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Title: Making filtered protozoan pellet medium (PPM).

Date: Friday 1st of April 2022

Aim: Making PPM, autoclaving it, filtering, and autoclaving it again. The PPM will be used for creating high-density monocultures.

Steps:

1. Label PPM bottles (PPM EG Today's date)
2. Fill bottles with water (Daphnia room, A65)
3. Add protist pellet to bottles (Daphnia room, A65) – Add 0.46 g of pellet/L of water
4. Autoclave bottles (program: P6)
5. Label **filtered** PPM bottles (filtered PPM EG Today's date)
6. Filter PPM using folded filters (Whatman, 10311651)
7. Autoclave filtered PPM bottles (Program: P6)

• 8 L PPM

Title = time to fill tube rack
Date = Sunday 3rd April 2022
Aim = seeing how it takes to fill the four tube racks + counting the numbers on them.

Results = around 20 minutes



CHECK HOW MUCH MATERIAL I HAVE (AUTOCLAVED†)

- Eppendorf tube racks = 5x
- Styrofoam boards = 4x
- Microwaving racks = 2x
- 15 ml falcon tube racks = 7x
- Eppendorf tubes (2 ml) > 1000x
- 15 ml Eppendorf tubes = 125x
- 50 ml " " = 125x
- 1 ml tips (blue) = 13x boxes (96 tips each)
- 200 µl tips (yellow) = 23x boxes (96 tips each) + 2 in climate chamber
- 10 µl tips (green) = 10x boxes (24 tips each) + 2x wash gloves (16 tipse each) + 3x 5 L Bechers (60 tips each)
- 500 ml Schott bottles* = 31x
- 5 L Schott bottles = 2x
- 1 L Schott bottles = 2x
- PPM = 8 L
- Demineralized water = 1 L

Mixing =
- 12x 500 ml Schott bottles w/ seeds
- 1x large bottle (at least 6.6 L) to mix PPM

† / autoclaved be autoclavable
* w/ two wheat seeds

Title: growing bacteria for protists.

Date: Sunday 3rd of April 2022

Aim: growing the bacteria mix (mix of *Bacillus subtilis*, *Bacillus brevis*, and *Serratia fonticuli*) to feed to the protists when growing monocultures to high densities.

Steps:

1. **Gather material**
   a. G floor
      - Pipette boy
      - 50 ml serological pipette (for the pipette boy)
   b. A floor
      - Filtered PPM (0.46 g/L Protozoan Pellet Medium by Carolina) created on Friday 1st April 2022
      - 2x Erlenmeyer flask – autoclaved with two wheat seeds
2. **Label flasks**
   *200 ml PPM + 2 aliquot bacteria mix* *EG* *Today's date*
3. **Fill bottles with 200 ml PPM** (using the pipette boy, pipetting 50 ml at the time)
4. **Add bacterial stock** (General lab, LA G60)
   a. Get metal box number 57 from the bottom drawer of the freezer (-80 °C ULT Freezer)
   b. Get stock number 3 from the metal box
   c. Get 4 bacterial stocks (=$N_{bac\ stocks}$) (3 ml bacterial solution containing *Bacillus subtilis (Cat.No.: 154865 - Carolina)*, *Bacillus brevis (Cat.No.: 154921 - Carolina)*, and *Serratia fonticuli (Cat.No.: DSM 4576 – DSMZ)* prepared on 09.05.2016).
   d. Hold bacteria stock until they melt (1-5 minutes)
   e. Pour bacterial stocks into flasks with PPM – 1 stock/100 ml (REMEMBER: WEAR GLOVES)
5. **Bring bacterial solution to the climate room (A73.2)**



Title: autoclaving nanoparticle water using small autoclave

Date: Monday 4th April 2022

Aim: autoclaving nanoparticle water using the small autoclave. This will be then used to flush the dispenser when creating high-density monocultures.

Steps:

1. **Fill 2x 1 L Schott bottles with nanoparticle water**
2. **Prepare bottles for autoclaving**
   a. Open bottles
   b. Add autoclaving strip
3. **Place sensor in the small autoclave**
   Fill a bottle with deionised water and insert the sensor in it. The bottle should be the same size as the one you are autoclaving.
4. **Add filtered water to the top of the small autoclave** *
5. **Run program P4 in the small autoclave**

* Needs to be turned on so that when water is enough it beeps.
We believe water from the outside container ( 8 ( ).

Title: growing protists to high concentration (creating protist monoculture with the addition of bacteria).

Date: Tuesday 5th of April 2022

Aim: growing the protists from the collection to high concentration to created mixed cultures next Wednesday.

Steps:

1. **Label 500 ml Schott bottles**

ID nr  Protist species  EG  Today's date

**All protists but Col, Lox, Tet**

2. **Mix PPM + bacs**
   Mix PPM and bacterial solution into the 5 L *"PPM + bacs mixing bottle"*
   a. Add 264 ml of bacterial solution made on 3/4/22
      (date)
      (measuring cylinder: 250 ml x1 + 7ml x2 (10 µl micropipette))
   b. Add 4.488 L of PPM made on 1/4/22
      (date)
      (measuring cylinder: 250 ml x17
      measuring cylinder: 230 ml x1
      micropipette: 8 ml x1)
3. **Add PPM + bacs**
   *Add 180 ml from the "PPM + bacs mixing bottle" to the autoclaved 500 ml bottles with two wheat seeds*
   (Measuring cylinder: 180 ml x1)
4. **Mix protists – see the cultures we mixed in "cultured_used" in Table 1 of additional information)**
5. **Add protists**
   For each *high-density monoculture bottle*, add 20 ml protists
   (10 ml micropipette: 6.67 ml x3)

**Col, Lox, Tet**

6. **Add PPM**
   a. Add 135 ml to the Col bottles (ID: 7,8,9)
      (Measuring cylinder: 135 ml x1)
   b. Add 145 ml to the first two Lox bottles (ID: 16,17)
      (Measuring cylinder: 145 ml x1)
   c. Add 100 ml to the third Lox bottle (ID: 18)
      (Measuring cylinder: 100 ml x1)
   d. Add 75 ml to the Tet bottles (ID: 31-34)
      (Measuring cylinder: 75 ml x1)
7. **Add bacs**



   a. Add 10 ml to Col (ID: 7,8,9) and Lox (ID: 16-18) bottles
      (10 ml micropipette: 5 ml x2)
   b. Add 5 ml to Tet (ID: 31-34)
      (10 ml micropipette: 5 ml x1)
8. **Mix protists**
   a. Col: mix 26.3.22 (3) into 26.3.22 (1)
   b. Lox: mix 28.2.22 into 18.2.22
9. **Add protists**
   a. Col (ID: 7-9): add 55 ml from Col 26.3.22 (1)
      (Pipette boy: 40 ml x1 + 15 ml x1)
   b. Lox (ID: 16-17): add 45 ml from Lox 18.2.22 Lox (ID: 18): added 80 ml* from Lox 2.4.22
      (Pipette boy: 45 ml x1)
   c. Tet (ID: 31-34): add 20 ml from Tet 2.4.22
      (10 ml micropipette: 6.67 ml x3)
10. **Bring the monocultures to the climate chamber (A73.2).**

*There weren't 90 ml, but only 80 ml! So I added only 80 ml. I also added 10 ml PPM to keep Lacs to 5%.

(12)

| ID | Species | culture_ml | protists_% | bacteria_% | PPM_% | Protists_ml | bacteria_ml | PPM_ml | cultured_used |
|---|---|---|---|---|---|---|---|---|---|
| 1 | Ble | 200 | 10 | 5 | 85 | 20 | 10 | 170 | 23.2.22 + 26.3.22 (3) |
| 2 | Ble | 200 | 10 | 5 | 85 | 20 | 10 | 170 | 23.2.22 + 26.3.22 (3) |
| 3 | Ble | 200 | 10 | 5 | 85 | 20 | 10 | 170 | 23.2.22 + 26.3.22 (3) |
| 4 | Cep | 200 | 10 | 5 | 85 | 20 | 10 | 170 | 18.2.22 + 26.3.22 (4) |
| 5 | Cep | 200 | 10 | 5 | 85 | 20 | 10 | 170 | 18.2.22 + 26.3.22 (4) |
| 6 | Cep | 200 | 10 | 5 | 85 | 20 | 10 | 170 | 18.2.22 + 26.3.22 (4) |
| 7 | Col | 200 | | 5 | | 55 | 10 | 135 | 26.3.22 (3) + 26.3.22 (1) |
| 8 | Col | 200 | | 5 | | 55 | 10 | 135 | 26.3.22 (3) + 26.3.22 (1) |
| 9 | Col | 200 | | 5 | | 55 | 10 | 135 | 26.3.22 (3) + 26.3.22 (1) |
| 10 | Eug | 200 | 10 | 5 | 85 | 20 | 10 | 170 | 16.2.22 + 28.2.22 |
| 11 | Eug | 200 | 10 | 5 | 85 | 20 | 10 | 170 | 16.2.22 + 28.2.22 |
| 12 | Eug | 200 | 10 | 5 | 85 | 20 | 10 | 170 | 16.2.22 + 28.2.22 |
| 13 | Eup | 200 | 10 | 5 | 85 | 20 | 10 | 170 | 18.2.22 + 25.2.22 |
| 14 | Eup | 200 | 10 | 5 | 85 | 20 | 10 | 170 | 18.2.22 + 25.2.22 |
| 15 | Eup | 200 | 10 | 5 | 85 | 20 | 10 | 170 | 18.2.22 + 25.2.22 |
| 16 | Lox | 200 | | 5 | | 45 | 10 | 145 | 18.2.22 + 28.2.22 |
| 17 | Lox | 200 | | 5 | | 45 | 10 | 145 | 18.2.22 + 28.2.22 |
| 18 | Lox | 200 | | 5 | | 80 | 10 | 160 | 2.4.22 |
| 19 | Pau | 200 | 10 | 5 | 85 | 20 | 10 | 170 | 18.2.22 + 26.3.22 (2) |
| 20 | Pau | 200 | 10 | 5 | 85 | 20 | 10 | 170 | 18.2.22 + 26.3.22 (2) |
| 21 | Pau | 200 | 10 | 5 | 85 | 20 | 10 | 170 | 18.2.22 + 26.3.22 (2) |
| 22 | Pca | 200 | 10 | 5 | 85 | 20 | 10 | 170 | 25.2.22 + 26.3.22 (3) |
| 23 | Pca | 200 | 10 | 5 | 85 | 20 | 10 | 170 | 25.2.22 + 26.3.22 (3) |
| 24 | Pca | 200 | 10 | 5 | 85 | 20 | 10 | 170 | 25.2.22 + 26.3.22 (3) |
| 25 | Spi | 200 | 10 | 5 | 85 | 20 | 10 | 170 | 16.2.22 + 28.2.22 |
| 26 | Spi | 200 | 10 | 5 | 85 | 20 | 10 | 170 | 16.2.22 + 28.2.22 |
| 27 | Spi | 200 | 10 | 5 | 85 | 20 | 10 | 170 | 16.2.22 + 28.2.22 |
| 28 | Spi te | 200 | 10 | 5 | 85 | 20 | 10 | 170 | 26.3.22 (1) |
| 29 | Spi te | 200 | 10 | 5 | 85 | 20 | 10 | 170 | 26.3.22 (1) |
| 30 | Spi te | 200 | 10 | 5 | 85 | 20 | 10 | 170 | 26.3.22 (1) |
| 31 | Tet | 100 | 20 | 5 | 75 | 20 | 5 | 75 | 2.4.22 |
| 32 | Tet | 100 | 20 | 5 | 75 | 20 | 5 | 75 | 2.4.22 |
| 33 | Tet | 100 | 20 | 5 | 75 | 20 | 5 | 75 | 2.4.22 |
| 34 | Tet | 100 | 20 | 5 | 75 | 20 | 5 | 75 | 2.4.22 |

Additional info:

• When making up mix of CCS + PPM 1 part MM and CCS when went over to Cacl2 the measuring cylinder was used when making MM x mixed 1 counter

• Forgot Phoe Coll CCS to Coll (1)

• 1 mistake have 9 mistakes in Eug (10)

• Pipetted only 6.67 x 2 Eugl have the same dos Col (7-9) and Lox (16-18) when pipetting

(13)

Title: collection control.

