaciens

1. Thawed Agrobacterium tumefaciens cells on wet ice
2. Combined 1 µL of pCB302-gfp-MBD plasmid DNA and 20 µL of cells in an Eppendorf tube
3. Pipetted the cells into a cuvette and electroporated
4. Added 1 mL of YM media that was prepared yesterday and transferred to a 15 mL falcon tube
5. The tubes were incubated at 30°C at 200 rpm for 3 hours (take out at 3:45 PM)
6. 100 µL of each culture was streaked onto a YM kanamycin plate.
7. 200 µL of each culture was also streaked onto a YM kanamycin plate.
8. The plates were incubated at 30°C for 65 hours (take out at 6:00 on Thursday 7/4/19)
9. The left transformation samples were stored in the fridge.

Results:

N/A

Conclusion:

N/A

Name: Chiara

Date: 7/2/19

Goal:

1. Make overnight cultures from pcb302 in E. Coli transformations

Materials:

- LB
- Kanamycin

Protocol:

Overnight Cultures

1. Added about 5-7 mL of LB to a 15 mL Falcon tube along with 5-7 µL of antibiotics
    a. Kanamycin
2. Using the tip of a toothpick, a colony was touched. This toothpick was then inserted into 10 µL of DI water. 1 µL of this DI water was inserted into the falcon tube
3. Incubated in the water bath at 37° C at 200 rpm for 16-18 hours

Results:

N/A

Conclusions:

N/A

Name: Chiara
Date: 7/2/19

Goal:

1. Colony PCR on pcb302 in E.Coli

Materials:

- Dream Taq PCR mastermix (2x)

Protocol:

C**olony PCR Protocol**

20 µL Reaction

1. Prepared a PCR concentration cocktail with the following proportions: 7 µL of diH2O, 10 µL Dream Taq PCR Mastermix (2x), 1 µL of the forward primer, and 1 µL of the reverse primer.
    - 7 reactions with primers 1 & 2
        - 150 µL/2, colony 1
        - 150 µL/2, colony 2
        - 75 µL/1, colony 1
        - 75 µL/1, colony 1
        - 75 µL/2, colony 1
        - 75 µL/1, colony 1
        - 150 µL/1, colony 1
    - 7 reactions with primers 1 & 4
        - 150 µL/1, colony 2
        - 75 µL/1, colony 3
        - 150 µL/1, colony 3
        - 150 µL/2, colony 3
        - 75 µL/2, colony 2
        - 75 µL/2, colony 3
        - 75 µL/2, colony 4
2. Added 19 µL of the concentration cocktail into a PCR tube.
3. Using the tip of a toothpick, a colony was touched. This toothpick was then inserted into 10 µL of DI water. 1 µL of this DI water was inserted into the PCR tube.
4. Placed PCR tube in the thermocycler at the following settings:
    1. 95° C for 3:00 minutes
    2. 95° C for 1:00 minute
    3. **45° C for 1:00 minute**
    4. 72° C for 1:00 minute
    5. 30X (Go to Step 2)
    6. 72° C for 5:00 minutes

Lid Temperature: 105° C

Results:

N/A

Conclusion:

We have been setting the annealing temperature for the PCR of pcb302 incorrectly. The primers' melting temperatures are either 51 or 52 degrees Celcius and the thermocycler has been running at 52. The annealing temperature should be at least 4 degrees below the Tm to avoid primer dimers. Because of this, today's temp. was set to 45.

Name: Sijia, Jiazi

Date: 7/2/19

Goal: verify algae life

Materials:

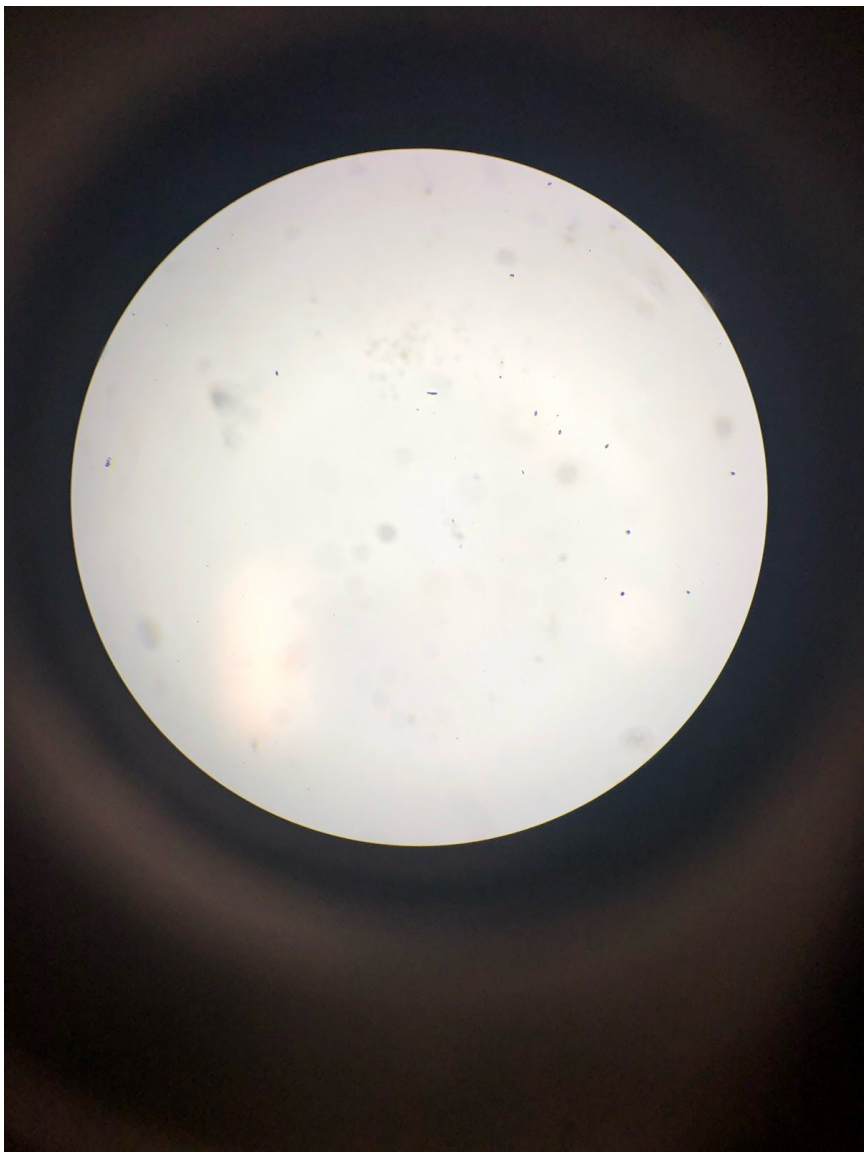
Algae samples

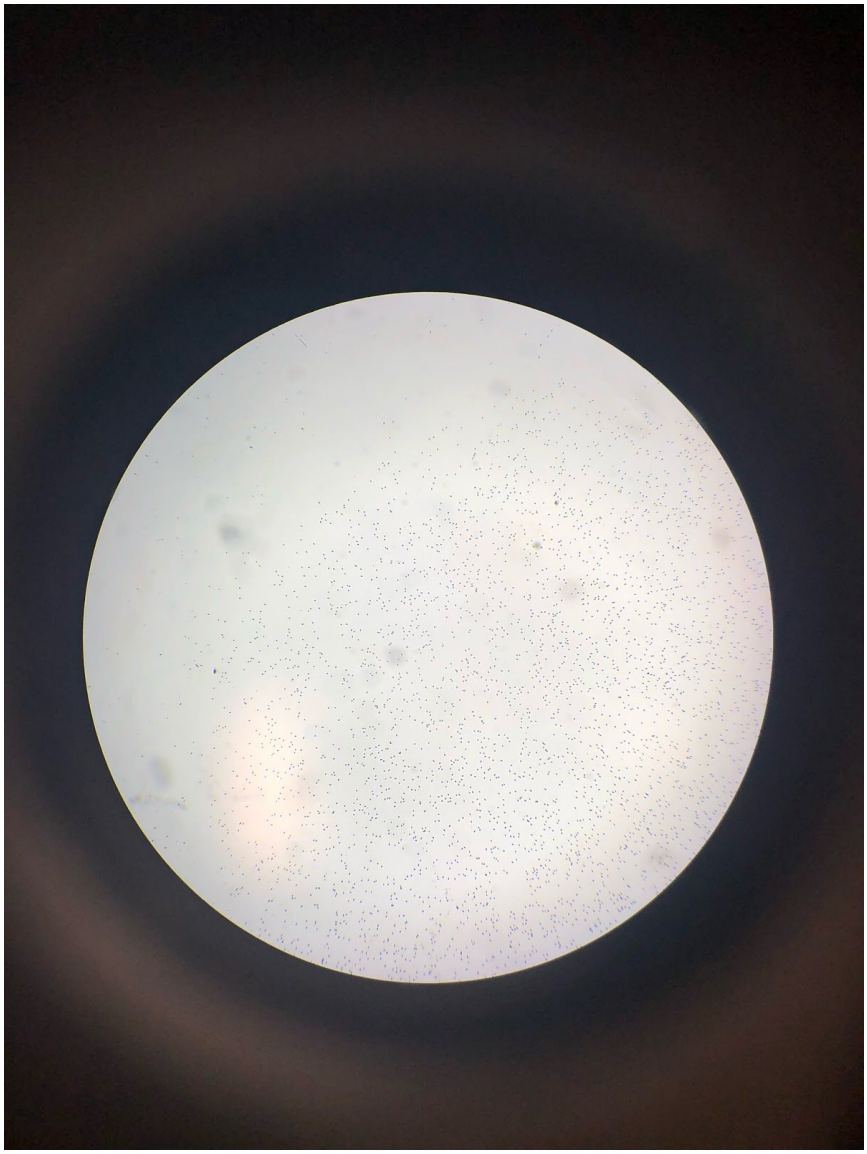
Protocol:

