Iron experiment (Karla, Raphael, Karin, Eva) p. 25 –

9/10/2018

Trace metal mix made with different Fe-concentrations

1. Combine 1L milliQ-water with all trace metals except for $FeCl_3 . 6H_2O$.

Trace metals are:

Na_2EDTA	4.36 g/L
$FeCl_3 . 6H_2O$	3.15 g/L
$CuSO_4 . 5H_2O$	0.01 g/L
$ZnSO_4 . 7H_2O$	0.022 g/L
$CoCl_2 . 6H_2O$	0.02 g/L
$MnCl_2 . 4H_2O$	0.18 g/L
$NaMoO_4 . 2H_2O$	0.006 g/L
H_3BO_3	1.00 g/L

2. Divided trace metal mix without $FeCl_3 . 6H_2O$ into 5 bottles à 200mL.
3. Added the following amounts of $FeCl_3 . 6H_2O$:
   Control: 0.63g
   F1: 0.315g
   F_2: 0.0315g
4. For F_3, 200µl of F_2 were diluted with 200mL-200µl trace metal mix without $FeCl_3 . 6H_2O$ to achieve an amount of 0.0000315g $FeCl_3 . 6H_2O$ in F_3.

200ml trace metal mix without $FeCl_3 . 6H_2O$ saved for later. All bottles stored in the fridge (4°C).

11/10/2018

Finish medium

1. Prepare sterile workplace: spray with 70% ethanol and wipe.
2. Prepare sterile workplace: start the ventilation to open gas valve. (10 minutes)
3. Fill 30-35 ml of metal mix into corresponding plastic vials (50ml).
4. Filter about 15 ml of each metal mix into corresponding plastic vials (15ml).
5. Get the 4 medium bottles.
6. Add 1ml of sterile-filtered vitamin mix to each medium bottle.
7. Add ~~1ml~~ 650µl of sterile-filtered selenium to each medium bottle.
8. Add ~~650µl~~ 1ml of the corresponding, sterile-filtered metal mix (Control, F1, F2, F3) to each medium bottle.

Check algae culture cell counts (Strain 1)

1. Clean sterile workbank with ethanol and start the burner.
2. Carefully turn algae bottle and open close to the flame. Turn the opening around close to the flame.
3. Pour a bit of the culture into a small beaker. Repeat for the other 2 algae bottles.
4. Set up the flow cam (see FlowCam Protocol) and calibrate the focus.
5. Add 1ml of medium to the flow cam to flush.
6. Carefully sway the beaker and add at least 2.5ml of algae culture to the flow cytometer.
7. Select „Autopicture" and let it run.
8. Sort out all non-Gonyo pictures and export the file to Excel. Check for cells/ml.
9. Repeat for the other 2 algae bottles.

Bottle 1: 1235, 808, 1104 → 1049 cells/ml
Bottle 2: 4413, 4388, 4500 → 4434 cells/ml ← This one we used
Bottle 3: 2807, 3478, 2982 → 3089 cells/ml

11/10/2018

Set up algae bottles with treatments (Strain 1)

1. Clean sterile workbank with ethanol.
2. Move medium and culture bottles into sterile workbank.
3. Starting from the medium with the lowest iron concentration (F3), add 85 ml medium to each culture bottle in 2 steps (45 and 40ml). You should have 5 culture bottles per medium (Crtl, Fe01, Fe02, Fe03).
4. Move algae cultures to the sterile workbank.
5. Start the burner.
6. Open the algae culture bottle close to the flame and turn the opening around close to the flame.
7. Carefully sway the algae culture bottle, then immediately take out 15ml with the 25ml pipette (sucking slowly is good).
8. Add 15ml algae culture to the first culture bottle with medium. Tip the culture bottles so that the algae culture runs down the side of the bottle. Use slow speed to avoid that the cells burst!
9. Repeat until all medium bottles have algae culture in them.
10. Turn the ~~cut~~ algae culture bottle opening close to the flame and close it.
11. Close all culture bottles. Remove everything from the sterile workbank, clean it and close the gas valve.
12. Return the medium, algae culture bottles and culture bottles to the constant room (20°C).
    Medium: black box Rest: shelves
13. Place culture bottles on the long, flat side. Carefully tilt them to get rid of drops on the bottles' sides.
14. Check culture bottles every 1-2 days to avoid condensation. In that case, carefully tilt the bottle.