Date: Thursday 7th of April 2022

Aim: checking the abundance of the Tet high-density monocultures.

Step:

1. **Check Tet**
   Check protist abundances of Tet high-density monocultures (ID: 31-34) and abundances of the Tet highest density monoculture from the culture collection (Tet created on 2.4.22).
2. **Fill the high-density monocultures with bacteria and PPM**
   For the monocultures that are doing well (> 40 individuals/drop) add 95 ml PPM created on 1.4.22 (date) and 5 ml of bacterial solution created on 2.4.2022.
   ~~a. Calculate how much to mix in a large bottle~~

   ~~PPM to mix = (nr of cultures to increase in volume * 95 ml) * 1.~~2
   ~~(4·95ml)·1.1 = 420 ml~~
   ~~(4x95 ml)·1.2 = 456 ml~~

   ~~Bacterial solution to mix = (nr of culture to increase in volume * 5 ml) * 1.~~2
   ~~(4·5ml)·1.1 = 22~~

   ~~b. Mix in an autoclaved bottle~~ PPM
   c. Add ~~100~~ 35 ml ~~from the autoclaved bottle~~ to each of the high-density monocultures a. Add 5 ml bacterial solution to each of the high-density monocultures
3. **Split Tet 2.4.2022** ~~[If TET 2.4.2022 grew enough]~~
   a. Label a 500 ml bottle where you will create a new high-density monoculture
      Tet 35 EG 7.4.22
   b. Take 50 ml out from Tet 2.4.2022 and pipette them into a new 500 ml Schott bottle
      (Pipette boy: 50ml x1)
   c. Add to the new high-density monoculture bottle (ID: 35) 45 ml of PPM
      (Pipette boy: 45 ml x1)
   d. " " " 5 ml of bacterial solution created on 2.4.2022.
      (10 ml micropipette: 5ml x1)
   e. Fill the Tet monoculture from the collection with 50 ml PPM
      (Pour from PPM bottle)



Checklist:

**Bringing upstairs**
- [x] **PPM**
- [x] **Tet cultures (high-density monocultures + Tet 2.4.2022)**
- [x] **Bacteria**

## Collection & High-density monocultures Control

| | | | | |
|---|---|---|---|---|
| Tet collection 26.03.2022 (1) | 35 | 38 | 35 → | 36 |
| Tet high density monoculture (ID: 31) | 46 | 50 | 40 → | 45 |
| Tet high density monoculture (ID: 32) | 37 | 34 | 43 → | 38 |
| Tet high density monoculture (ID: 33) | ~~48~~ 54 | 48 | 48 → | 50 |
| Tet high density monoculture (ID: 34) | 48 | 34 | 44 → | 42 |

Title: Making protozoan pellet medium (PPM).

Date: 10/4/2022

Aim: Making protozoan protist medium (PPM) using the protist pellet (Protozoa Pellet by Carolina) and then autoclaving it.

Steps:

1. **Label PPM bottles**
   (PPM EG Today's date)
2. **Gather material from A floor (Altermatt cupboard)**
   - ☑ Ground protozoan pellet (provided by Carolina, Biological Supply Company, Burlington NC, USA)
   - ☑ Spoon and tray to weight protist pellet
3. **Fill bottles with water** (in the Daphnia room, A65)
   Fill with cold water, which you let run for 60 seconds before.
4. **Add protist pellet to bottles** (Daphnia room, A65)
   Add 0.46 g of ground protozoan pellet/L
5. **Autoclave bottles** ~~(program: P6)~~ (program 4 in small autoclave)

Litres of PPM made: 3

Title: growing bacteria for protists.

Date:

Aim: growing a bacterial solution from a bacteria mix (mix of *Bacillus subtilis*, *Bacillus brevis*, and *Serratia fonticuli*) to feed to the protists.

Steps:

1. **Gather material A floor**
   - ☑ filtered/~~unfiltered~~ PPM (0.46 g/L Protozoan Pellet Medium by Carolina) created on 1.4.2022 (creation date)
   - ☑ Erlenmeyer flasks – autoclaved with two wheat seeds
2. **Label flasks**
3. **Fill bottle with medium**
   ~~(Pipette boy: 50 ml at the time)~~
4. **Add bacterial stock** (General lab, LA G60)
   a. Get metal box number 57 from the bottom drawer of the freezer (-80 °C ULT Freezer)
   b. Get bacterial stock number 3 from the metal box (3 ml bacterial solution containing *Bacillus subtilis (Cat.No.: 154865 - Carolina)*, *Bacillus brevis (Cat.No.: 154921 - Carolina)*, and *Serratia fonticuli (Cat.No.: DSM 4576 – DSMZ)* prepared on 09.05.2016).
   c. Hold bacteria stock until they melt (1-5 minutes)
   d. Pour bacterial stocks into flasks with PPM – 1 stock/100 ml (REMEMBER: WEAR GLOVES)
5. **Bring medium with bacterial solution to the climate room (A73.2)**

Litres of bacterial solution made: 0.4 L

Title: high-density monocultures control.

Date: 10/4/2022

Aim: checking how high-density monocultures are doing (and the best Tet in the collection).

## High-density monocultures Control

| | | | | |
|---|---|---|---|---|
| Ble (ID: 1) | 6 | 6 | 4 | → 5 |
| Ble (ID: 2) | 12 | 6 | 10 | → 9 |
| Ble (ID: 3) | 5 | 2 | 7 | → 5 |
| Cep (ID: 4) | 15 | 9 | 11 | → 12 |
| Cep (ID: 5) | 11 | 8 | 11 | → 10 |
| Cep (ID: 6) | 13 | 13 | 13 | → 13 |
| Col (ID: 7) | 75 | 57 | 68 | → 67 |
| Col (ID: 8) | 80 | 72 | 61 | → 71 |
| Col (ID: 9) | 73 | 65 | 76 | → 71 |
| Eug (ID: 10) | 100 | 110 | 130 | → 113 |
| Eug (ID: 11) | 120 | 110 | 110 | → 113 |
| Eug (ID: 12) | 200 | 170 | 200 | → 190 |
| Eup (ID: 13) | 28 | 17 | 20 | → 22 |
| Eup (ID: 14) | 20 | 24 | 25 | → 23 |
| Eup (ID: 15) | 23 | 26 | 21 | → 23 (Recount on 11.4.22 = 24 \| 24 \| 18 → 22) |
| Lox (ID: 16) | 75 | 52 | 60 | → 62 |
| Lox (ID: 17) | 60 | 47 | 48 | → 52 |
| Lox (ID: 18) | 22 | 23 | 27 | → 24 |
| Pau (ID: 19) | 29 | 33 | 36 | → 33 |
| Pau (ID: 20) | 52 | 30 | 45 | → 42 |
| Pau (ID: 21) | 26 | 40 | 42 | → 36 |
| Pca (ID: 22) | 35 | 24 | 37 | → 32 |
| Pca (ID: 23) | 40 | 39 | 30 | → 36 |
| Pca (ID: 24) | 32 | 35 | 35 | → 34 |



| | | | | |
|---|---|---|---|---|
| Spi (ID: 25) | 2 | 4 | 2 | → 3 |
| Spi (ID: 26) | 1 | 2 | 4 | → 2 |
| Spi (ID: 27) | 6 | 0 | 4 | → 3 |
| Spi te (ID: 28) | 12 | 6 | 5 | → 8 |
| Spi te (ID: 29) | 8 | 8 | 10 | → 9 |
| Spi te (ID: 30) | 7 | 7 | 6 | → 7 |
| Tet (ID: 31) | 60 | 50 | 40 | → 50 |
| Tet (ID: 32) | 75 | 50 | 45 | → 57 |
| Tet (ID: 33) | 80 | 32 | 47 | → 39 |
| Tet (ID: 34) | 40 | 30 | 40 | → 37 |
| Tet (ID: 35) | 25 | 25 | 25 | → 25 |

RECOUNT: 2 2 2 → 2; 1 1 2 → 1; 1 1 3 → 2

**Collection control**

Tet 26.3.2022 (1) 25 | 10 | 10 → 15

Tet 8.4.22 90 | 90 | 95 → 92

~~Note~~

Best high-density monocultures

| | ID | | |
|---|---|---|---|
| Ble | 2 | 9 | LOW |
| Cep | 6 | 13 | LOW REALLY! |
| Col | 9 | 71 | |
| Eug | 12 | 190 | |
| Eup | 15 | 23 | LOW |
| Lox | 16 | 62 | |
| Pau | 20 | 42 | |
| Pca | 23 | 36 | |
| Spi | 27 | 2.5 | LOW REALLY! |
| Spi te | 29 | 9 | LOW REALLY! |
| Tet | 32 | 57 | |

Title: quantifying evaporation

Date: Sunday 10/4/2022

Aim: determining how much water evaporates when microwaving cultures.

Steps: 1. Measure evaporation from 5.25 ml of demonized water.

a. Fill 15 tubes (50 ml falcon tubes) with 5.25 ml of demonized water

b. Put them in rack

c. Microwave ~~1 m 30 sec~~ 3 minutes

d. Measure mass left of the demonized water

Results = (grams)

~~4.79 4.94 4.83 4.81 4.84 5.07~~
~~4.87 4.92 4.97 4.73 4.78 4.73~~ Rack 1
~~4.74 4.77 4.72~~

~~4.89 4.89 4.94 4.83 5.06 5.13~~
~~5.08 5.19 5.27 5.34 5.35 4.71~~ Rack 2
~~5.19 5.20 5.33~~

The tare of the scale didn't work, so retract 49.52 from the following values.



5.25 ml rack 1: grams left

53.25 53.74 53.89 53.93 54.10
53.63 54.04 54.37 53.99 54.14
54.30 54.02 53.92 53.78 53.45

5.25 ml rack 2

53.93 54.34 53.85 53.97 54.27 54.99
54.17 54.54 54.11 53.81 54.09 53.87
53.77 53.88 53.80

5.25 ml rack 3

53.94 53.92 53.87 53.72 54.14
54.02 54.13 54.39 54.20 53.78
54.21 53.72 53.96 53.98 53.97

6.75 ml rack 1

55.00 55.27 55.45 55.47 55.85
55.40 55.14 55.78 56.04 55.41
55.78 55.36 55.47 55.77 55.00

6.75 ml rack 2

54.95 55.79 55.33 54.60 55.51
54.40 55.31 55.86 55.54 55.32
55.76 55.39 55.35 54.88 54.72

6.75 ml rack 3

54.29 54.73 54.77 55.17 55.80 55.72
55.35 56.11 54.73 55.85 56.05 55.90
55.72 55.30 54.61

Discussion: some of the water overflowed outside from the falcon tubes.