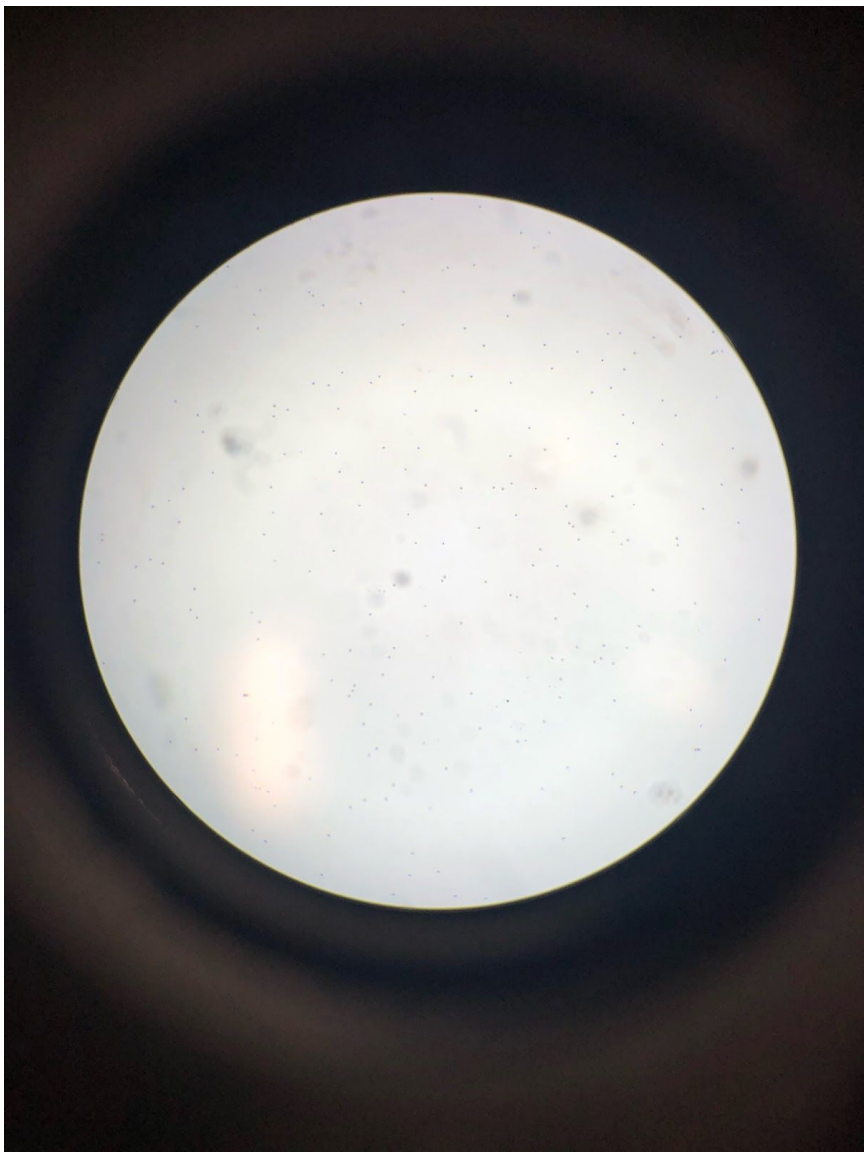
Used microscope to verify algae life

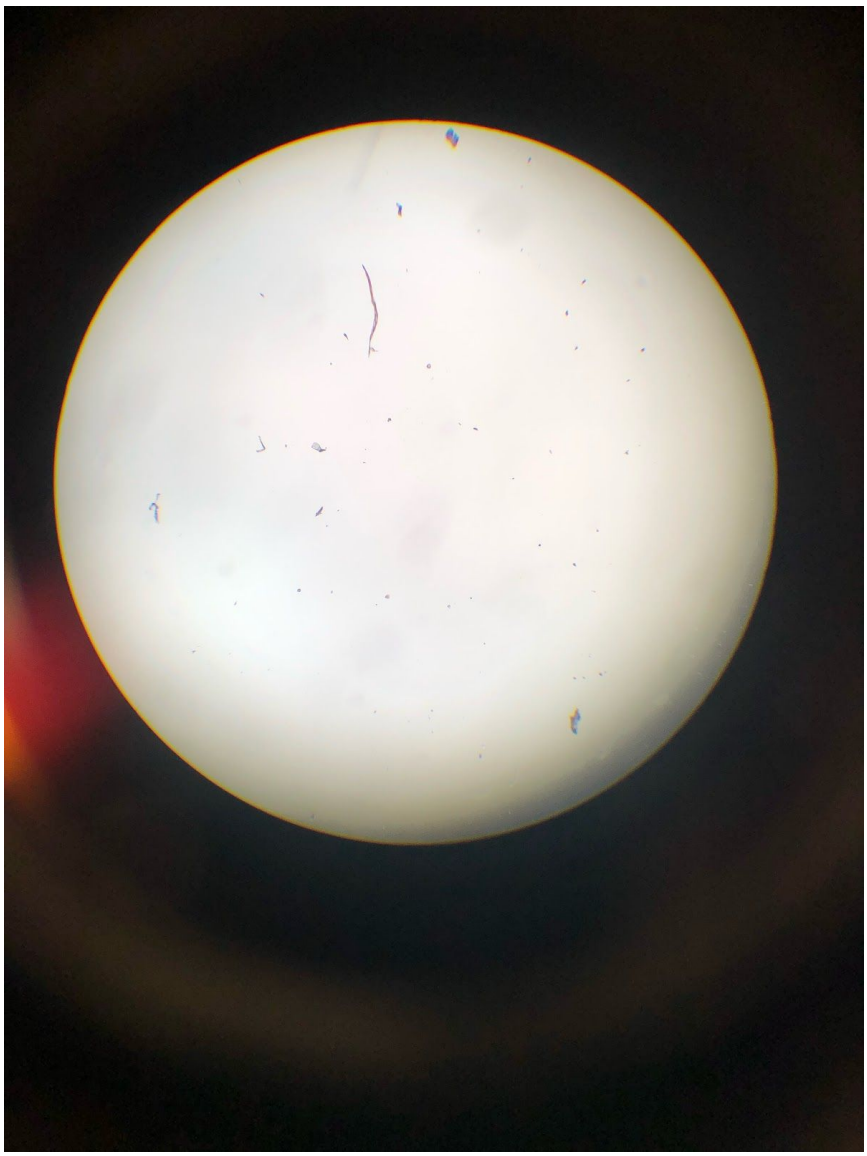
Results:

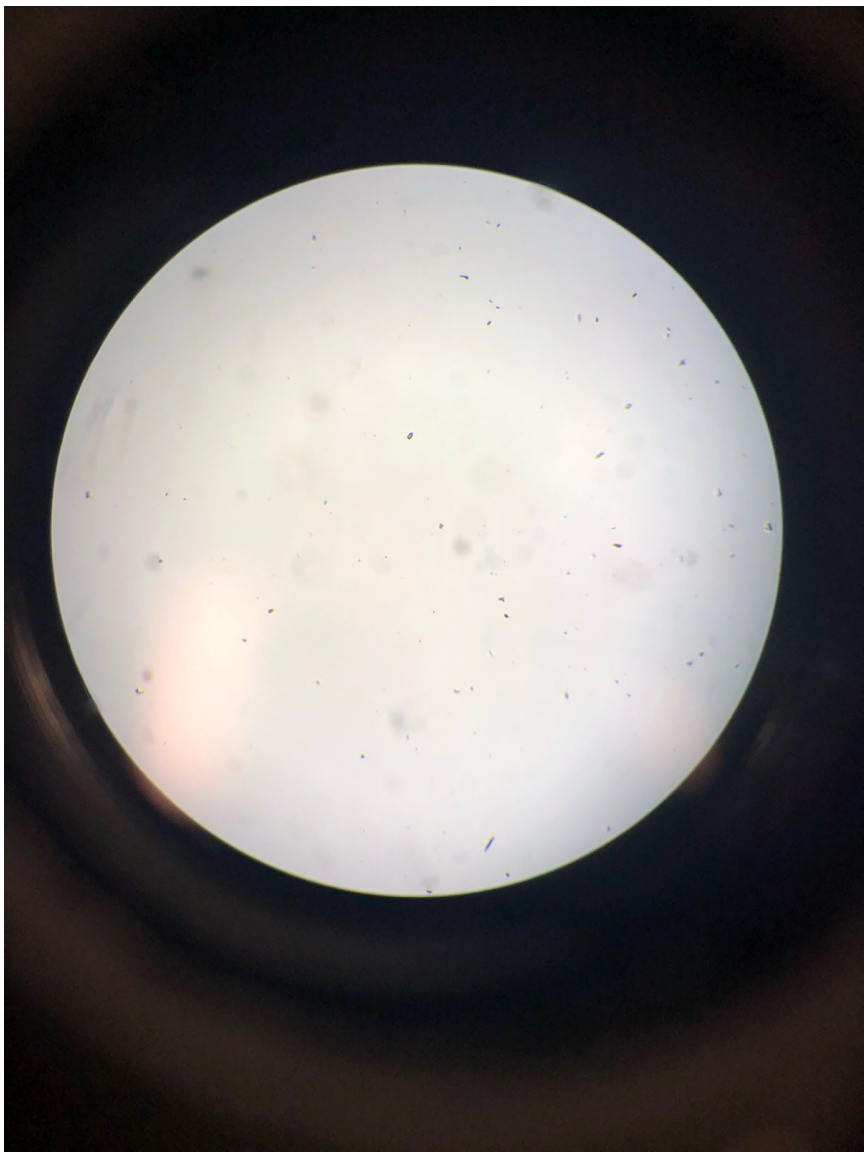
More details are in algae log.