Light:
Temperature: 20°C

18/10/2018
Measuring cell count in Culture bottles (Strain 1).
→ see flow cam protocol

1. Clean sterile workbank with ethanol and start the ventilation.
2. Start the burner.
3. Start. Carefully turn the culture bottle.
4. Open bottle around flame.
5. Pour ca. 10 ml into the corresponding vial.
6. Turn bottle opening close to flame and close.
7. Repeat for all culture bottles.
8. Set up flow cam (see flow cam protocol).
9. Carefully turn ~~algae~~ vial.
10. Add at least 2.5 ml to the flow cytometer. Measure.
11. Repeat for the other 4 bottles of that treatment.
12. Rinse 2x with MilliQ-water.
13. Repeat for other 3 treatments.

Preliminary (unsorted) results:
Control: 1135 particles/ml (average)
Fe01: 941 particles/ml (average)
Fe02: 616 particles/ml (average)
Fe03: 472 particles/ml (average)

AquaPen measurements (Strain 1)
1. Put the vials into a box so they are in the dark. Leave for at least 20 minutes.
2. Connect AquaPen to PC and clean 3 cuvettes for use.
3. Take first vial from box and close it again. Carefully turn vial to mix algae.
4. Pour liquid from vial into cuvette so that cuvette is almost full.
5. Clean out side of cuvette and put it into the AquaPen. Close.
6. Measure & download to the PC.

18/10/2018
AquaPen measurements
[...]
7. Remove cuvette from AquaPen and rinse 3x with milli-Q water.
8. Repeat for all other vials.

19/10/2018
Check algae culture ~~strain~~ cell counts (Strain 2)
→ see p. 26 for procedure

Bottle 1: 1229 cells/ml (average) ← This one we used
Bottle 2: 812,578 cells/ml
Bottle 3: 190 cells/ml

20/10/2018
Set up algae bottles with treatments (Strain 2)
done by Raphael
see p. 27 for procedure
BUT: 50 ml algae culture
50 ml medium

25/10/2018
Measuring cell count in culture bottles (Strain 1)
done by Marie, see p. 28 for procedure

AquaPen Measurements (Strain 1)
done by Marie, see p. 28 for procedure

28/10/2018

Measuring cell count in culture bottles (Strain 2)
done by Raphael, see p. 28 for procedure

Aqua Pen measurements (Strain 2)
done by Raphael, see p. 28 for procedure

30/10/2018

Checked cell count from 25/10/2018
Preliminary (unsorted) results:

Control:	2305	particles/ml	(average)
Fe01:	1963	particles/ml	(average)
Fe02:	680	particles/ml	(average)
Fe03:	462	particles/ml	(average)

1/11/2018

Measuring cell count in culture bottles (Strain 1)
see p. 28 for procedure
Preliminary (unsorted) results:

Control:	3502	particles/ml	(average)
Fe01:	3123	particles/ml	(average)
Fe02:	394	particles/ml	(average)
Fe03:	150	particles/ml	(average)

~~1/11/~~/Aqua Pen measurements (Strain 1)
see p. 28 for procedure

2/11/2018

Measuring cell count in culture bottles (Strain 2)
see p. 28 for procedure
Preliminary (unsorted) results:

Control:	225	particles/ml	(average)
Fe01:	287	particles/ml	(average)
Fe02:	279	particles/ml	(average)
Fe03:	287	particles/ml	(average)

Aqua Pen measurements (Strain 2)
see p. 28 for procedure

5/11/2018

Making medium (7l)
1. Fill all bottles with 1L milliQ-water.
2. Add 0.115 g/L TES buffer to each bottle.
3. Add 1ml of each stock solution (1,2,3,4,5,6) to each bottle.
4. Auto-clave 20 minutes at 115°C. Make sure the bottles don't touch each other or the sides of the oven. Leave bottle caps a little open! Add autoclave-tape (green should turn black).

Sorting cells from FlowCam measurements
18/10/18 data
double-Gonyo-pictures: Fe01-01 2x; Fe01-03 2x; Fe01-04 1x; Fe01-05 2x; Fe02-06 1x; Crtl-17 1x; Crtl-19: 1x; Crtl-20: 1x

5/11/2018
[...]

