* Zooplankton list

IG predators

*Stentor*

*Brachionus calyciflorus*

*Blepharisma* (only feed on bacteria)

IG preys

*Euplotes*

*Frontonia*

*Onychodromus*

*Colpidium* (only feed on bacteria)

* Bacteria species

*Serratia marcescens,*

*Bacillus cereus,*

*Bacillus subtilis,*

*Proteus vulgaris*

* Culture 1 protist species in different media and on food resources

1. In Woods Hole MBL Medium and on Chlamydomonas and bacteria (unknown…)

**Modified Woods Hole MLB Medium**:

Stock solution g/L

1. CaCl2\*2H2O (Calcium chloride dihydrate) 36.80
2. MgSO4\*7H2O (Magnesium Sulfate Heptahydrate) 37.00
3. NaHCO3 (Sodium Hydrogen Carbonate) 12.60
4. K2HPO4\*3H2O (Potassium Hydrogen Phosphate Trihydrate) 11.40
5. NaNO3 (Sodium Nitrate) 85.00
6. Na2SiO3\*5H2O (Sodium Metasilicate Pentahydrate) 21.20
7. Combined trace elements

Na2EDTA 4.36

FeCl3\*6H2O 3.15

CuSO4\*5H2O 0.01

ZnSO4\*7H2O 0.022

CoCl2\*6H2O 0.01

MnCl2\*4H2O 0.18

Na2MoO4\*H2O 0.006

H3BO3 1.00

1. TRIS buffer 0.115
2. Vitamin Mix 0.5g/50mL
3. mg/L

Thiamin HCl (B1) 0.1

Biotib (H) 0.0005

Cyanocobalamin (B12) 0.0005

- Add 1 mL of each stock solution (1-7) and dry buffer to 750 mL distilled water.

- Allow to stir until TRIS buffer is dissolved and bring final medium volume to 1.0 L with distilled water.

- Autoclave media flask.

- Add 1mL vitmin mix (9) with syringe filter when medium is cool.

- (For species which cannot use nitrate substitute 1mL of NH4Cl made up to 5.4 g /L H2O) Adjust pH to 7.2 with HCl. Autoclave at 121°C (18 PSI for 45 mins).

- Add 1 mL of each of the stocks to 750mL of water, then add 1 mL of the trace elements you ordered, lastly dissolve TRIS buffer.

1. In Protozoa Pellets media (PPV) on 3 bacteria sp.

**Protozoa Pellets media (PPV)**

1. Well water 1400ml
2. Protozoa pellets 0.35g
3. vitamin mix (HERPTIVITE Multivitamin) 0.07g

- Mix (1) to (3) and autoclave on standard liquid cycle (45 mins; 121℃; 17psi)

- After PPV cool down, inoculate 3 bacteria sp. into the PPV

1. In Protozoa Pellets media (PPV) on 4 bacteria sp. + Chlamydomonas
2. In Protozoa Pellets media (PPV) on 4 bacteria sp. + 2 phytoplankton sp.
3. In media of Brad’s algae culture

**Protozoa list**

IG predator: *Blepharisma*

IG prey: *Colpidium*

Basal prey: *Bacillus cereus, Bacillus subtilis, Proteus vulgaris*

**Protozoa Pellets media (PPV) medium**

1. DI water 1400ml
2. Protozoa pellets 0.35g
3. vitamin mix (HERPTIVITE Multivitamin) 0.07g

- Mix (1) to (3) and autoclave on standard liquid cycle (45 mins; 121℃; 17psi)

- After PPV cool down (maybe 1-2 hours or could be over night), inoculate 3 bacteria sp. into the PPV

**Nutrient agar**

1. DI water 1000ml
2. Carolina nutrient agar 23g

**UROP/undergraduate student works**

WK1 (Oct.3-Oct.7): building 20 special made glass bottles for the first stage

- To make one unit of special mage glass bottle, cut off the bottom of two Qorpak glass bottles and glue two opening parts together with Nitex mesh (about 7.5 cm by 7.5 cm) in between.

- need 15 units with 250 μm Nitex mesh and 5 units of 10 μm Nitex mesh for the first stage of the experiments

WK2 (Oct.10-Oct.14):

- Finishing 20 units of 250-mesh special made glass bottles

- Prepare PPV media (5L)

- ID protozoa

WK3 (Oct.17-Oct.21):

- Finish all experimental units, including autoclave

- Prepare PPV media and nutrient agar (for slant test tube)

2\* 1.4L PPV / person

6 slant test tubes / person

- inoculate bacteria to slant test tube

- ID protozoa (Thu/Fri)

recognize the two protozoa sp.

Autoclave experimental units and 5 normal glass bottles

- inoculate protozoa (Thu/Fri)

suck them out with the caterpillar tube (one ind. at a time) to a normal glass jar (NOT an experimental unit)

WK4 (Oct. 24-Oct. 28)

- Start the first stage experiment (subsample every other day)

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**Method and materials**

…of experiments starts on June 22 (niche partitioning estimation)

**Microcosms**

To estimate the resource partitioning between *Colpidium striatum* and *Blepharisma americanum*, the two ciliate species were cultured separately with 4 bacterial prey species, *Serratia marcescens*, *Bacillus cereus*, *Bacillus subtilis*, and *proteus vulgaris*. Replicates of the two food web configurations were established in 125 mL plastic shaker flasks containing 100 mL of medium. The flasks were capped with 0.22 μm PTFE pore caps to allow air exchange but prevent contamination. The design used five replicates of each distinct food web for a total of 10 microcosms. Flasks and media were autoclaved before use. Replacement of 10% of the total volume with fresh medium every 7 d provided additional nutrients.

Community assembly involved additions of 20 individuals of each species into microcosms containing freshly prepared medium with four bacterial prey species as the standardized initial bacterial species composition. *Colpidium* and *Blepharisma* grow readily on a diet of these bacteria.

**Data**

To estimate ciliate population dynamics, microcosms were subsampled every 3 days for the 36-day duration. Subsampling involved withdrawing 0.50 mL of medium from a thoroughly mixed microcosm with a sterile pipette. Ciliates were identified and counted live with the aid of a stereoscopic microscope. Each sample consisted of 10~0.04 mL subsamples (drops) placed on a tared plastic petri plate. The sample volume was rapidly determined by mass (to 0.0001 g precision) using an electronic balance. Counts standardized per unit volume provided estimates of average protist densities. Sampled medium was replaced with an equal volume of fresh sterile medium. On average, only ~0.5% of the entire community was removed in each sample. Microcosms were subsampled

To estimate the grazing impacts of each ciliate on bacterial prey, plate counts of serially diluted subsamples were conducted. The abundance of bacterial prey before grazing were estimated by plate counting the bacteria density before the addition of the ciliates. The abundance of bacterial prey after grazing were estimated by plat counting the bacteria density after ciliate reached stable population density, as determined by ciliate population estimation mentioned before.