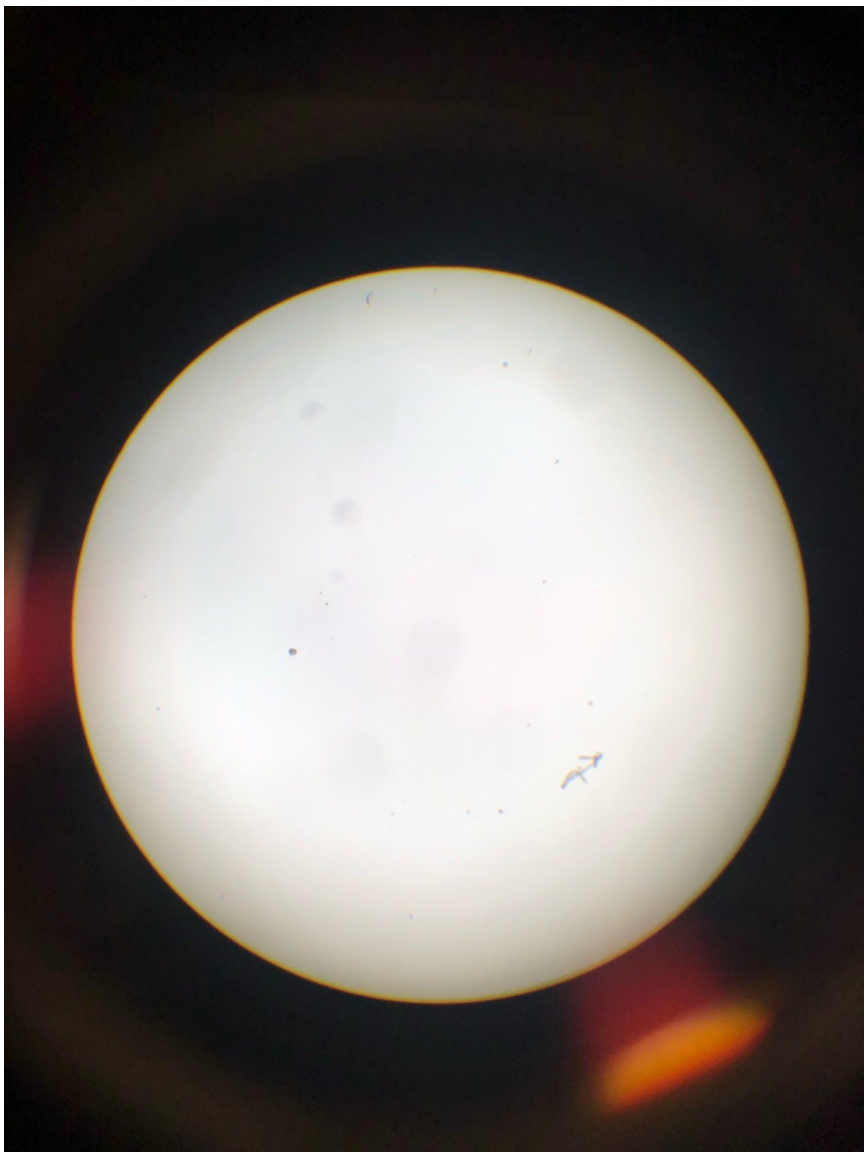
O. Marina	

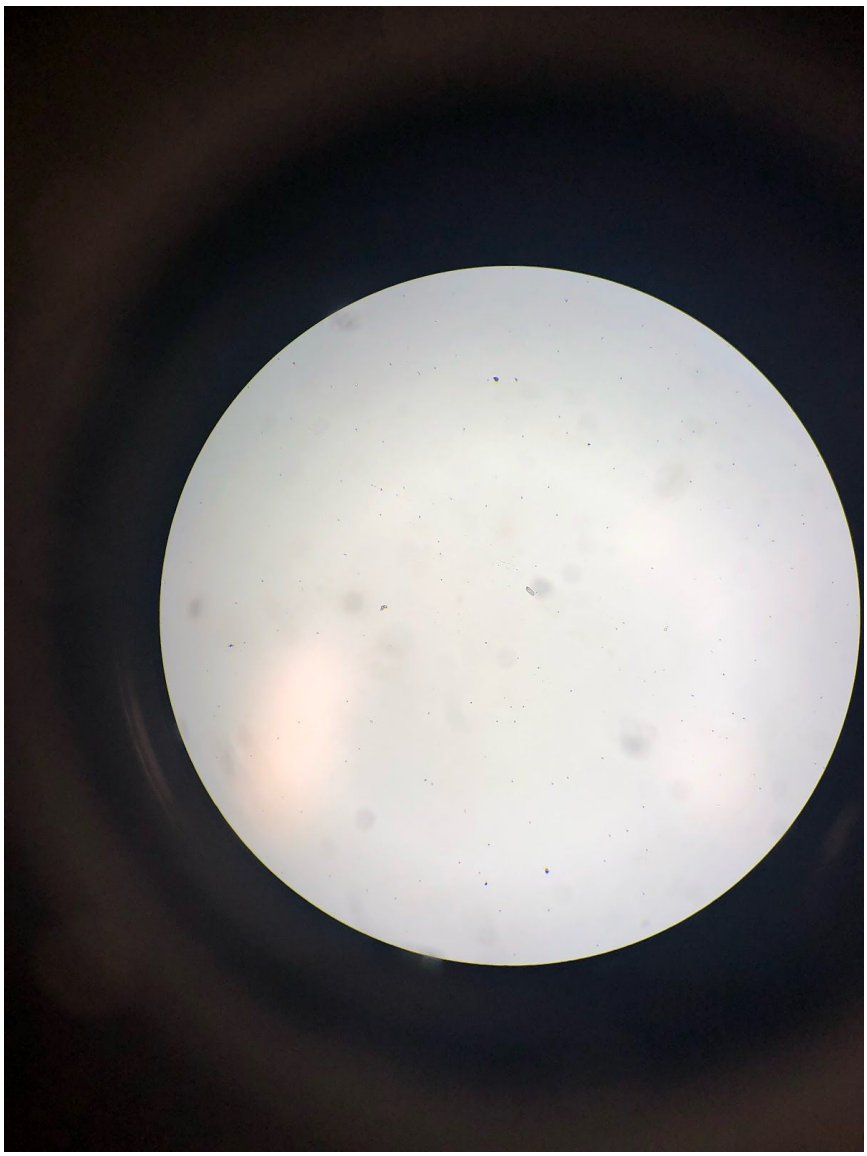
D. Tertiolecta	

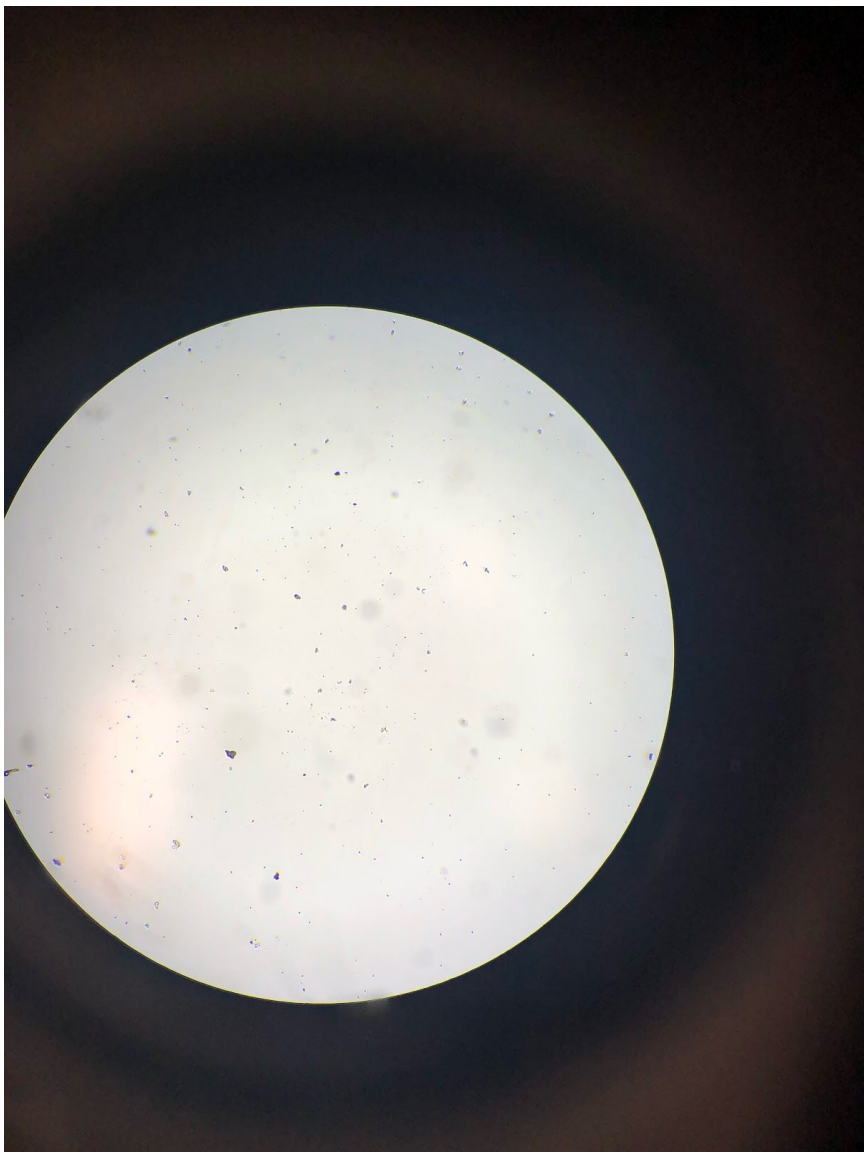
S. Microadriaticum	

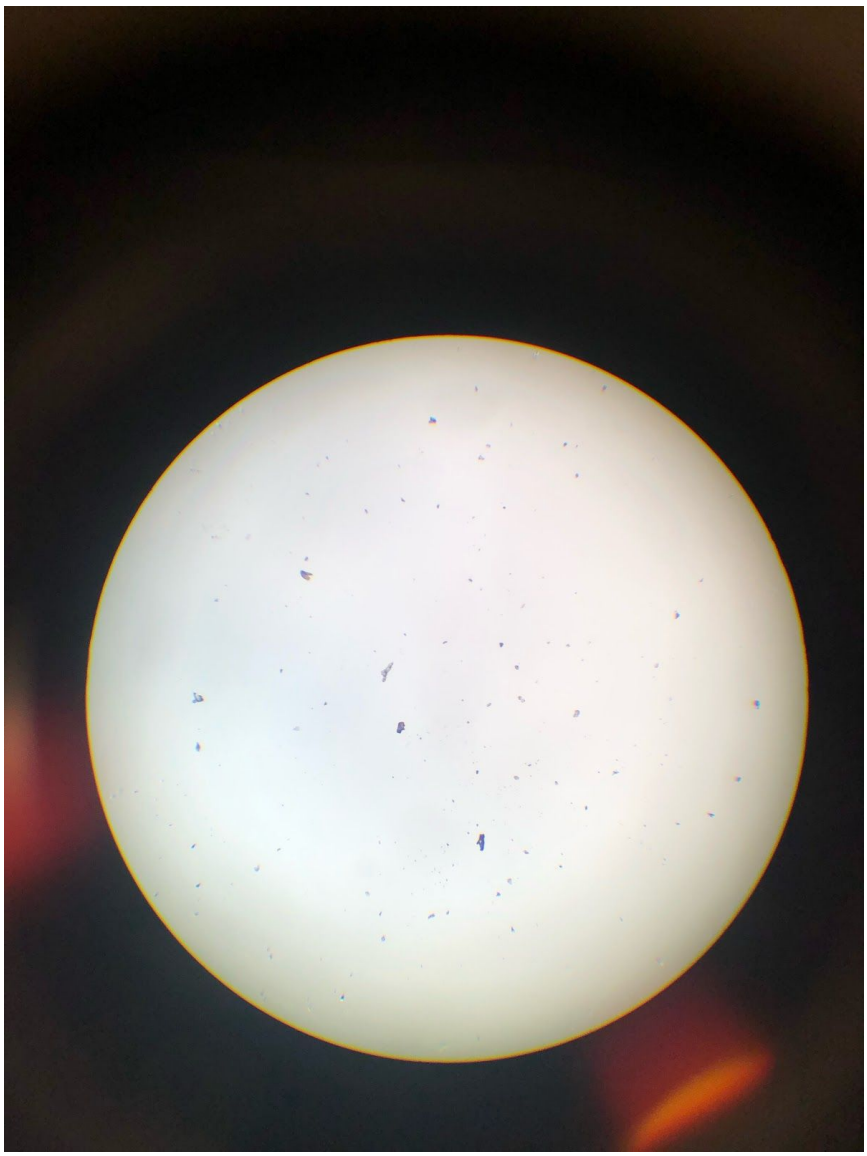
O. Marina f/2 **75ml seawater**	

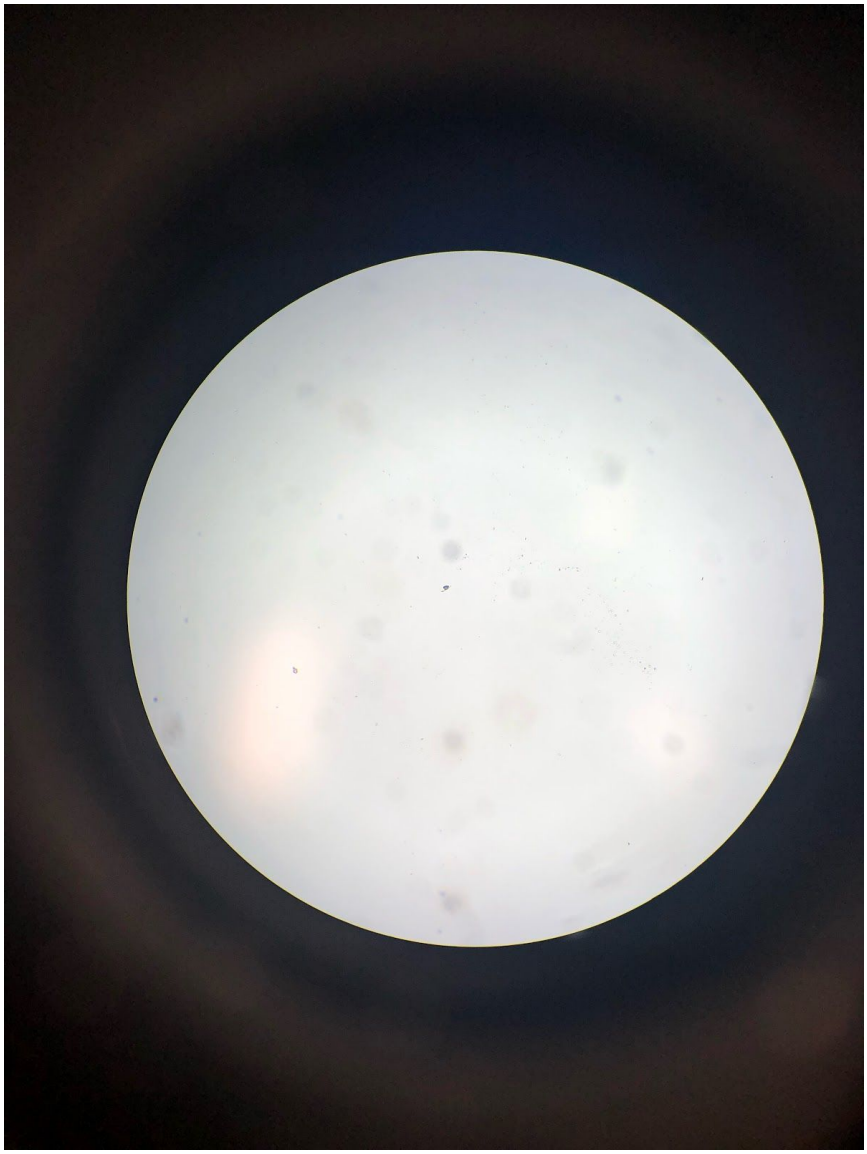
D. Tertiolecta f/2 **75ml seawater**	

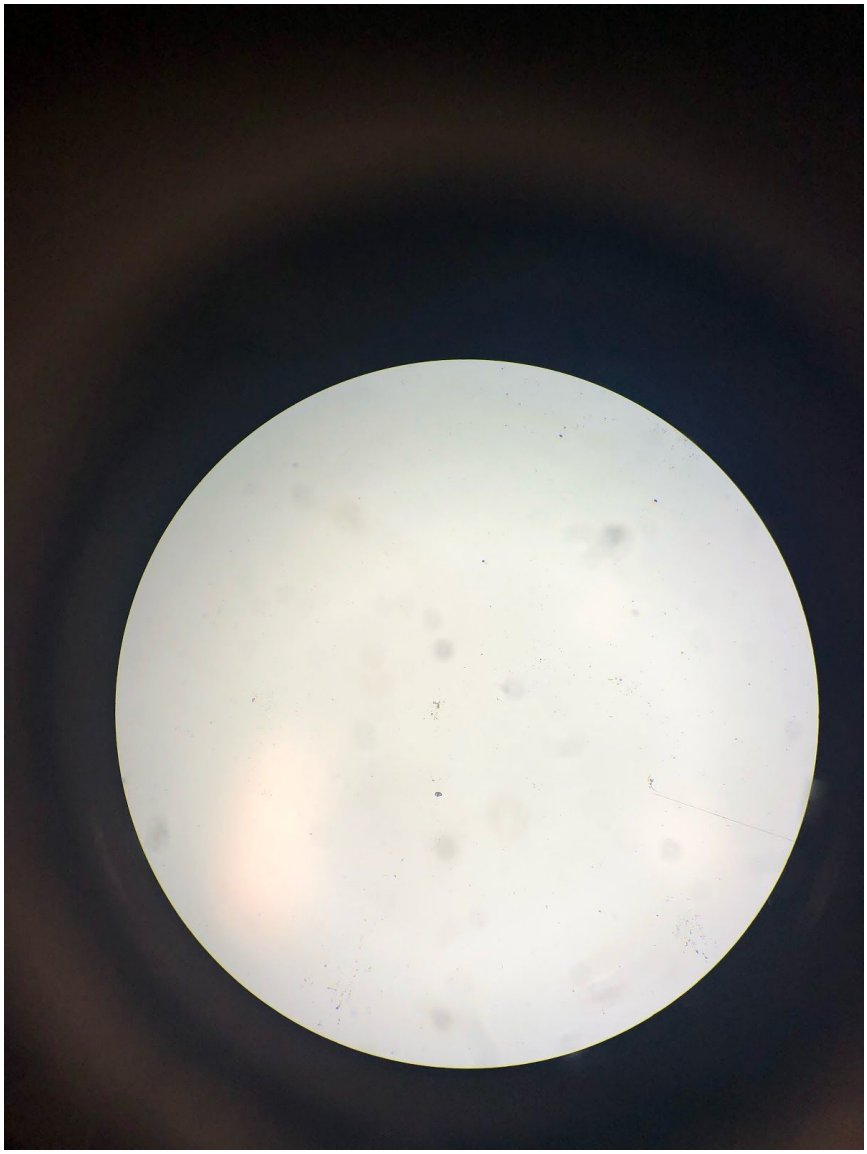