Title: Creation of new high-density monocultures from other high-density monocultures

Date: Monday 11th April 2022

Aim: growing mutants to high concentration

Steps:

1. Label the following 15 bottles (500 ml) which have been autoclaved with two wheat seeds inside

| Volume of bottle(L) | | |
|---|---|---|
| 1 | ID: [redacted]36 | Ble |
| 1 | ID: [redacted] 37 | Ble |
| 2 | ID: [redacted] 38 | Ble |
| 1 | ID: [redacted] 39 | Cep |
| 1 | ID: [redacted] 40 | Cep |
| 1 | ID: [redacted] 41 | Cep |
| 1 | ID: [redacted] 42 | Eup |
| 1 | ID: [redacted] 43 | Eup |
| 1 | ID: [redacted] 44 | Eup |
| 20 | ID: [redacted] 45 | Spi |
| 2 | ID: [redacted] 46 | Spi |
| 1 | ID: [redacted] 47 | Spi |
| 5 | ID: [redacted] 48 | Spi te |
| 1 | ID: [redacted] 49 | Spi te |
| 1 | ID: [redacted] 50 | Spi te |

2. Add 45 mL of PPM to each of the previous bottles (PPM created yesterday)

3. Add 5 ml of Cachiral solution to each of the previous bottles (Cachiral solution created yesterday, see page 18)



4. Transfer 50 mL mutants from the Best culture to the corresponding bottle (Best cultures: see page 18)

~~Best high-density monoculture for...~~

~~Ble~~:

5. Refill the Best cultures with
   - 135 ml PPM
   - [redacted] mL Cachiral solution
   - 7.5

Title = creation of high-density monocultures from protist collection

Date = Monday 11th April 2022

Aim: creating high-density monocultures from the best cultures in my personal collection

Steps: 1. Label autoclaved 500 ml bottles with two wheat seeds as follows:

ID: 51 Ble (38 ml from 23.2.22, 2 ml from 26.3.22)
ID: 52 Ble (40 ml from 26.3.22(1))
ID: 53 Cep (25.65 ml from 18.2.22, 21.85 ml from 26.3.22(2))
ID: 54 Cep (40 ml from 26.3.2022(2))
ID: 55 Eup
ID: 56 Eup (40 ml from mix 18.2.22 + 26.3.22)
ID: 57 Spi
ID: 58 Spi (40 ml from mix 16.2.22 + 26.3.22(1))
ID: 59 Spi te
ID: 60 Spi te (40 ml from mix 26.3.22(1) + 2.4.22)

2. Add 55 mL of PPM to the bottles 40 mL

3. Add protists from these monoculture stocks in my personal collection

Ble: 23.2.22, then 26.3.2022 (1)
Cep: 18.2.22, then 26.3.2022(2)
Eup: 18.2.22, then 26.3.2022(4)
Spi: 16.2.22, then 26.3.2022(1)
Spi te: 26.3.22(1), then 2.4.2022

mix culture on the right of the comma IN the one on the left on the comma

4. Bring to the Climate chamber (A73.2)



Title: Making protozoan pellet medium (PPM).

Date: Tuesday 12th April 2022

Aim: Making protozoan protist medium (PPM) using the protist pellet (Protozoa Pellet by Carolina) and then autoclaving it.

Steps:

1. **Label PPM bottles**
   (PPM EG Today's date)
2. **Gather material from A floor (Altermatt cupboard)**
   - ☑ Ground protozoan pellet (provided by Carolina, Biological Supply Company, Burlington NC, USA)
   - ☑ Spoon and tray to weight protist pellet
3. **Fill bottles with water** (in the Daphnia room, A65)
   Fill with cold water, which you let run for 60 seconds before.
4. **Add protist pellet to bottles** (Daphnia room, A65)
   Add 0.46 g of ground protozoan pellet/L
5. **Autoclave bottles (program: P6)**
6. Filter 7. Reautoclave (program: P6)

Litres of PPM made: 3

Title: creation of jars with PPM + demineralized water

Date: 12.4.2022 Tuesday

Aim: creating bottles with PPM and demineralized water.

## SMALL PERTURBATION

Before every nutrance exchange I'll add 0.73 ml of demineralized water + 0.175 ml of PPM.

$$\text{Percentage PPM} = \frac{0.175}{0.73+0.175} \cdot 100 = 19.3\%$$

$$\text{Percentage demineralized water} = \frac{0.73}{0.73+0.175} \cdot 100 = 80.7\%$$

⇓

Create small perturbation bottle with =

807 ml demineralized water
193 ml PPM

## LARGE PERTURBATION

Before every nutrance exchange I'll add 0.86 ml of demineralized water + 0.175 ml of PPM

$$\text{Percentage PPM} = \frac{0.175}{0.86+0.175} \cdot 100 = 16.9\%$$

$$\text{Percentage demineralized water} = \frac{0.86}{0.86+0.175} \cdot 100 = 83\%$$

⇓

Create large perturbation bottle with =

831 ml demineralized water
169 ml PPM



**Title:** training videos

**Date:** 12.4.22 Tuesday

**Aim:**

1. Selecting the best monocultures from which to assemble the experiment
2. Taking videos of the monocultures so that they can be used for training the BEMOVI

**Steps:**

1. **Check high-density monocultures**

## High-density monocultures control

| | | | |
|---|---|---|---|
| Ble (ID: 1) 200 ml | 3 | 17 | 8 → 9 |
| Ble (ID: 2) 200 ml | [illegible] | 0 | 3 → 2 |
| Ble (ID: 3) 200 ml | 11 | 7 | 6 → 8 |
| Cep (ID: 4) 200 ml | 16 | 15 | 23 → 18 |
| Cep (ID: 5) 200 ml | 25 | 18 | 15 → 19 |
| Cep (ID: 6) 200 ml | 4 | 3 | 4 → 4 |
| Col (ID: 7) 200 ml | 100 | 70 | 65 → 78 |
| Col (ID: 8) 200 ml | 100 | 60 | 60 → 73 |
| Col (ID: 9) 200 ml | 55 | 62 | 70 → 62 |
| Eug (ID: 10) 200 ml | 220 | 250 | 250 → 270 |
| Eug (ID: 11) 200 ml | 250 | 300 | 220 → 290 |
| Eug (ID: 12) 200 ml | 300 | 270 | 310 → 293 |
| Eup (ID: 13) 200 ml | 30 | 22 | 22 → 25 |
| Eup (ID: 14) 200 ml | 28 | 22 | 23 → 24 |
| Eup (ID: 15) 200 ml | 3 | 4 | 8 → 5 |
| Lox (ID: 16) 200 ml | 15 | 70 | 55 → 47 |
| Lox (ID: 17) 200 ml | 20 | 36 | 25 → 27 |
| Lox (ID: 18) 200 ml | 19 | 12 | 13 → 15 |
| Pau (ID: 19) 200 ml | 32 | 32 | 40 → 35 |
| Pau (ID: 20) 200 ml | 41 | 38 | 40 → 40 |
| Pau (ID: 21) 200 ml | 21 | 29 | 31 → 27 |
| Pca (ID: 22) 200 ml | 27 | 28 | 27 → 27 |
| Pca (ID: 23) 200 ml | 38 | 45 | 28 → 37 |
| Pca (ID: 24) 200 ml | 31 | 47 | 31 → 36 |
| Spi (ID: 25) 200 ml | 3 | 2 | 4 → 3 |
| Spi (ID: 26) 200 ml | 3 | 3 | 3 → 3 |
| Spi (ID: 27) 200 ml | 0 | 1 | 0 → 0 |
| Spi te (ID: 28) 200 ml | 7 | 0 | 7 → 8 |
| Spi te (ID: 29) 200 ml | 4 | 2 | 4 → 3 |
| Spi te (ID: 30) 200 ml | 5 | 16 | 12 → 11 |
| Tet (ID: 31) 200 ml | 29 | 37 | 32 → 33 |
| Tet (ID: 32) 200 ml | 34 | 39 | 21 → 31 |

| | | | | |
|---|---|---|---|---|
| Tet (ID: 33) 200 ml | 22 | 28 | 31 | → 27 |
| Tet (ID: 34) 100 ml | 52 | 33 | 32 | → 39 |
| Tet (ID: 35) 100 ml | 17 | | 15 | → 14 |
| Ble (ID: 36) 100 ml | 8 | 4 | 4 | → 5 |
| Ble (ID: 37) 100 ml | 6 | 9 | 3 | → 6 |
| Ble (ID: 38) 100 ml | 7 | 9 | 4 | → 7 |
| Cep (ID: 39) 100 ml | 15 | 10 | 17 | → 14 |
| Cep (ID: 40) 100 ml | 12 | 5 | 13 | → 10 |
| Cep (ID: 41) 100 ml | 11 | 16 | 10 | → 12 |
| Eup (ID: 42) 100 ml | 20 | 16 | 13 | → 16 |
| Eup (ID: 43) 100 ml | 12 | 13 | 15 | → 13 |
| Eup (ID: 44) 100 ml | 16 | 7 | 15 | → 13 |
| Spi (ID: 45) 100 ml | 0 | 0 | 0 | → 0 |
| Spi (ID: 46) 100 ml | 1 | 0 | 1 | → 1 |
| Spi (ID: 47) 100 ml | 3 | 1 | 0 | → 1 |
| Spi te (ID: 48) 100 ml | 4 | 7 | 3 | → 5 |
| Spi te (ID: 49) 100 ml | 5 | 3 | 5 | → 4 |
| Spi te (ID: 50) 100 ml | 6 | 8 | 8 | → 7 |
| Ble (ID: 51) 100 ml | 6 | 10 | 8 | → 8 |
| Ble (ID: 52) 100 ml | 5 | 6 | 6 | → 6 |
| Cep (ID: 53) 100 ml | 47 | 52 | 52 | → 50 |
| Cep (ID: 54) 100 ml | 48 | 40 | 40 | → 43 |
| Eup (ID: 55) 100 ml | 25 | 27 | 30 | → 27 |
| Eup (ID: 56) 100 ml | 30 | 30 | 24 | → 28 |
| Spi (ID: 57) 100 ml | 3 | 3 | 2 | → 3 |
| Spi (ID: 58) 100 ml | 3 | 4 | 4 | → 4 |
| Spi te (ID: 59) 100 ml | 8 | 20 | 17 | → 15 |
| Spi te (ID: 60) 100 ml | 15 | 11 | 9 | → 12 |

2. **Create the experimental monocultures.**
   Mix into the best culture the second best until having 220 ml (if the first and second best are only 100 ml, then add also from the third best).
3. **Transfer 1 ml of each culture into a 2 ml microtube**
4. **Set up microscope and computer (see Altermatt lab protocol nr 18)**
   Frames/second = 25, Total frames = 125, delay between frames = 40 msec
5. **Take videos of the cultures (Microscope = Volterra, Camera = *Hamamatsu Orca Flash 4*)** (AT LEAST 100 INDIVIDUALS/SPECIES CAPTURED ON VIDEO)
6. **Transfer videos to the hard drive.**
   a. Write the time and that the videos have been taken into the description file
   b. Copy the video folder Desktop -> Ema -> PatchSizePilot -> training into the hard drive "EMA 2" in the folder PatchSizePilot
   c. Repeat for the hard drive "EMA 1"
7. **Analyse videos in the hard drive "EMA 2"**
   a. Download the file "Felix_script.R" from https://github.com/Emanuele-Giacomuzzo/bemovi/blob/master/Felix_script.R and save it in the "EMA 2" hard drive in the folder PatchSizePilot -> training
   b. Set working directory to the source file location

c. Run Felix script



BEST (IND/DROP)[ml], SECOND BEST (IND/DROP)[ml], THIRD BEST (IND/DROP)[ml]

| | | | |
|---|---|---|---|
| = Ble | 1 (9) [200] | 3 (8) [200] | |
| ↑ Cep | 53 (50) [100] | 54 (43) [100] | 5 (19) [200] |
| ↑ Col | 7 (78) [200] | 8 (73) [200] | |
| ↑ Eug | 12 (293) [200] | 11 (290) [200] | |
| ↓ Eup | 56 (28) [100] | 55 (27) [100] | 13 (25) [200] |
| = Lox | 16 (97) [200] | 17 (27) [200] | |
| ↑ Pam | 20 (40) [200] | 19 (35) [200] | |
| ↑ Pca | 23 (32) [200] | 24 (36) [200] | |
| ↓ Spi | 58 (4) [100] | 26 (3) [200] | |
| ↓ Spi k | 59 (15) [100] | 60 (12) [100] | 30 (11) [200] |
| ↑ Tet | 34 (39) [200] | 31 (33) [200] | |