25/10/18 data

double-Gonyo-pictures:				
	Fe01-01	2x	Crtl-16	2x
	Fe01-02	5x	Crtl-17	3x
	Fe01-03	1x	Crtl-18	1x
	Fe01-05	3x	Crtl-19	2x
	Fe02-07	2x	Crtl-20	2x
	Fe02-09	1x		

28/10/18 data

double-Gonyo-pics:				
	Fe02-07	1x	Fe03-14	1x
	Fe02-08	1x		

2/11/18 data

double Gonyo-pics:				
	Fe02-27	1x	Fe01-24	1x
	Fe02-28	1x		

1/11/18 data

double-Gonyo-pics:				
	Fe03-13	1x	Fe01-05	5x
	Fe01-01	2x	Crtl-16	1x
	Fe01-02	4x	Crtl-17	6x
	Fe01-02(2)	5x	Crtl-18	6x
	Fe01-04	3x	Crtl-19	3x
	Fe01-03	4x	Crtl-20	3x

6/11/2018
Finish medium
see p. 26 for procedure
vitamin mix + selenitem added to all 7 bottles
Metal mix (Crtl, F1, F2, F3) added to 4 bottles

7/11/2018
Measuring cell count in culture bottles (strain 2,3,4)
see p. 28 for procedure
cell counts

QSPS (Poland)

745 cells/ml
975 cells/ml
914 cells/ml

NO-018 (US)

①	②	③
2801	1654	1352
2850	1722	
2809		

↳ used for strain 3 culture bottles

BO-182 (Sweden)

①	②
66	25

8/11/2018
Measuring cell count in culture bottles (Strain 1)
see p. 28 for procedure
Preliminary (unsorted) results:

Control:	6834	particles/ml	(average)
Fe01:	6864	particles/ml	(average)
Fe02:	182	particles/ml	(average)
Fe03:	50	particles/ml	(average)

Aqua Pen measurements (Strain 1)
see p. 28 for procedure

Filtering for Fe, N, P, C analysis (Strain 1)
Preparations
- Filters (
- vials (50ml, acid-washed)
- 2x plastic filter holders (
- plastic syringe (50ml)
- alu-foil rectangles (

8/11/2018

[...]

Filtration into a ~~35~~ 50 ml vial

1. Filter 25 ml. Make sure the filter holder is properly closed to avoid leaking.
2. Remove filter with tweezers and put onto alufoil-rectangle. Carefully fold in half so that the inner side of the filter is „closed".
3. Mark alufoil and put into freezer.
4. Close 50ml vial, mark and put into freezer.
5. Repeat for 2nd filter for sample.
6. Repeat for all samples. Carefully rinse filter holder + syringe between treatments with milliQ water.

Filter 1 for Fe02-08: filter leaked!

9/11/2018

Measuring cell count in culture bottles (strain 2)
see p. 28 for procedure

Preliminary (unsorted) results:

Control:	170	particles/ml
Fe01:	169	particles/ml
Fe02:	208	particles/ml
Fe03:	288	particles/ml

Fe03-31: 1st measurement with wrong context settings

Aqua Pen measurements (strain 2)
see p. 28 for procedure

→ skip measuring this strain on 16/11, as it grows so little

9/11/2018

Start a Microcystis cultivation
Strain: Toxic Reference strain (

preparation / set-up culture bottles (see p. 27 for procedure)

1. 1L bottle medium, addes 650µg of metal mix Control.
2. Fill 260ml culture bottle with 150 ml control-medium. Do 5 bottles.
3. Add 750µg Microcystis culture per bottle. All work in sterile work bench.
4. Bottles moved to Gonyo-constant room under same light regime.

ALSO: strain 3 st...

12/11/2018

Start a Chlamydomonas cultivation
Strain:

~~Set up~~ Preparation / set-up of culture bottles (see p. 27)

1. Add 650µg of metal mix Control to 1L bottle medium
2. Fill 260 ml culture bottle with 150 ml control-medium. Do 6 bottles.
3. Add 500µl Chlamydomonas culture per bottle. All work in sterile bench.
4. Bottles moved to Gonyo-constant room under same light regime.

Sorting cells from FlowCam measurements

9-11-2018 data: double Gonyo pictures

Fe02-06: 1x	Fe01-04: 16x	Crtl-18: 11x
Fe01-01: 31x	Fe01-05: 23x	Crtl-19: 15x
Fe01-02: 19x	Crtl-16: 17x	Crtl-20: 21x
Fe01-03: 11x	Crtl-17: 30x	

16/11/2018
Measuring cell count in culture bottles (Strain 3)
see p28 for procedure
Preliminary (unsorted) results:
Control: 1147 particles/ml (average)
FeO1 : 1248 particles/ml (average)
FeO2 : 1002 particles/ml (average)
FeO3 : 902 particles/ml (average)

AquaPen measurements (Strain 3)
see p. 28 for procedure

21/11/2018
Measuring cell counts in culture bottles CSP2
cell counts: 863
791 -> not enough to set up the strain...
1032
1310