S. Microadriaticum f/2 75ml seawater	

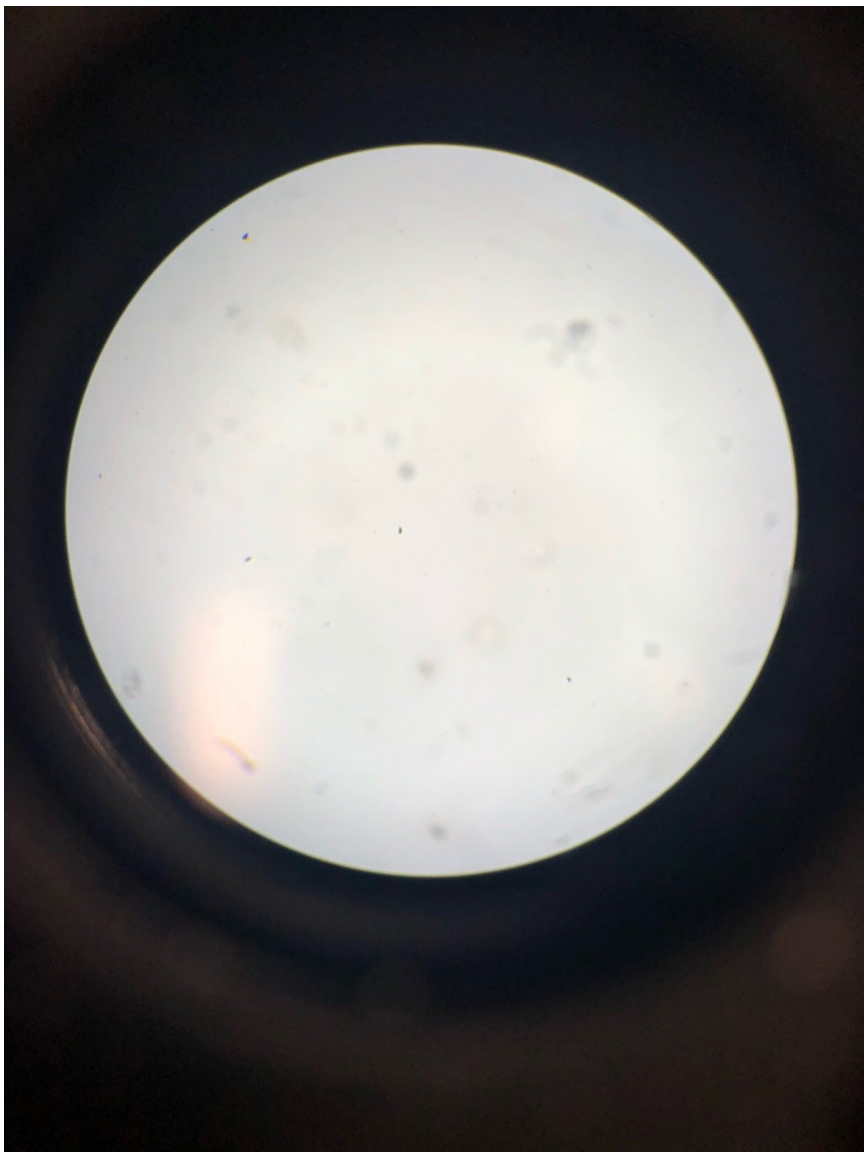
O. Marina **ASP-8A 75ml**	

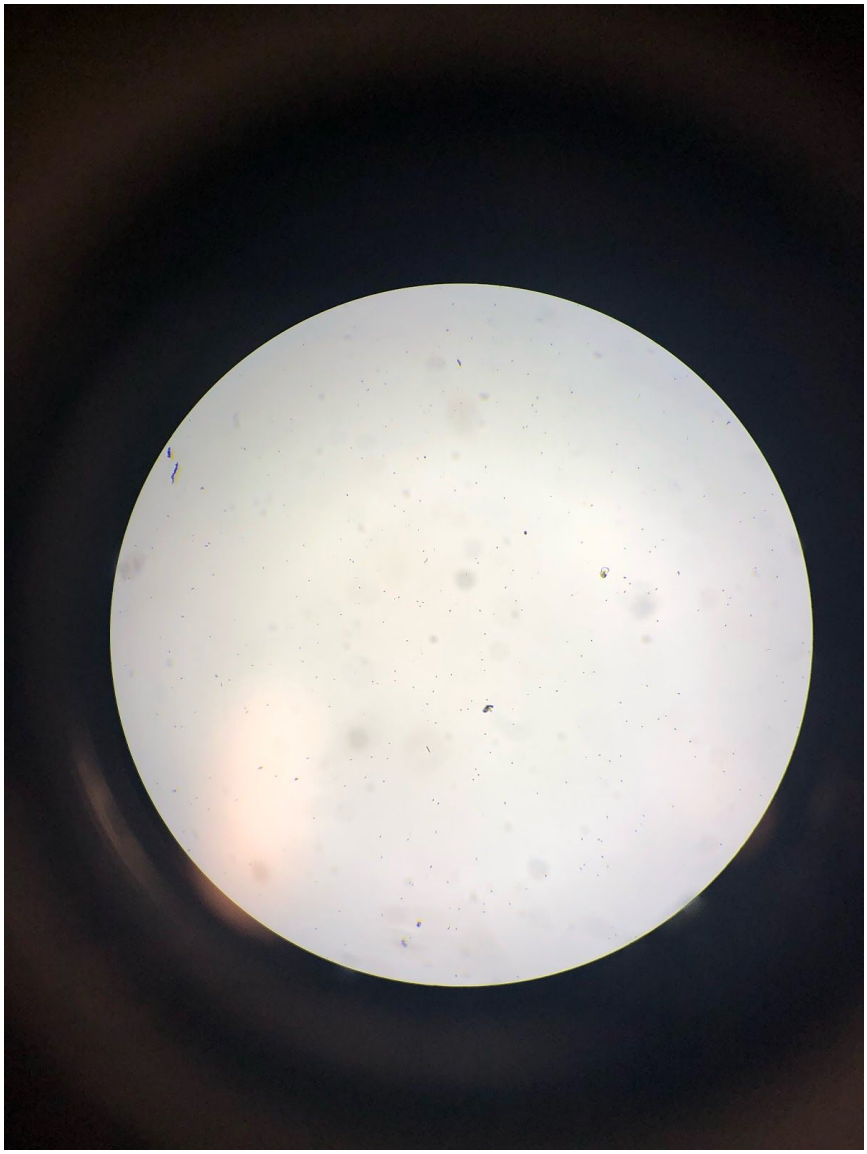
D. Tertiolecta **ASP-8A 75ml**	

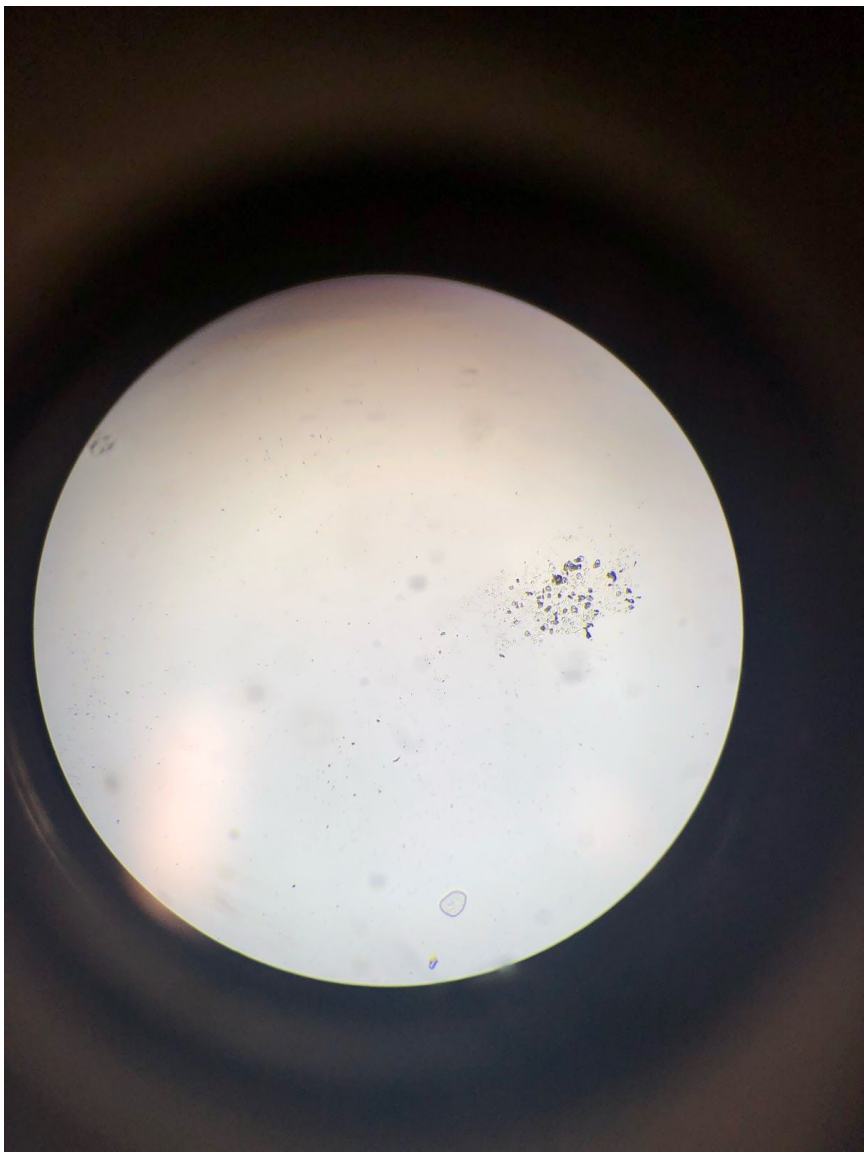
S. Microadriaticum ASP-8A 75ml	

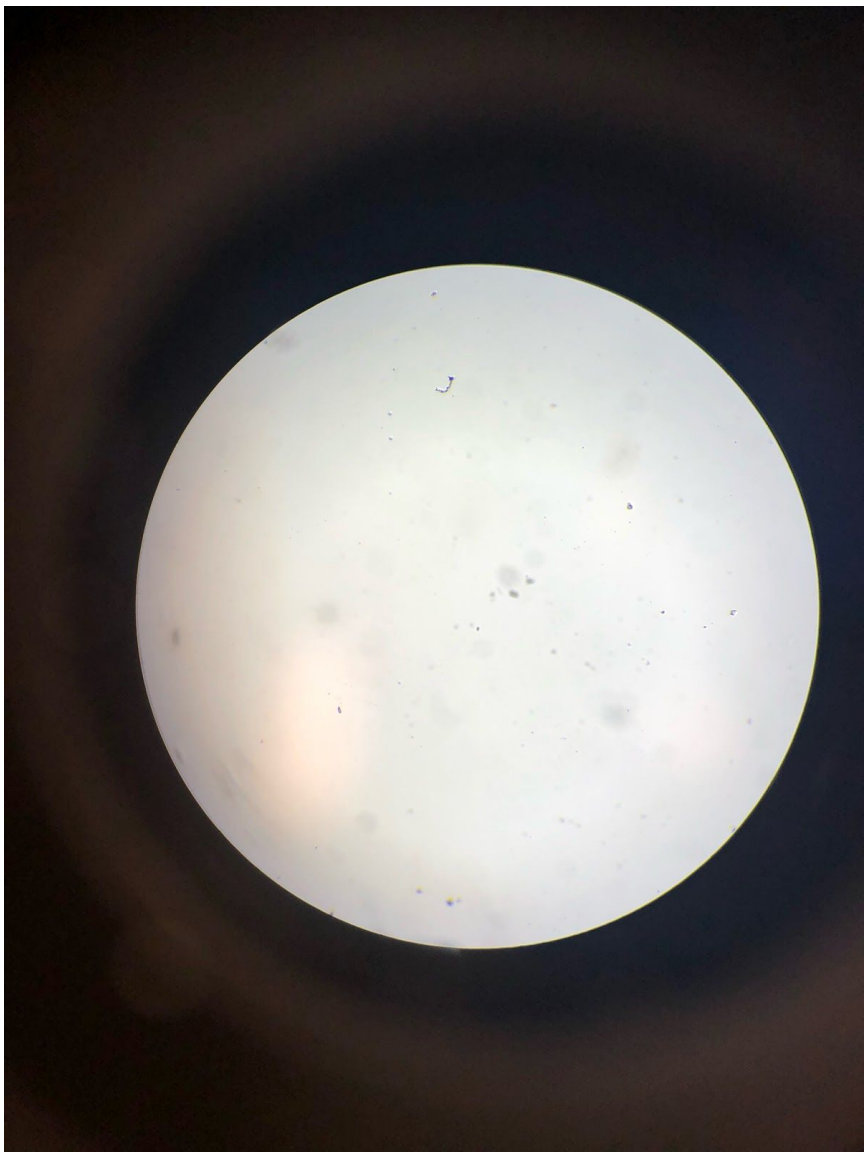
O. Marina f/2 **25ml seawater**	