| SPECIES | FILE_NR | INDIVIDUALS | |
|---|---|---|---|
| BLE | 1 | 2 | |
| BLE | 2 | 5 | |
| | 3 | 6 | |
| | 4 | 8 | |
| | 5 | 3 | |
| | 6 | 4 | |
| | 7 | 5 | |
| | 8 | 3 | |
| | 9 | 5 | Tot = 41 |
| | 10 | 6 | 47 |
| | 11 | 7 | 54 |
| | 12 | 8 | 62 |
| | 13 | 5 | 67 |
| | 14 | 7 | 74 |
| | 15 | 1 | 75 |
| | 16 | 7 | 82 |
| | 17 | 4 | 86 |
| | 18 | 4 | 90 |
| | 19 | 2 | 92 |
| | 20 | 4 | 96 |
| | 21 | 5 | 101 |

| | | |
|---|---|---|
| CEP | 22 | 20 |
| " | 23 | 17 |
| | 24 | 20 |
| " | 25 | 25 |
| | 26 | 30 |
| COL | 27 | 30 |
| " | 28 | 30 |
| " | 29 | 30 |
| | 30 | 30 |
| EUG | 31 | 50 |
| " | 32 | 50 |
| " | 33 | 50 |
| EUP | 34 | 22 |
| " | 35 | 22 |
| | 36 | 25 |
| | 37 | 20 |
| | 38 | 20 |
| Lox | 39 | 15 |
| | 40 | 15 |
| " | 41 | 20 |
| | 42 | 20 |
| | 43 | 20 |
| | 44 | 20 |
| Pan | 45 | 17 |
| | 46 | 17 |
| " | 47 | 17 |
| | 48 | 17 |
| | 49 | 17 |
| | 50 | 17 |
| | 51 | 17 |
| | 52 | 17 |
| Pco | 53 | 17 |
| | 54 | 17 |
| | 55 | 15 |
| | 56 | 13 |



| | | | |
|---|---|---|---|
| " | 57 | 15 | |
| | 58 | 15 | |
| | 59 | 20 | |
| Spi | 60 | 2 | |
| | 61 | 2 | |
| | 62 | 2 | |
| | 63 | 3 | |
| | 64 | 3 | |
| " | 65 | 2 | |
| | 66 | 4 | |
| | 67 | 2 | |
| | 68 | 1 | |
| | 69 | 3 | 29 |
| | 70 | 2 | 26 ← delete (can see borders) |
| | 71 | 1 | 27 |
| | 72 | 4 | 31 |
| | 73 | 4 | 35 |
| | ~~74~~ | ~~4~~ | ~~39~~ |
| | 74 | 3 | 38 |
| | 75 | 3 | 41 |
| Spi te | 76 | 5 | |
| | 77 | 7 | |
| | 78 | 2 | |
| | 79 | 6 | |
| " | 80 | 6 | 26 |
| | 81 | 5 | 29 |
| | 82 | 6 | 32 |
| | 83 | 3 | 40 |
| | 84 | 6 | 46 |
| | 85 | 8 | 54 |
| | 86 | 4 | 58 |
| | 87 | 7 | 62 |
| | 88 | 8 | 70 |
| | 89 | 5 | 75 |
| | 90 | 5 | 80 |

| | | | |
|---|---|---|---|
| Sy | 91 | 7 | |
| | 92 | 10 | |
| Tet | 93 | 20 | |
| | 94 | 20 | |
| | 95 | 20 | |
| | 96 | 20 | |
| | 97 | 20 | |
| | 98 | 20 | |
| Spl | 99 | 3 | 42 |
| | 100 | 3 | 45 |
| | 101 | 2 | 47 |
| | 102 | 2 | 49 |
| | 103 | 2 | 51 |
| | 104 | 2 | 53 |
| | 105 | 3 | 56 |
| | 106 | 7 | 63 |
| | 107 | 3 | 66 |
| | 108 | 1 | 67 |
| | 109 | 6 | 73 |
| | 110 | 3 | 76 |
| | 111 | 2 | 78 |
| | 112 | 3 | 81 |
| | 113 | 5 | 86 |
| | 114 | 3 | 89 |
| | 115 | 3 | 92 |
| | 116 | 5 | 97 |
| | 117 | 3 | |



Title: assembly of the experiment.

Date: Wednesday 13th of April 2022

Aim: assembling the experiment.

Steps:

1. **Bring experimental high-density monocultures to the Protista Lab**
   Bring upstairs the experimental high-density monocultures you will use to create the mixed cultures
2. **Mix monocultures together**
   Mix 200 ml from each of the experimental monocultures together into a 5 L autoclaved Schott bottle.
   (Autoclaved measuring cylinder: 220 ml x1)
3. **Add 600 ml of filtered PPM to the 5 L bottle**
4. **Separate between small cultures, medium cultures and large cultures**

SMALL (7.5 ml):
1-5
16-25
46, 48, 50, 52, 54
56-60
71-80
101, 103, 105, 107, 109

MEDIUM (22.5 ml):
6-10
26-35
61-65
81-90

LARGE (37.5 ml)
11-15
36-45
47, 49, 51, 53, 55
66-70
91-100
102, 104, 106, 108, 110

5. **Micropipette from the large bottle into the experimental cultures**
   a. Add 7.5 ml to the small cultures
   b. Add 22.5 ml to the medium cultures
   c. Add 37.5 ml to the large cultures
6. **Randomise cultures on foamboard**
7. **Bring foamboard to the climate chamber (A73.2)**

Title: videos of t0

Date: Wednesday 13th of April 2022

Aim: taking videos of the experiment at t0

Steps:

1. **Set up microscope and computer (see Altermatt lab protocol nr 18)**
   Frames/second = 25, Total frames = 125, delay between frames = 40 msec
2. **Take 12 videos from the large bottle where you mixed all the protist cultures used for the experiment (Microscope = Volterra, Camera = *Hamamatsu Orca Flash 4*) (see Altermatt lab protocol nr. 18)**
3. **Transfer videos to the hard drive**
   a. Write time
      Write time at which videos have been taken into the description file
   b. Copy into EMA 2
      Copy the video folder Desktop -> Ema -> PatchSizePilot -> t0 into the hard drive "EMA 2" in the folder PatchSizePilot
   c. Copy into EMA 1
      Repeat for the hard drive "EMA 1"

Title: evaporation test

Date: Thursday 14th of April 2022

Aim: measuring how much water evaporates from the falcon tubes during the resource exchange.

1. Label the falcon tubes

**Small perturbation**

5.25ml
2. Weight the tubes with and without water.
   *For each falcon tube:*
   a. Weight the falcon tube
   b. Add **5.25** ml of deionised water
   c. Reweight the falcon tube
3. Microwave all the 15 falcon tubes on the rack (3 minutes)
4. Tare a Becker
5. Weight the water poured into the Becker
6. Cool down and dry falcon tubes
7. Repeat point 2-6 for a total of three times

**Large perturbation**

8. Weight the tubes with and without water.
   *For each falcon tube:*
   a. Weight the falcon tube
   b. Add **6.75** ml of deionised water
   c. Reweight the falcon tube
9. Microwave all the 15 falcon tubes on the rack (3 minutes)
10. Tare a Becker
11. Weight the water poured into the Becker
12. Cool down and dry falcon tubes
13. Repeat point 8-12 for a total of three times

Falcon tube disposition on rack:

| | | | | |
|---|---|---|---|---|
| 1 | 2 | 3 | 4 | 5 |
| 6 | 7 | 8 | 9 | 10 |
| 11 | 12 | 13 | 14 | 15 |



## Small perturbation (5.25 ml)

### Rack 1

| Falcon tube | FALCON TUBE g (empty) | FALCON TUBE g (with water) | Weight water (measured in Becker) AFTER MICROWAVING |
|---|---|---|---|
| 1 | 12.97 | 18.11 | 3.85 |
| 2 | 14.53 | 19.66 | 4.11 |
| 3 | 14.41 | 19.53 | 4.32 |
| 4 | 14.50 | 19.67 | 3.99 |
| ~~4~~ | ~~13.05~~ | | |
| 5 | 13.05 | 18.16 | 4.01 |
| 6 | 13.07 | 18.27 | 3.97 |
| 7 | 13.11 | 18.25 | 3.36 |
| 8 | 13.12 | 18.22 | 4.26 |
| 9 | 14.65 | 19.73 | 3.80 |
| 10 | 14.52 | 19.61 | 3.85 |
| 11 | 13.12 | 18.22 | 2.86 |
| 12 | 13.11 | 18.23 | 2.89 |
| 13 | 14.64 | 19.79 | 4.13 |
| 14 | 13.03 | 18.15 | 2.96 |
| 15 | 13.10 | 18.24 | 3.88 |

### Rack 2

| Falcon tube | FALCON TUBE g (empty) | FALCON TUBE g (with water) | Weight water (measured in Becker) AFTER MICROWAVING |
|---|---|---|---|
| 1 | 13.52 | 18.54 | 4.07 |
| 2 | 15.19 | 20.02 | 4.20 |
| 3 | 15.26 | 20.09 | 4.39 |
| 4 | 13.61 | 18.49 | 3.41 |
| ~~4~~ | | | |
| 5 | 13.69 | 18.56 | 7.48-3.41= |
| 6 | 13.65 | 18.45 | 3.80 |
| 7 | 13.62 | 18.46 | 4.03 |
| 8 | 15.01 | 19.87 | 4.35 |
| 9 | 13.69 | 18.58 | 4.10 |
| 10 | 13.61 | 18.30 | 4.00 |
| 11 | 13.62 | 18.49 | 3.87 |
| 12 | 13.68 | 18.63 | 3.80 |
| 13 | 15.03 | 20.00 | 4.19 |
| 14 | 14.89 | 19.88 | 3.78 |
| 15 | 15.04 | 19.95 | 4.03 |

## Rack 3

| Falcon tube | FALCON TUBE g (empty) | FALCON TUBE g (with water) | Weight water (measured in Becker) |
|---|---|---|---|
| 1 | 13.47 | 18.39 | 4.47 |
| 2 | 14.91 | 19.87 | 8.79 - 4.47 = |
| 3 | 14.16 | 19.09 | 4.49 |
| 4 | 13.00 | 18.03 | 4.44 |
| 4 | | | |
| 5 | 13.63 | 18.57 | 4.45 |
| 6 | 14.13 | 18.96 | 8.55 - 4.45 = |
| 7 | 15.24 | 20.12 | 4.56 |
| 8 | 14.61 | 19.58 | 5.08 |
| 9 | 14.53 | 19.43 | 4.97 |
| 10 | 13.62 | 18.51 | 4.37 |
| 11 | 14.83 | 19.70 | 8.82 - 4.37 = |
| 12 | 16.11 | 21.01 | 4.30 |
| 13 | 15.87 | 20.87 | 4.48 |
| 14 | 15.99 | 20.86 | 2.43 |
| 15 | 16.15 | 21.18 | 4.47 |