22/11/2018
Sorting cells from FlowCam measurements
16-11-2018 data: double Conyo pictures
Crtl-56(2): 2x FeO3-53: 1x
Crtl-57 : 2x FeO2-49: 1x
Crtl-58 : 1x FeO1-45: 1x

Counting Microcystis-cells from culture bottles
Dilution: 100x Count until 400 cells are reached
Bottle 1: 12 12 12 5 6 6 13 8 12 9
cell counts 7 8 7 8 10 12 13 13 20 11
5 10 6 9 7 6 11 8 10 9
6 9 13 9 10 9 8 12 14 9
} 400 cells in 40 quadrat
-> 10 cells/quadrat
10 cells x 1000 (µg/L) x 100 (dilution) =
1,000,000 cells/ml

22/11/2018
[...]
Bottle 2: 7 8 6 6 6 9 6 60 16 11
cell counts 6 13 11 3 7 8 8 7 7 4
7 8 7 8 9 13 7 9 16 16
16 8 9 10 5 7 10 11 8 9
} 401 cells in 40 quadrats
10,1 cells x 1000 x 100 =
1,010,000 cells/ml

Bottle 3: 6 21 16 9 10 7 12 9 10 12
cell counts 11 13 8 14 11 10 11 8 10 11
15 30 9 8 5 4 8 6 9 9
7 10 17 6 10 8 7 6 8 3
} 414 cells in 40 quadrats
10,35 cells x 1000 x 10 =
1,035,000 cells/ml

23/11/2018
Measuring cell count in culture bottles (Strain 3)
see p. 28 for procedure
Preliminary (unsorted) results:
Control: 1515 particles/ml (average)
FeO1: 1659 particles/ml (average)
FeO2 : 906 particles/ml (average)
FeO3: 545 particles/ml (average)

AquaPen measurements (Strain 3)
see p. 28 for procedures

Measuring cell count in culture bottles (Strain 2)
see p. 28 for procedure
Preliminary (unsorted) results:
Control: 125 particles/ml (average)
FeO1 : 168 particles/ml (average)
FeO2 : 177 particles/ml (average)
FeO3 : 247 particles/ml (average)

23/11/2018
[...]
AquaPen measurements (Strain 2)
see p. 28 for procedure

24/11/2018
Sorting cells from FlowCam measurements
22/11/2018 - data
Strain 2: no double Gonyo pictures

25/11/2018
Sorting cells from FlowCam measurements
22-11-2018 data: double Gonyo pictures
Fe03-54: 1x Fe01-43: 2x Crtl-58: 1x
Fe02-49: 1x Fe01-45: 1x
Fe01-41(2): 1x Crtl-56(2): 1x

26/11/2018
Counting Microcystis cells from culture bottles Dilution: 10x
M1: 78 110 82 70 88
112 81 107 92 97 } 917 cells in 10 quadrats
92 x 1000 x 10 = 920,000 cells/ml

M2: 121 125 115 121 98 } 116 cells in 5 quadrats
116 x 1000 x 10 = 1,160,000 cells/ml

M3: 122 122 109 118 } 471 cells in 4 quadrats
118 x 1000 x 10 = 1,180,000 cells/ml

26/11/2018
[...]
M4: 130 124 119 105 } 478 cells in 4 quadrats
119.5 x 1000 x 10 = 1,195,000 cells/ml

M5: 135 109 132 131 } 507 cells in 4 quadrats
126.75 x 1000 x 10 = 1,267,500 cells/ml

For Microcystis culture set-up:
1) combined M2, M3, M4 and M5
2) initial cell concentration should be around 366,000 cells/ml
-> 75ml medium + 25ml Microcystis culture
see p. 27 1)-11)
Stored standing at same conditions as G. semen

also: 4x 70ml bottles with 30ml medium + 100µl Microcystis
-> see how it grows with initial culture set-up volumes (-> photos)

27/11/2018
Count Chlamydomonas cell count with FlowCam (unsorted)
Chl 1: 445,718 particles/ml
Chl 2: 573,299 particles/ml
Chl 3: 583,896 particles/ml
Chl 4: 596,546 particles/ml
Chl 5: 665,180 particles/ml
Chl 6: 628,039 particles/ml
initial cell concentration should be ~16,000 cells/ml
-> set up Chlamydomonas from Chl 4
2.5 ml Chlamydomonas culture + 97.5ml medium
see p. 27 1)-11) for procedure
Stored standing at same conditions as G. semen

28/11/2018
Metal stocks: crystals visible in CrtL and F1, a bit in F2, ~~too~~
crystals remained at room temperature + after stirring