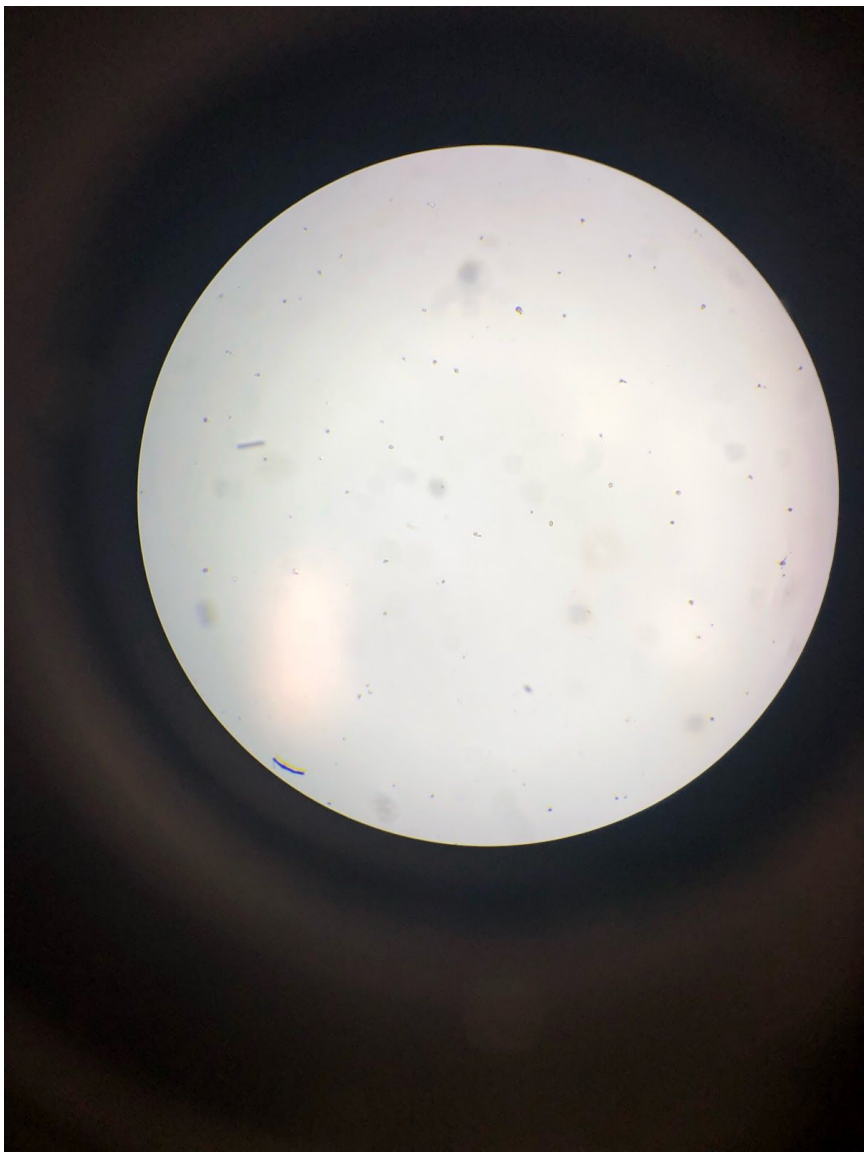
D. Tertiolecta f/2 **25ml seawater**	

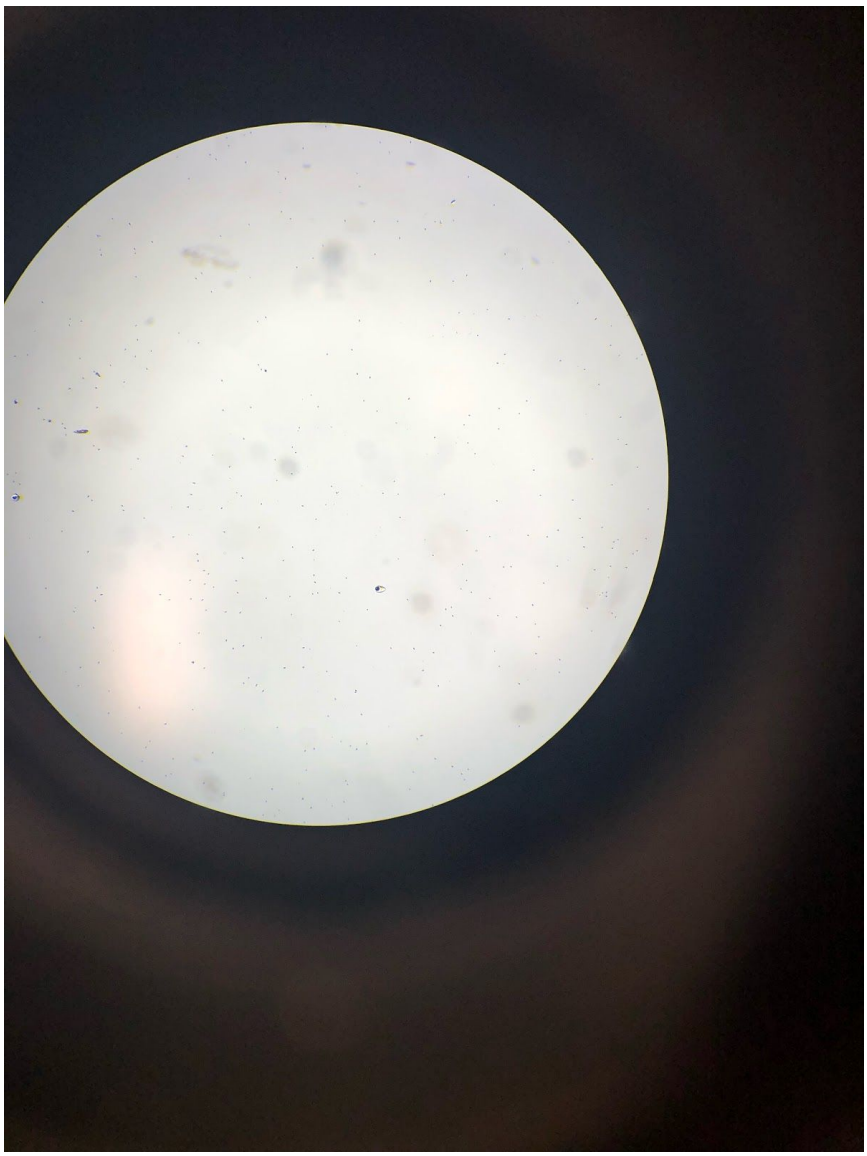
S. Microadriaticum f/2 25ml seawater	

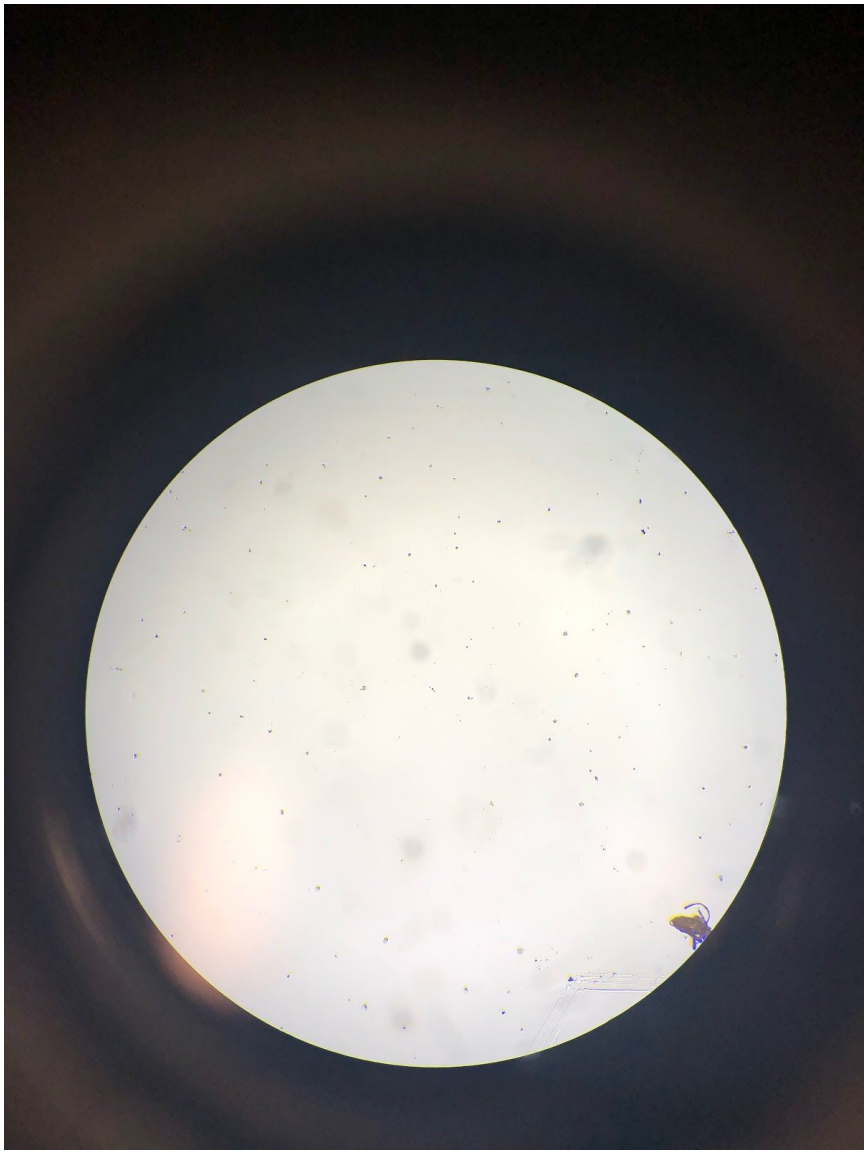
O. Marina **ASP-8A 25ml**	

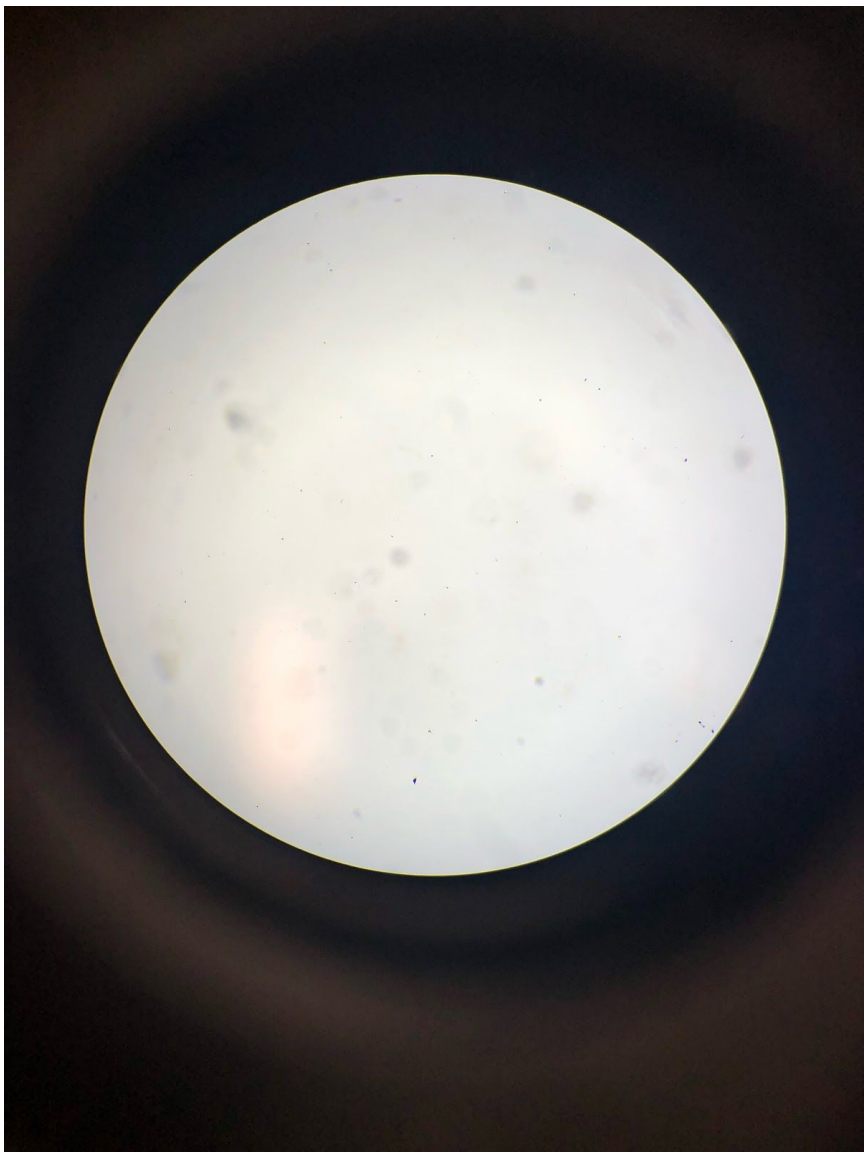
D. Tertiolecta **ASP-8A 25ml**	

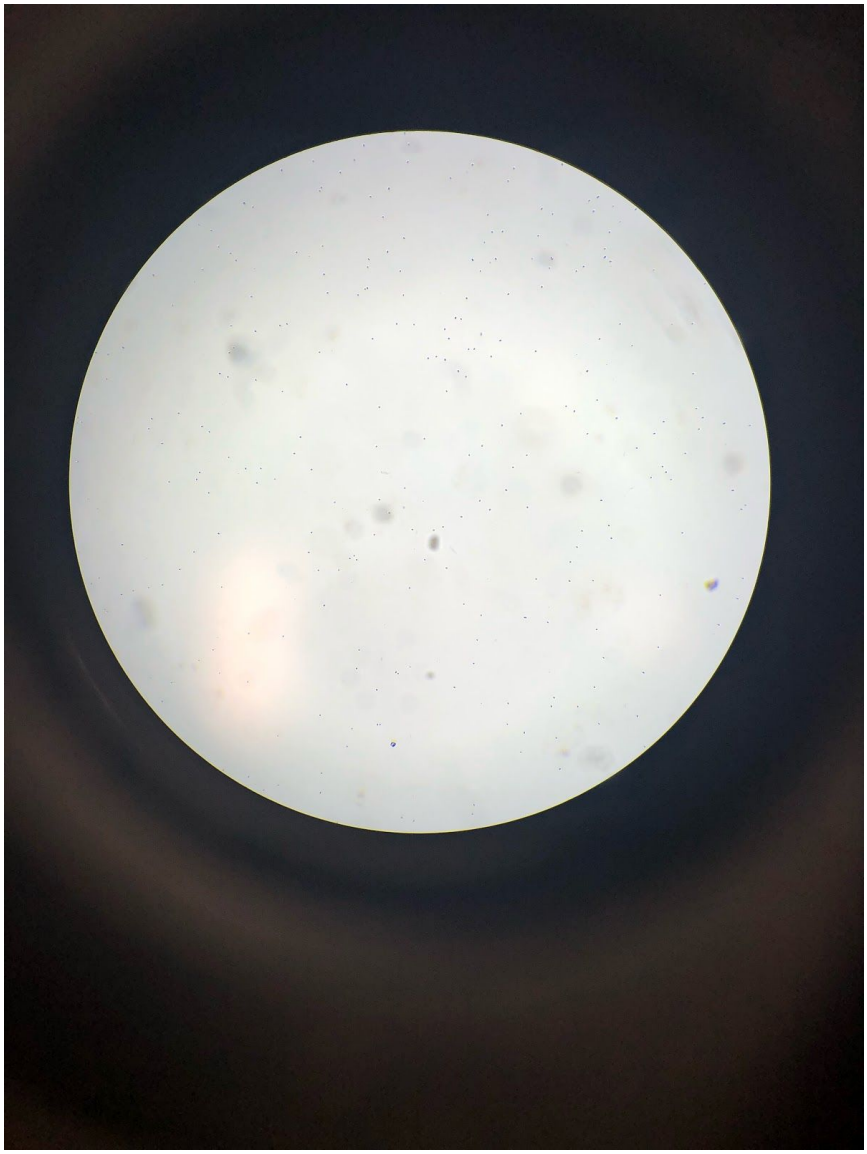
S. Microadriaticum ASP-8A 25ml	