## Large perturbation (6.75 ml)

## Rack 1

| Falcon tube | FALCON TUBE g (empty) | FALCON TUBE g (with water) | Weight water (measured in Becker) |
|---|---|---|---|
| 1 | 15.08 | 21.54 | 5.31 |
| 2 | 15.68 | 22.10 | 5.88 |
| 3 | 13.53 | 20.04 | 5.13 |
| 4 | 13.66 | 20.01 | 5.76 |
| 4 | | | |
| 5 | 13.72 | 20.25 | 5.99 |
| 6 | 13.69 | 20.11 | 5.52 |
| 7 | 15.09 | 21.62 | 5.85 |
| 8 | 15.15 | 21.67 | 6.32 |
| 9 | 13.71 | 20.24 | 6.19 |
| 10 | 14.40 | 20.81 | 12.49 - 6.19 = |
| 11 | 14.70 | 21.18 | 5.22 |
| 12 | 13.51 | 20.00 | 5.54 |
| 13 | 13.53 | 20.01 | 4.96 |
| 14 | 14.84 | 21.33 | 10.33 - 4.96 = |
| 15 | 13.50 | 20.00 | 5.52 |

## Rack 2

| Falcon tube | FALCON TUBE g (empty) | FALCON TUBE g (with water) | Weight water (measured in Becker) |
|---|---|---|---|
| 1 | 14.03 | 20.50 | 5.82 |
| 2 | 13.56 | 20.02 | 5.87 |
| 3 | 15.38 | 21.46 | 5.38 |
| 4 | 15.03 | 21.38 | 6.53 |
| 4 | | | |
| 5 | 14.95 | 21.41 | 5.47 |
| 6 | 15.14 | 21.43 | 6.24 |
| 7 | 14.07 | 20.02 | 5.71 |
| 8 | 13.46 | 19.78 | 5.80 |
| 9 | 13.53 | 19.94 | 5.84 |
| 10 | 13.43 | 19.86 | 6.45 |
| 11 | 15.23 | 21.16 | 4.54 |
| 12 | 13.49 | 19.06 | 4.87 |
| 13 | 13.48 | 20.08 | 5.37 |
| 14 | 15.13 | 21.62 | 5.51 |
| 15 | 13.59 | 20.05 | 5.15 |

## Rack 3

| Falcon tube | FALCON TUBE g (empty) | FALCON TUBE g (with water) | Weight water (measured in Becker) |
|---|---|---|---|
| 1 | 19.76 | 21.26 | 4.74 |
| 2 | 13.40 | 19.92 | 5.34 |
| 3 | 13.62 | 20.11 | 6.00 |
| 4 | 15.10 | 21.64 | 11.81-6= |
| ~~4~~ | | | |
| 5 | 15.22 | 21.73 | 5.45 |
| 6 | 13.42 | 19.87 | 5.36 |
| 7 | 13.53 | 19.90 | 5.70 |
| 8 | 13.60 | 20.07 | 6.02 |
| 9 | 13.57 | 19.87 | 5.73 |
| 10 | 15.17 | 21.61 | 5.76 |
| 11 | 15.11 | 21.63 | 10.91-5.26= |
| 12 | 14.96 | 21.46 | 5.60 |
| 13 | 13.38 | 18.90 | 4.95 |
| 14 | 13.34 | 19.84 | 10.18 - 4.95= |
| 15 | 13.48 | 19.89 | 5.43 |



Title: Making protozoan pellet medium (PPM).

Date: Friday 15th of April 2022

Aim: Making protozoan protist medium (PPM) using the protist pellet (Protozoa Pellet by Carolina) and then autoclaving it. The PPM is filtered so that there's less background noise in the videos and can be analysed in shorter time.

Steps:

1. **Label PPM bottles**
   (PPM EG Today's date)
2. **Gather material from A floor (Altermatt cupboard)**
   - ☐ Ground protozoan pellet (provided by Carolina, Biological Supply Company, Burlington NC, USA)
   - ☐ Spoon and tray to weight protist pellet
3. **Fill bottles with water** (in the Daphnia room, A65)
   Fill with cold water, which you let run for 60 seconds before.
4. **Add protist pellet to bottles** (Daphnia room, A65)
   Add 0.46 g of ground protozoan pellet/L
5. **Autoclave bottles** ~~(program: P6)~~ SMALL AUTOCLAVE = P4
6. **Filter PPM**
   Place funnel on the top of the bottle with inside a filter (Folded filter, Whatman, 10311651) and then pour autoclaved PPM. Change filter when PPM can't run through anymore.
7. **Add "filtered" on the bottle labels**
8. **Autoclave filtered PPM bottles** ~~(Program: P6)~~ SMALL AUTOCLAVE = P4

Litres of PPM made: 2

Title: bottle with PPM & deionised water.

Date: ~~Friday 15th~~ SUNDAY 17th of April 2022

Aim: creating a bottle with PPM and deionised water that I can use to refill the cultures before the resource exchange.

Steps:

1. Gather material
   - 2x autoclaved 1 litre bottles
   - 500 ml filtered PPM (12.4.22)
   - 2 L autoclaved deionised water
2. Label bottles
3. To each bottle add 833.3 ml autoclaved deionised water
   (measuring cylinder: 250 ml x3
   measuring cylinder: 80 ml x1
   10 ml micropipette: 3.3 ml x1)
4. To each bottle add 166.7 ml of filtered PPM
   (measuring cylinder: 160 ml x1
   10 ml micropipette: 6.7 ml x1)

Calculations:

Each resource exchange events we add 1 ml deionised water + 0.2 ml filtered PPM.
Therefore, when adding water and PPM, they are in the following relative concentration:

Water percentage = (1 ml / 1 ml + 0.2 ml) * 100 = 83.33%
PPM percentage = (0.2 ml / 1 ml + 0.2 ml) * 100 = 16.67%

Therefore, we should make a 1 L bottle with:

Water volume = 1 L * 0.8333 = 833.3 ml
PPM volume = 1 L * 0.1667 = 166.7 ml



Title: videos

Date: SUNDAY 17th APRIL 2022

Aim: taking videos of the experiment at t1 (time point)

Steps:

1. **Transfer 0.2 ml of each culture into a 2 ml Eppendorf microtube (in the climate chamber) where the microtube rack mirrors the foamboard** (set micropipette to 0.2 ml)n (spin the cultures) (3 h)
2. **Reorder the tubes from 1 to 110** (10 min)
3. **Set up microscope and computer (see Altermatt lab protocol nr 18)**
   Frames/second = 25, Total frames = 125, delay between frames = 40 msec
4. **Take videos of the cultures (Microscope = Volterra, Camera = *Hamamatsu Orca Flash 4*) (see Altermatt lab protocol nr. 18)** (every 10 videos check that you took the right number of videos)
5. **Transfer videos to the hard drive**
   a. Write time
      Write time at which videos have been taken into the description file
   b. Copy into EMA 2
      Copy the video folder Desktop -> Ema -> PatchSizePilot -> t1 into the hard drive "EMA 2" in the folder PatchSizePilot
   c. Copy into EMA 1
      Repeat for the hard drive "EMA 1"

- When going down with the cart =
  2x Blue tip boxes + micropipette
  Eppendorf racks (w/ and w/o tubes)
  Ethanol + paper
  Extra tubes
  Waste Beaker

- Video 22: I first had the door sliding under the metal, but then I managed to get a video from a door not sliding under the metal (I recreated the door).
  also video 47

- Video 68: I had to take out some debris that otherwise would have covered the whole video

Title: resource exchange 1

Date: Monday 18th April 2022

Aim: exchanging resources between ecosystems by microwaving.

Steps:

9AM **1. Bring cultures up** (close lids) incubator (in A61)
Bring experimental cultures from the ~~climate chamber (A73.2)~~ to the Protista lab (G74) and dispose them in order on the falcon tube racks

9:25 **2. Add deionised water + PPM**
Add 1.2 l (1 ml deionised water + 0.2 ml PPM) to all cultures
(10 ml micropipette: 1.2)

10:25 **3. Pipette from cultures to microwaving tubes** (flip tubes)

2 h

a. Green tubes (small perturbation)
Pipette 5.25 ml from the cultures to tubes that will be microwaved (e.g., from culture 1 to microwaving tube nr 1, from culture 2 to microwaving tube nr 2). After you have micropipetted between two tubes, move them to empty racks (an empty rack for cultures and an empty rack for microwaving tubes).
(10 ml micropipette: 5.25)

b. Red tubes (large perturbation)
Same as in previous point, but pipetting 6.75 ml
(10 ml micropipette: 6.75)

13:30 **4. Bring cultures back**
Bring cultures to the climate chamber (A73.2, 20 °C) (close lids) and rearrange them in random order on the foam boards (open lids)

14:45 **5. Microwave** IN BATCHES OF 15
Microwave the microwaving tubes for 3 minutes (open lids ¾)

3h30m

15:35 **6. Let tubes cool down**
Wait for 1 hours and 15 minutes that the tubes have cooled down.

17:05 **7. Bring microwaving tubes to the incubator room (A61)** (close lids)

12:10 **8. Pour cultures**
Pour microwaved material into the corresponding number (where the arrow points at – if there's no arrow, pour back into the same number) (check openness of lids)

19:15 **9. Wash and dry microwaving tubes**
Wash microwaving tubes with distilled water and let them dry on a rack. Let the caps dry on a tray with paper towels with their number up.



- Exchanging resources 1-3 I forgot to ~~put~~ flip the tubes, so I repeat the culture 1 each and redone it. Culture 2 and 3 I ~~did~~ flip the tubes.
- When pouring 60 each I spilled it out
- The liquid ~~going into 105 was really little~~ coming from tubes 106-110 seems to be little when pouring back

Title: evaporation test for only 5 tubes

Date: Wednesday 20th April 2022

Aim: testing how much water evaporates in 5 tubes if they are in the microwave with tubes with or without water.

Steps:

WITHOUT WATER IN OTHER TUBES

1. Weight tubes with and without water

| Falcon tube | g falcon tube | g falcon tube |
|---|---|---|
| 1 | 14.46 | 21.15 + water (6.75 ml) |
| 2 | 13.08 | 19.80 |
| 3 | 14.50 | 21.17 |
| 4 | 13.05 | 19.73 |
| 5 | 13.12 | 19.77 |

2. Dispose on a rack with the 10 empty falcon tubes
3. Microwave rack for 3 minutes
4. Take a Becher
5. Weight the remaining water from the Tubes

| Falcon tube | g water left |
|---|---|
| 1 | 2.10 |
| 2 | 1.97 |
| 3 | 2.01 |
| 4 | 1.61 |
| 5 | 2.20 |



WITH WATER IN OTHER TUBES

As in point 1-5. This time have microwave the 5 tubes with the other tubes on the rack filled with 6.75 g of water.

| Tube | g tube | g tube + water | water left g |
|---|---|---|---|
| 1 | 14.60 | 21.20 | 4.82 |
| 2 | 14.68 | 21.33 | 5.56 |
| 3 | 13.24 | 19.83 | 5.07 |
| 4 | 13.18 | 19.80 | 5.04 |
| 5 | 13.23 | 19.84 | 4.80 |

(See excel sheet)

Difference in grams evaporated with or without water in the other tubes = 3.15 g

Title: videos

Date: Thursday 21st April 2022

Aim: taking videos of the experiment at $t_2$ (time point)

Steps:

1. **Transfer 0.2 ml of each culture into a 2 ml Eppendorf microtube (in the climate chamber) where the microtube rack mirrors the foamboard** (set micropipette to 0.2 ml)n (flip the cultures)
2. **Reorder the tubes from 1 to 110**
3. **Set up microscope and computer (see Altermatt lab protocol nr 18)**
   Frames/second = 25, Total frames = 125, delay between frames = 40 msec
4. **Take videos of the cultures (Microscope = Volterra, Camera = *Hamamatsu Orca Flash 4*) (see Altermatt lab protocol nr. 18)** (every 10 videos check that you took the right number of videos)
5. **Transfer videos to the hard drive**
   a. Write time
      Write time at which videos have been taken into the description file
   b. Copy into EMA 2
      Copy the video folder Desktop -> Ema -> PatchSizePilot -> $t_2$ into the hard drive "EMA 2" in the folder PatchSizePilot
   c. Copy into EMA 1
      Repeat for the hard drive "EMA 1"



Title: resource exchange 2

Date: Friday 22nd April 2022

Aim: exchanging resources between ecosystems by microwaving.