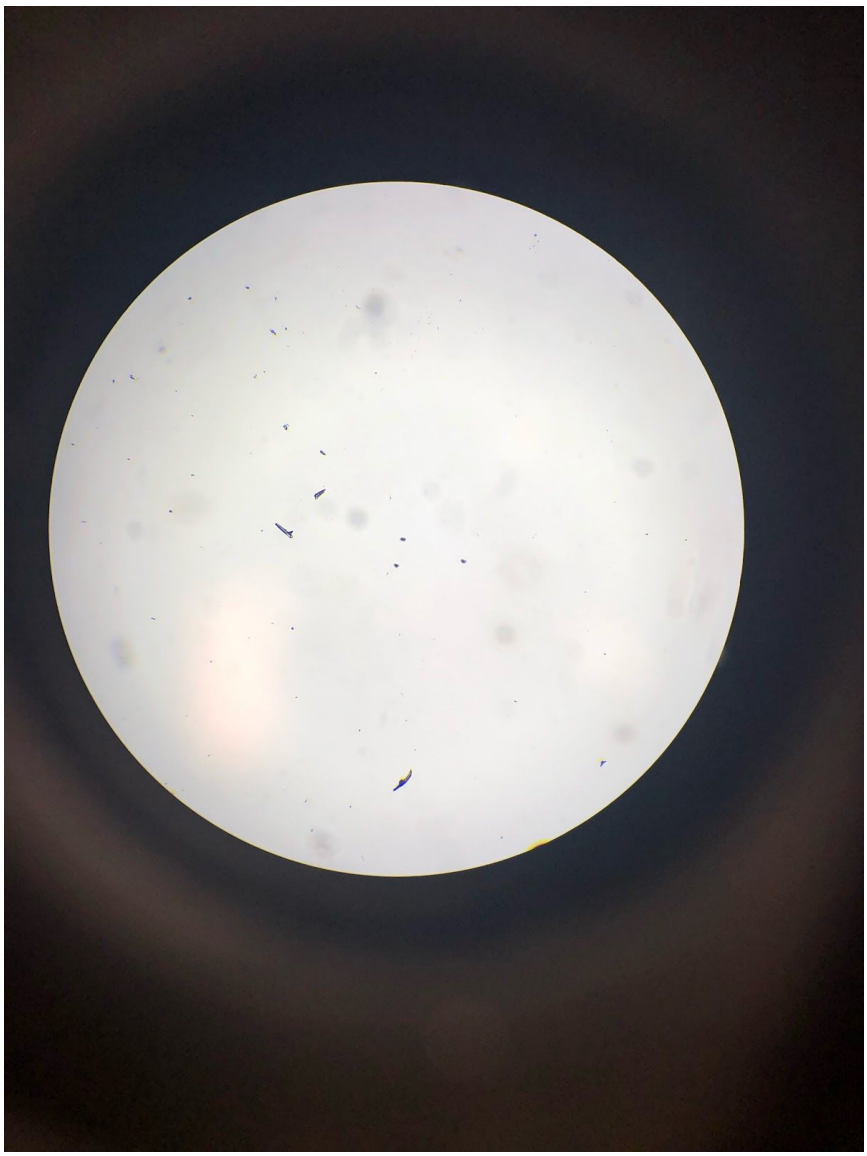
O. Marina f/2 **9ml seawater**	

D. Tertiolecta f/2 **9ml seawater**	

S. Microadriaticum f/2 9ml seawater	

O. Marina **ASP-8A 9ml**	

D. Tertiolecta **ASP-8A 9ml**	

S. Microadriaticum ASP-8A 9ml	

Name: Amirah

Date: 7/2/19

Goal: Transform chemically competent E. Coli cells with codon optimized RFP

Materials

DH5 alpha max efficiency cells

Codon optimized RFP in pUCIDT

pUC19 control

TE buffer

Protocol:

Heat Shock

1. Thawed One Shot TOP10 chemically competent cells on ice.
2. Added 2 µL of DNA sample into competent cells
3. Incubated the cells on ice for 35 minutes.
4. After the ice incubation, placed the samples into a 42° C water bath for 30 seconds.
5. **Quickly** took them out and **immediately** added 250µL of SOC medium
6. Placed the samples into a 37° C shaking water incubator for 1 hour at 200 rpm.
7. After shaking for 1 hour, smeared 150 µL of the solution onto an agar plate with the respective antibiotics.
    a. 1000x Ampicillin
8. Incubated plates at 37°C for at least 24 hours.