Steps:

1. **Bring cultures up** (close lids)
   Bring experimental cultures from the climate chamber (A73.2) to the Protista lab (G74) and dispose them in order on the falcon tube racks *
2. **Add deionised water + PPM**
   Add 1.2 l (1 ml deionised water + 0.2 ml PPM) to all cultures
   (10 ml micropipette: 1.2)
3. **Pipette from cultures to microwaving tubes** (flip tubes)
   a. Green tubes (small perturbation)
      Pipette 5.25 ml from the cultures to tubes that will be microwaved (e.g., from culture 1 to microwaving tube nr 1, from culture 2 to microwaving tube nr 2). After you have micropipetted between two tubes, move them to empty racks (an empty rack for cultures and an empty rack for microwaving tubes).
      (10 ml micropipette: 5.25)
   b. Red tubes (large perturbation)
      Same as in previous point, but pipetting 6.75 ml
      (10 ml micropipette: 6.75)
4. **Bring cultures back**
   Bring cultures to the climate chamber (A73.2, 20 °C) (close lids) and rearrange them in random order on the foam boards (open lids)
5. **Microwave** IN BATCHES OF 15
   Microwave the microwaving tubes for 3 minutes (open lids ¼)
6. **Let tubes cool down**
   Wait for 1 hours and 15 minutes that the tubes have cooled down.
7. **Bring microwaving tubes to the incubator room (A61)** (close lids)
8. **Pour cultures**
   Pour microwaved material into the corresponding number (where the arrow points at – if there's no arrow, pour back into the same number) (check openness of lids)
9. **Wash and dry microwaving tubes**
   Wash microwaving tubes with distilled water and let them dry on a rack. Let the caps dry on a tray with paper towels with their number up.

* top up 106 - 100 with 3.15 ml of water

Culture 56: I pipetted out almost all culture. I also don't remember if I flipped the culture. Also: 57

Culture 60: the culture had less than 6.75 mL
105



Title: videos

Date: Monday 25th April 2022

Aim: taking videos of the experiment at t3 (time point)

Steps:

9:45 1. **Transfer 0.2 ml of each culture into a 2 ml Eppendorf microtube (~~in the climate chamber~~) where the microtube rack mirrors the foamboard** (set micropipette to 0.2 ml)n (flip the cultures) One foam board /tube

2. **Reorder the tubes from 1 to 110**

10:10 3. **Set up microscope and computer (see Altermatt lab protocol nr 18)**
Frames/second = 25, Total frames = 125, delay between frames = 40 msec

4. **Take videos of the cultures (Microscope = Volterra, Camera = *Hamamatsu Orca Flash 4*) (see Altermatt lab protocol nr. 18)** (every 10 videos check that you took the right number of videos)

5. **Transfer videos to the hard drive**
   a. Write time
   Write time at which videos have been taken into the description file
   b. Copy into EMA 2
   Copy the video folder Desktop -> Ema -> PatchSizePilot -> t4 into the hard drive "EMA 2" in the folder PatchSizePilot
   c. Copy into EMA 1
   Repeat for the hard drive "EMA 1"

Title: resource exchange 3

Date: Tuesday 26th April 2022

Aim: exchanging resources between ecosystems by microwaving.

Steps:

1. **Bring cultures up** (close lids)
   Bring experimental cultures from the climate chamber (A73.2) to the Protista lab (G74) and dispose them in order on the falcon tube racks
2. **Add deionised water** 9-11:30
   Add deionised water the to the cultures until they reach the original culture volume (7.5, 22.5, or 37.5 ml)
3. **Pipette from cultures to microwaving tubes** (flip tubes)
   a. Green tubes (small perturbation)
      Pipette 5.25 ml from the cultures to tubes that will be microwaved (e.g., from culture 1 to microwaving tube nr 1, from culture 2 to microwaving tube nr 2). After you have micropipetted between two tubes, move them to empty racks (an empty rack for cultures and an empty rack for microwaving tubes).
      (10 ml micropipette: 5.25)
   b. Red tubes (large perturbation)
      Same as in previous point, but pipetting 6.75 ml
      (10 ml micropipette: 6.75)
4. **Bring cultures back**
   Bring cultures to the climate chamber (A73.2, 20 °C) (close lids) and rearrange them in random order on the foam boards (open lids)
5. **Microwave** IN BATCHES OF 15
   Microwave the microwaving tubes for 3 minutes (open lids ¼) (make of three positions e.g. tube 1 in position 3)
6. **Let tubes cool down**
   Wait for 1 hours and 15 minutes that the tubes have cooled down.
7. ~~**Bring microwaving tubes to the incubator room (A61)** (close lids)~~
8. **Pour cultures**
   Pour microwaved material into the corresponding number (where the arrow points at – if there's no arrow, pour back into the same number) ~~(check openness of lids)~~
9. **Bring cultures up** (close lids)
   Bring experimental cultures from the climate chamber (A73.2) to the Protista lab (G74)
10. **Add ~~PPM, then~~ deionised water**
    Add ~~0.2 ml of PPM and~~ enough deionised water to reach the original culture volume (7.5, 22.5, or 37.5 ml) (1 ml micropipette: 0.2)
11. **Wash and dry microwaving tubes**
    Wash microwaving tubes with distilled water and let them dry on a rack. Let the caps dry on a tray with paper towels.

POINT 2 =



| Culture | Deionised water added (ml) | |
|---|---|---|
| 30 | 1.4 | |
| 37 | 2 | |
| 92 | 2.5 | |
| 103 | 2.2 | |
| 62 | 0.25 ×6 | |
| 20 | 2.0 | |
| 105 | 2.5 | |
| 71 | 2 | |
| 76 | 3 | |
| 15 | 2 | |
| 56 | 3.5 | |
| 54 | 1.5 | |
| 86 | 1 | |
| 38 | 1.5 ← | double water add |
| 32 | 1 ← | |
| 3 | 1 ← | |
| 104 | 3 ← | |
| 38 | 0.5 ← | |
| 32 | 0.5 ← | |
| 3 | 0 ← | |
| 107 | 0 ← | |
| 65 | 2 | |
| 25 | 2 | |
| 70 | 2 | |
| 91 | 2.5 | |
| 69 | 2 | |
| 79 | 2 | |
| 35 | 2.5 | |
| 109 | 2 | |
| 27 | 4 | |

Started : 9 AM

| | |
|---|---|
| 102 | 3 |
| 97 | 2 |
| 90 | 1.5 |
| 63 | 2.5 |
| 87 | 1.5 |
| 59 | 2.5 |
| 13 | 2.5 |
| 21 | 2 |
| 23 | 2.5 |
| 82 | 2.5 |
| 34 | 2.5 |
| 70 | 3.5 |
| 73 | 2 |
| 11 | 4.5 |
| 17 | 2 |
| 75 | 2 |
| 28 | 2.5 |
| 95 | 2.5 |
| 12 | 3.5 |
| 57 | 3.5 |
| 55 | 2 |
| 46 | 2 |
| 1 | 2.5 |
| 99 | 2.5 |
| 9 | 2 |
| 36 | 2 |
| 47 | 2 |
| 94 | 3 |



| | |
|---|---|
| 16 | 2 |
| 60 | 5 |
| 93 | 2.5 |
| 88 | 2.5 |
| 49 | 2 |
| 26 | 3.5 |
| 42 | 2.5 |
| 48 | 3 |
| 22 | 1 |
| 52 | 1 |
| 58 | 3 |
| 8 | 0.5 |
| 101 | 2.5 |
| 108 | 2 |
| 66 | 2.5 |
| 18 | 2.5 |
| 31 | 2.5 |
| 6 | 3 |
| 53 | 1.5 |
| 39 | 1 |
| 98 | 2 |
| 2 | 3 |
| 81 | 1.5 |
| 41 | 2.5 |
| 96 | 2 |
| 29 | 2.5 |
| 110 | 3 |
| 43 | 3.5 |
| 62 | 2 |
| 77 | 2.5 |

| | | |
|---|---|---|
| 19 | 1.5 | |
| 45 | 3.5 | |
| 72 | 3 | |
| 44 | 3.5 | |
| 68 | 2.5 | |
| 84 | 1.5 | |
| 64 | 2.5 | |
| 24 | 1.5 | ← double water add |
| 5 | 3 | |
| 61 | 2.5 | |
| 7 | 1.5 | |
| 89 | 2 | |
| 78 | 2.5 | |
| 33 | 3 | |
| 10 | 2.5 | |
| 80 | 2.5 | |
| 24 | 0 | ← double water add |
| 85 | 3 | |
| 107 | 2.5 | |
| 100 | 2 | |
| 14 | 3.5 | |
| 51 | 3 | |
| 83 | 1.5 | |
| 74 | 3 | |
| 50 | 1.5 | |
| 4 | 2 | |
| 106 | 1.5 | |

· When pipetting 1 Rougot to flip culture 1

POINT 10 =

Culture Desonnaz cofer added (ml)

| | | |
|---|---|---|
| 30 | 2 | 2 |
| 92 137 | 1.5 | |
| 103 | 2 | |
| 67 | 1.5 | |
| 20 | 2 | |
| 105 | 2 | |
| 71 | 2 | |
| 76 | 2 | |
| 15 | 2 | |
| 56 | 2.5 | |
| 54 | 1.5 | |
| 86 | 2.5 | |
| 38 | 2 | |
| 32 | 1 | |
| 3 | 2 | |
| 104 | 2 | |
| 65 | 1 | |
| 25 | 2.5 | |
| 70 | 2.5 | |
| 91 | 2 | |
| 69 | 1.5 | |
| 79 | 1.5 | |
| 35 | 1.5 | |
| 109 | 2.5 | |
| 27 | 2.5 | |



| | |
|---|---|
| 102 | 2 |
| 97 | 2 |
| 90 | 2 |
| 63 | 2 |
| 87 | 1.5 |
| 59 | 2.5 |
| 13 | 1.5 |
| 21 | 1 |
| 23 | 3 |
| 82 | 1 |
| 34 | 1 |
| 40 | 3 |
| 73 | 1.5 |
| 11 | 2 |
| 17 | 2 |
| 75 | 2 |
| 28 | 2 |
| 95 | 1.5 |
| 12 | 2.5 |
| 57 | 2.5 |
| 55 | 2.5 |
| 46 | 1.5 |
| 1 | 2.5 |
| 99 | 2 |
| 9 | 2 |
| 36 | 1.5 |
| 47 | 1.5 |
| 94 | 1 |

| | |
|---|---|
| 16 | 2 |
| 60 | 2 |
| 93 | 1.5 |
| 88 | 2 |
| 49 | 1.5 |
| 26 | 2.5 |
| 42 | 1.5 |
| 48 | 1.5 |
| 22 | 2 |
| 52 | 2 |
| 58 | 1.5 |
| 8 | 2.5 |
| 101 | 1.5 |
| 108 | 1.5 |
| 66 | 1 |
| 18 | 1 |
| 31 | 1 |
| 6 | 1 |
| 53 | 1 |
| 39 | 2 |
| 98 | 1.5 |
| 2 | 1.5 |
| 81 | 1.5 |
| 41 | 1.5 |
| 96 | 1 |
| 29 | 1 |
| 110 | 2 |
| 43 | 2 |
| 62 | 1.5 |
| 77 | 1.5 |

| | |
|---|---|
| 19 | 1 |
| 45 | 2 |
| 72 | 1.5 |
| 44 | 1 |
| 68 | 1.5 |
| 84 | 2 |
| 64 | 0.5 |
| 5 | 1 |
| 61 | 1.5 |
| 7 | 2 |
| 89 | 1.5 |
| 78 | 1.5 |
| 33 | 1.5 |
| 10 | 1 |
| 80 | 1 |
| 24 | 1 |
| 85 | 1 |
| 107 | 2.5 |
| 100 | 1 |
| 14 | 2.5 |
| 51 | 0.5 |
| 83 | 1.5 |
| 74 | 1.5 |
| 50 | 1 |
| 4 | 1 |
| 106 | 1.5 |

Title: topping up with PPM

Date: Thursday 28th ~~Wednesday 27th~~ of April 2022

Aim: topping up all cultures with 0.2 ml PPM

Steps:

1. **Bring cultures up** (close lids)
   Bring experimental cultures from the incubator (A61) to the Protista lab (G74)
2. **Add PPM** (Created : 15/9/22)
   Add 0.2 ml of PPM to all cultures
3. **Bring cultures back**
   Bring cultures to the incubator (A61) (close lids)



Title: videos

Date: Friday 28th April 2022

Aim: taking videos of the experiment at t4

Steps:

1. **Bring upstairs foam board** (close lids)
2. **Transfer 0.2 ml of each culture into a 2 ml Eppendorf microtube (in the climate chamber) where the microtube rack mirrors the foamboard** (set micropipette to 0.2 ml) (flip the cultures)
3. **Repeat steps 1-2 for each of the foam boards**
4. **Reorder the tubes from 1 to 110**
5. **Set up microscope and computer (see Altermatt lab protocol nr 18)**
   Frames/second = 25, Total frames = 125, delay between frames = 40 msec
6. **Take videos of the cultures (Microscope = Volterra, Camera = *Hamamatsu Orca Flash 4*) (see Altermatt lab protocol nr. 18)** (every 12 videos check that you took the right number of videos)
7. **Transfer videos to the hard drive**
   a. Write time
      Write time at which videos have been taken into the description file
   b. Copy into EMA 2
      Copy the video folder Desktop -> Ema -> PatchSizePilot -> t4 into the hard drive "EMA 2" in the folder PatchSizePilot
   c. Copy into EMA 1
      Repeat for the hard drive "EMA 1"

Title: resource exchange 4

Date: Saturday 30th April 2022

Aim: exchanging resources between ecosystems by microwaving.

Steps:

1. **Bring cultures up** (close lids)
   Bring experimental cultures from the incubator (A61) to the Protista lab (G74) and dispose them in order on the falcon tube racks
2. **Pipette from cultures to microwaving tubes** (flip tubes)
   a. Green tubes (small perturbation)
      Pipette 5.25 ml from the cultures to tubes that will be microwaved (e.g., from culture 1 to microwaving tube nr 1, from culture 2 to microwaving tube nr 2). After you have micropipetted between two tubes, move them to empty racks (an empty rack for cultures and an empty rack for microwaving tubes).
      (10 ml micropipette: 5.25)
   b. Red tubes (large perturbation)
      Same as in previous point, but pipetting 6.75 ml
      (10 ml micropipette: 6.75)

Add 0.2 ml PPM to all cultures

3. **Rearrange cultures in random order on foam boards**
4. **Bring cultures back to the incubator** (close lids)
5. **Microwave**
   Microwave the microwaving tubes for 1 minutes (open lids ¼) in batches of 4
6. **Let tubes cool down**
   Wait for 1 hours and 15 minutes that the tubes have cooled down.
7. **Bring cultures up** (close lids)
   Bring experimental cultures from the incubator (A61) to the Protista lab (G74)
8. **Pour cultures**
   Pour microwaved material into the corresponding number (where the arrow points at – if there's no arrow, pour back into the same number)
9. **Add ~~PPM and~~ deionised water**
   Add ~~0.2 ml of PPM and~~ enough deionised water to reach the original culture volume (7.5, 22.5, or 37.5 ml) (1 ml micropipette: 0.5)
10. **Wash and dry microwaving tubes**
    Wash microwaving tubes with distilled water and let them dry on a rack. Let the caps dry on a tray with paper towels.

Bring cultures back to A61



How much water I added in point 9 =

| Culture | Deionized water add (ml) | Culture | Deionized water add (ml) |
|---|---|---|---|
| 30 | / | 102 | / |
| 37 | / | 97 | / |
| 92 | / | 90 | 0.5 |
| 103 | / | 63 | / |
| 67 | / | 87 | 1.5 |
| 20 | / | 59 | 1 |
| 105 | 1.5 | 13 | 1 |
| 71 | 1.5 | 21 | 0.5 |
| 76 | 0.5 | 23 | 1 |
| 15 | 1.5 | 82 | 1 |
| 56 | 1.5 | 34 | 0.5 |
| 54 | / | 40 | 1 |
| 86 | / | 73 | 1.5 |
| 38 | / | 11 | 1.5 |
| 32 | / | 17 | 0.5 |
| 3 | 1.5 | 75 | 1.5 |
| 104 | 0.5 | 28 | 1 |
| 65 | / | 95 | 1 |
| 25 | 0.5 | 12 | 1 |
| 70 | / | 57 | 1 |
| 91 | 1.5 | 55 | 1 |
| 69 | / | 46 | 1 |
| 79 | / | 1 | 1 |
| 35 | 1.5 | 99 | 1 |
| 109 | / | 9 | 1 |
| 27 | 0.5 | 36 | 1 |
| | | 47 | 1 |
| | | 94 | 1.5 |

| | |
|---|---|
| 16 | 0.5 |
| 60 | 1 |
| 93 | 1 |
| 88 | 1.5 |
| 49 | 1 |
| 26 | 1 (1 put too much (should have put 0.5) |
| 42 | 1 |
| 48 | 0.5 |
| 22 | 0.5 |
| 52 | 0.5 |
| 58 | 1 |
| 8 | 1 |
| 101 | 1 |
| 108 | 1 |
| 66 | 0.5 |
| 18 | 1 |
| 31 | 1 |
| 6 | 1 |
| 53 | 1 |
| 39 | 1 |
| 98 | 1.5 |
| 2 | 1 |
| 81 | 1 |
| 41 | 1 |
| 96 | 1.5 |
| 29 | 1 (too much water) |
| 110 | 1.5 |
| 43 | 1 |
| 62 | 1 |
| 77 | 1 |



| | |
|---|---|
| 19 | 1.5 |
| 45 | 1 |
| 72 | 0.5 |
| 44 | 1 |
| 68 | 1 |
| 87 | 1 |
| 69 | 1 |
| 5 | 1 |
| 61 | 1 |
| 7 | 1.5 |
| 89 | 2 |
| 78 | 1 |
| 33 | 1 |
| 10 | 1.5 |
| 80 | 1 |
| 24 | 1 |
| 85 | 1 |
| 107 | 0.5 |
| 100 | 1 |
| 14 | 0.5 |
| 51 | 1 |
| 83 | 1 |
| 74 | 0.5 |
| 50 | 0.5 |
| 4 | 1 |
| 100 | 1 |

Title: videos

Date: Tuesday 3rd May 2022

Aim: taking videos of the experiment at t5

Steps: ✓ Bring cultures upstairs

1. **Transfer 0.2 ml of each culture into a 2 ml Eppendorf microtube ~~(in the climate chamber)~~ where the microtube rack mirrors the foamboard** (set micropipette to 0.2 ml)n (flip the cultures) ✓ Bring cultures back to incubator
2. ✓ **Reorder the tubes from 1 to 110**
3. ✓ **Set up microscope and computer (see Altermatt lab protocol nr 18)**
   Frames/second = 25, Total frames = 125, delay between frames = 40 msec
4. ✓ **Take videos of the cultures (Microscope = Volterra, Camera = *Hamamatsu Orca Flash 4*) (see Altermatt lab protocol nr. 18)** (every 12 videos – end of a microtube row – check that you took the right number of videos)
5. **Transfer videos to the hard drive**
   a. ✓ Fill time, date, and data point in the video description file
   b. Copy into EMA 2
      Copy the video folder Desktop -> Ema -> PatchSizePilot -> t5 into the hard drive "EMA 2" in the folder PatchSizePilot
   c. Copy into EMA 1
      Repeat for the hard drive "EMA 1"

Title: resource exchange 5

Date: Wednesday 4th May 2022

Aim: exchanging resources between ecosystems by microwaving.

Steps:

1/2h ✓ **1. Bring cultures up** (close lids)
Bring experimental cultures from the incubator (A61) to the Protista lab (G74) and dispose them in order on the falcon tube racks

1 1/2 h ✓ **2. Pipette from cultures to microwaving tubes** (flip tubes)

a. Green tubes (small perturbation)
Pipette 5.25 ml from the cultures to tubes that will be microwaved (e.g., from culture 1 to microwaving tube nr 1, from culture 2 to microwaving tube nr 2). After you have micropipetted between two tubes, move them to empty racks (an empty rack for cultures and an empty rack for microwaving tubes).
(10 ml micropipette: 5.25)

b. Red tubes (large perturbation)
Same as in previous point, but pipetting 6.75 ml
(10 ml micropipette: 6.75)

15 m ✓ **3. Rearrange cultures in random order on foam boards**

15 m ✓ **4. Bring cultures back to the incubator** (close lids)

1/2 h ✓ **5. Microwave**
Microwave the microwaving tubes for 1 minute (open lids ¼) in batches of 4

✓ **6. Let tubes cool down**
Wait for 1 hours and 15 minutes that the tubes have cooled down.

15 m **7. Bring cultures up** (close lids)
Bring experimental cultures from the incubator (A61) to the Protista lab (G74)

1 h 15 m ✓ **8. Pour cultures**
Pour microwaved material into the corresponding number (where the arrow points at – if there's no arrow, pour back into the same number)

2 h ✓ **9. Add deionised water**
Add enough deionised water to reach the original culture volume (7.5, 22.5, or 37.5 ml) (1 ml micropipette: 0.5)

30 m ✓ **10. Add PPM**
Add 0.2 ml of PPM to all cultures.

15 m ✓ **11. Bring cultures back to the incubator** (close lids)

15 m **12. Wash and dry microwaving tubes**
Wash microwaving tubes with distilled water and let them dry on a rack. Let the caps dry on a tray with paper towels.



Add deionised water

| Culture | Water (ml) | Culture | Water (ml) | Culture | Water (ml) | Culture | Water (ml) |
|---|---|---|---|---|---|---|---|
| 30 | 1.5 | 102 | 1.5 | 16 | 1 | 19 | 1.5 |
| 37 | 1 | 97 | 2 | 60 | 1 | 45 | 1 |
| 92 | 1.5 | 90 | 1.5 | 93 | 1.5 | 72 | 1.5 |
| 103 | 1.5 | 63 | 1 | 88 | 1 | 44 | 1 |
| 67 | 1.5 | 87 | 1 | 49 | 1.5 | 68 | 1.5 |
| 20 | 1 | 59 | 1 | 26 | 1 | 87 | 1.5 |
| 105 | 1 | 13 | 1.5 | 42 | 1.5 | 64 | 1 |
| 71 | 1 | 21 | 1 | 48 | 1.5 | 5 | 1 |
| 76 | 1.5 | 23 | 0.5 | 22 | 1.5 | 61 | 1.5 |
| 15 | 1.5 | 82 | 1 | 52 | 1.5 | 7 | 1.5 |
| 56 | 1.5 | 34 | 1.5 | 58 | 1.5 | 89 | 1 |
| 54 | 1 | 40 | 1.5 | 8 | 1.5 | 78 | 1 |
| 86 | 1 | 73 | 1 | 101 | 1.5 | 33 | 1.5 |
| 38 | 1.5 | 11 | 1.5 | 108 | 2 | 10 | 1.5 |
| 32 | 0.5 | 17 | 1 | 66 | 1.5 | 80 | 1.5 |
| 3 | 1 | 75 | 1 | 18 | 1 or 1.5 | 24 | 1.5 |
| 104 | 2 | 28 | 1 | 31 | 1.5 | 85 | 2 |
| 65 | 1.5 | 95 | 1 | 6 | 1.5 | 107 | 1.5 |
| 25 | 1.5 | 12 | 1.5 | 53 | 1.5 | 100 | 2 |
| 70 | 1.5 | 57 | 1.5 | 39 | 1.5 | 14 | 1.5 |
| 91 | 1 | 55 | 1 | 98 | 1.5 | 51 | 2 |
| 69 | 1.5 | 46 | 1 | 2 | 2 | 83 | 1.5 |
| 79 | 1.5 | 1 | 1.5 | 81 | 1.5 | 74 | 1.5 |
| 35 | 1 | 99 | 1.5 | 41 | 1.5 | 50 | 1.5 |
| 109 | 1.5 | 9 | 1.5 | 96 | 1.5 | 4 | 1.5 |
| 27 | 1.5 | 36 | 1 | 29 | 1.5 | 106 | 2 |
| | | 47 | 1.5 | 110 | 1.5 | | |
| | | 94 | 1.5 | 43 | 2 | | |
| | | | | 62 | 2 (1 should have put 0.5) | | |
| | | | | 77 | 1.5 | | |

Title = counting community abundance

Date = Thursday 5th May 2022

Aim = counting manually the community abundance in the cultures from the videos of t5

| Column 1: Culture | Column 2: Community abundance | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 2 | 28 | 21 | 55 | 18 | | 82 | 9 | 109 | 5 | |
| 2 | 2 | 29 | 14 | 56 | 0 | | 83 | 11 | 110 | 16 | |
| 3 | 5 | 30 | 21 | 57 | 2 | | 84 | 10 | | | |
| 4 | 5 | 31 | 8 | 58 | 2 | ok | 85 | 9 | | | |
| 5 | 3 | 32 | 16 | 59 | 0 | | 86 | 6 | | | |
| 6 | 5 | 33 | 14 | 60 | 2 | | 87 | 9 | | | |
| 7 | 6 | 34 | 21 | 61 | 6 | | 88 | 8 | | | |
| 8 | 7 | 35 | 16 | 62 | 6 | | 89 | 9 | | | |
| 9 | 8 | 36 | 15 | 63 | 12 | | 90 | 15 | | | |
| 10 | 6 | 37 | 25 | 64 | 9 | | 91 | 16 | | | |
| 11 | 12 | 38 | 14 | 65 | 11 | | 92 | 12 | | | |
| 12 | 15 | 39 | 12 | 66 | 13 | | 93 | 11 | | | |
| 13 | 18 | 40 | 12 | 67 | 18 | | 94 | 13 | | | |
| 14 | 10 | 41 | 12 | 68 | 11 | | 95 | 23 | ok | | |
| 15 | 19 | 42 | 28 | 69 | 19 | | 96 | 12 | | | |
| 16 | 5 | 43 | 10 | 70 | 24 | ok | 97 | 19 | | | |
| 17 | 3 | 44 | 17 | 71 | 1 | | 98 | 11 | | | |
| 18 | 4 | 45 | 15 | 72 | 5 | | 99 | 10 | | | |
| 19 | 4 | 46 | 7 | 73 | 4 | | 100 | 9 | | | |
| 20 | 4 | 47 | 8 | 74 | 4 | | 101 | 5 | ok | | |
| 21 | 8 | 48 | 9 | 75 | 1 | ok | 102 | 10 | | | |
| 22 | 4 | 49 | 12 | 76 | 3 | | 103 | 6 | | | |
| 23 | 8 | 50 | 7 | 77 | 6 | | 104 | 9 | | | |
| 24 | 5 | 51 | 14 | 78 | 1 | | 105 | 1 | | | |
| 25 | 7 | 52 | 12 | 79 | 1 | ok | 106 | 7 | | | |
| 26 | 8 | 53 | 11 | 80 | 4 | | 107 | 4 | | | |
| 27 | 5 | 54 | 7 | 81 | 6 | | 108 | 4 | | | |



Title = checking cultures

Date = Fri 6th May 2022

Cultures checked 41, 96 (took out 10 µl from each).

Title: videos

Date: Saturday 7th May 2022

Aim: taking videos of the experiment at t6

Steps:

1. **Bring experiment from the incubator in A61 to the Protist Lab (G74)**
2. **Transfer 0.4 ml of each culture into a 2 ml Eppendorf microtube where the microtube rack mirrors the foamboard** (set micropipette to 0.4 ml) (flip the cultures)
3. **Bring experiment back to the incubator in A61**
4. **Reorder the tubes from 1 to 110**
5. **Set up microscope and computer (see Altermatt lab protocol nr 18)**
   Frames/second = 25, Total frames = 125, delay between frames = 40 msec
6. **Take videos of the cultures from 1-110 and then again (Microscope = Volterra, Camera = *Hamamatsu Orca Flash 4*) (see Altermatt lab protocol nr. 18)** (every 12 videos check that you took the right number of videos)
7. **Transfer videos to the hard drive**
   a. Fill time, date, and data point in the video description file
   b. Copy into EMA 2
      Copy the video folder Desktop -> Ema -> PatchSizePilot -> t6 into the hard drive "EMA 2" in the folder PatchSizePilot
   c. Copy into EMA 1
      Repeat for the hard drive "EMA 1"



Title: resource exchange 6

Date: Sunday 8th May 2022

Aim: exchanging resources between ecosystems by microwaving.

Steps:

1. **Bring cultures up** (close lids)
   a. Bring experimental cultures from the incubator (A61) to the Protista lab (G74)
   b. Dispose cultures in order on the falcon tube racks
2. **Pipette from cultures to microwaving tubes** (flip tubes)
   a. Green tubes (small perturbation)
      Pipette 5.25 ml from the cultures to tubes that will be microwaved (e.g., from culture 1 to microwaving tube nr 1, from culture 2 to microwaving tube nr 2). After you have micropipetted between two tubes, move them to empty racks (an empty rack for cultures and an empty rack for microwaving tubes).
      (10 ml micropipette: 5.25)
   b. Red tubes (large perturbation)
      Same as in previous point, but pipetting 6.75 ml
      (10 ml micropipette: 6.75)
3. **Rearrange cultures in random order on foam boards**
4. **Bring cultures back to the incubator** (close lids)
5. **Microwave**
   Microwave the microwaving tubes for 1 minute in batches of 4 (open lids ¼)
6. **Let tubes cool down**
   Wait for 1 hours and 15 minutes that the tubes have cooled down.
7. **Wash large micropipette tips**
   By washing them now, you free up the container where you gather them so you can use it again for the microwave tubes after you have poured their content.
8. **Bring cultures up** (close lids)
   Bring experimental cultures from the incubator (A61) to the Protista lab (G74)
9. **Pour cultures**
   Pour microwaved material into the corresponding number (where the arrow points at – if there's no arrow, pour back into the same number)
10. **Add deionised water**
    Add enough deionised water to reach the original culture volume (7.5, 22.5, or 37.5 ml) and write down how much you added (1 ml micropipette: 0.5)
11. ~~**Add PPM**~~
    ~~Add 0.2 ml of PPM to all cultures.~~
12. **Bring cultures back to the incubator** (close lids)
13. **Wash and dry microwaving tubes**
    Wash microwaving tubes with distilled water and let them dry on a rack. Let the caps dry on a tray with paper towels.

Not added because next video day is the last day.

# Add deionized water

| Culture | Water (ml) | Culture | Water (ml) | Culture | Water (ml) | Culture | Water (ml) |
|---|---|---|---|---|---|---|---|
| 30 | 1 | 102 | 1 | 16 | 1 | 19 | 1 |
| 37 | 1.5 | 97 | 1 | 60 | 1.5 | 45 | 1 |
| 92 | 1.5 | 90 | 1.5 | 93 | 1.5 | 72 | 1 |
| 103 | 1.5 | 63 | 1.5 | 88 | 1.5 | 44 | 1 |
| 67 | 0.5 | 87 | 1.5 | 49 | 1 | 68 | 1.5 |
| 20 | 1 | 59 | Spilled half, 1.25 | 26 | 0.5 | 84 | 1 |
| 105 | 1 | 13 | 1.5 | 42 | 1 | 64 | 1.5 |
| 71 | 1 | 21 | 1.5 | 48 | 1 | 5 | 1.5 |
| 76 | 1.5 | 23 | 2 | 22 | 0.5 | 61 | 1.5 |
| 15 | 1.5 | 82 | 1.5 | 52 | 1 | 7 | 1 |
| 56 | 2 | 34 | 1.5 | 58 | 2 | 89 | 1.5 |
| 54 | 1.5 | 40 | 1.5 | 8 | 1.5 | 78 | 1.5 |
| 86 | 2 | 73 | 1.5 | 101 | 1.5 | 33 | 0.5 |
| 38 | 1 | 11 | 1.5 | 108 | 2 | 10 | 1.5 |
| 32 | 1.5 | 17 | 1 | 66 | 1.5 | 80 | 0.5 |
| 3 | 2 | 75 | 1.5 | 18 | 1.5 | 24 | 0.5 |
| 104 | 1.5 | 28 | 1 | 31 | 1.5 | 85 | 1 |
| 65 | 1.5 | 95 | 2 | 6 | 1.5 | 107 | 1 |
| 25 | 1 | 12 | 1.5 | 53 | 1 | 100 | 1.5 |
| 70 | 1.5 | 57 | 1.5 | 39 | 1.5 | 14 | 1.5 |
| 91 | 1.5 | 55 | 1.5 | 98 | 1.5 | 51 | 1.5 |
| 69 | 1.5 | 46 | 1 | 2 | 1.5 | 83 | 1.5 |
| 79 | 1.5 | 1 | 1.5 | 81 | 1.5 | 74 | 1.5 |
| 35 | 1.5 | 99 | 1.5 | 41 | 1.5 | 50 | 1 |
| 109 | 1.5 | 9 | 1 | 96 | 1.5 | 4 | 1.5 |
| 27 | 1.5 | 36 | 1 | 29 | 1 | 106 | 2.5 |
| | | 47 | 1.5 | 110 | 1.5 | | |
| | | 94 | 1.5 | 43 | 1.5 | | |
| | | | | 62 | 1 | | |
| | | | | 77 | 1.5 | | |



Title: videos

Date: Wednesday 11th May 2022

Aim: taking videos of the experiment at t7

Steps:

1. **Bring experiment from the incubator in A61 to the Protist Lab (G74)**
2. **Transfer 0.4 ml of each culture into a 2 ml Eppendorf microtube where the microtube rack mirrors the foamboard** (set micropipette to 0.4 ml)n (flip the cultures)
3. **Reorder the tubes from 1 to 110**
4. **Set up microscope and computer (see Altermatt lab protocol nr 18)**
   Frames/second = 25, Total frames = 125, delay between frames = 40 msec
5. **Take videos of the cultures**
   (Microscope = Volterra, Camera = *Hamamatsu Orca Flash 4*) (see Altermatt lab protocol nr. 18) (every 12 videos check that you took the right number of videos).
   Take two videos for each culture.
6. ~~**Bring experiment back to the incubator in A61**~~
7. **Transfer videos to the hard drive**
   a. Fill time, date, and data point in the video description file
   b. Copy into EMA 2
      Copy the video folder Desktop -> Ema -> PatchSizePilot -> t7 into the hard drive "EMA 2" in the folder PatchSizePilot
   c. Copy into EMA 1
      Repeat for the hard drive "EMA 1"