Microcystis cell count (microscope)
sample taken: ~ 6ml
↳ from that: 1.8 ml tab water + 0.2ml sample for microscope (10x dilution)

Average cell counts:

CrtL:	62.96	cells/~~sq~~squ		629,571.4 cells/ml
Fe01:	57.05	cells/~~sq~~squ	x1000	570,500 cells/ml
Fe02:	52.62	cells/~~sq~~squ	x10	526,178.8 cells/ml
Fe03:	51.45	cells/~~sq~~squ		514,500 cells/ml

AquaPen Measurements (Microcystis)
see p. 28 for procedure
at 455 and 630 nm wavelength
done by Raphael

29/11/2018
Measure cell count in culture bottles (Chlamydomonas)
sample taken: ~ 9ml (6ml for AquaPen; 2ml for Flow Cam)
Flow Cam: Manual Focus with sample (Fe01-01) 100-flow cell
Preliminary (unsorted) results:

CrtL:	23,880 ~~&~~ particles/~~ml~~ml	(average)
Fe01:	~~51,863~~ 18,827 particles/ml	(average)
Fe02:	24,542 particles/ml	(average)
Fe03:	33,265 particles/ml	(average

25/11/2018
Note: often a 1s-delay at the beginning of measurement x until the flow gets going, resulting in multiple counts of the same cell

AquaPen measurements (Chlamydomonas)
see p. 28 for procedure
both 455 and 630nm wavelength

30/11/2018
Measure cell count in culture bottles (Strain 3)
Preliminary (unsorted) results:

CrtL:	2851	particles/ml	(average)
Fe01:	2871	particles/ml	(average)
Fe02:	950	particles/ml	(average)
Fe03:	340	particles/ml	(average)

AquaPen measurement (Strain 3)
see p. 28 for procedure

1/12/2018
Sorting cells from FlowCam measurements &
30/11/2018 data: double-Konyo pictures

Fe02-47: 2x	Fe01-44: 4x	CrtL-58(2): 1x
Fe01-41(2): 2x	Fe01-45: 2x	CrtL-59(2): 5x
Fe01-42: 5x	CrtL-56(2): 2x	CrtL-60: 2x
Fe01-43 5x	CrtL-57(2): 2x	

Note: Fe01-43 - Fe01-45 sorted ~~12~~ 2/12/2018
CrtL-56(2) - CrtL-60 sorted 5/12/2018

1/12/2018
Microcystis cell count (microscope)
10x dilution for microscope
Average cell counts:

Crtl:	104.7 cells/~~ml~~squ		1,047,000 cells/ml
Fe01:	104.7 cells/~~ml~~squ	x1000	1,047,000 cells/ml
Fe02:	99.025 cells/~~ml~~squ	x10	990,250 cells/ml
Fe03:	90.3 cells/~~ml~~squ		903,000 cells/ml

AquaPen measurement (Microcystis)
see p.28 for procedure
Note: Fe01-03 first 455nm measurement is wrong; use ~~Fe~~ M-Fe01-03-a-2 for analysis!

Measure cell count in culture bottles (Chlamydomonas)
Preliminary (unsorted) results:
Crtl: 51,317 particles/ml (average)
Fe01: 91,869 particles/ml (average)
Fe02: ~~23~~ 99,819 particles/ml (average)
Fe03: 72,878 particles/ml (average)

AquaPen measurement (Chlamydomonas)
see p.28 for procedure

2/12/2018
Measure cell count in culture bottles (Chlamydomonas)
Preliminary (unsorted) results:
Crtl: 111,566 particles/ml (average)
Fe01: 138,427 particles/ml (average)
Fe02: 185,401 particles/ml (average)
Fe03: 149,262 particles/ml (average)

2/12/2018
AquaPen measurement (Chlamydomonas)
see p.28 for procedure

3/12/2018
Microcystis cell count (microscope)
dilution: 1980µl tabwater + 20µl samle → 100x
Average cell counts:

Crtl:	39.84632 cells/~~ml~~squ		3,984,632 cells/ml
Fe01:	44.4 cells/~~ml~~squ	x1000	4,440,000 cells/ml
Fe02:	45.90278 cells/~~ml~~squ	x100	4,590,278 cells/ml
Fe03:	46.16389 cells/~~ml~~squ		4,616,389 cells/ml

AquaPen measurement (Microcystis)
see p.28 for procedure

4/12/2018
Measure cell count in culture bottles (Chlamydomonas)
cell count stopped after 5000 counts!
Preliminary (unsorted) results:
Crtl: 174,139 particles/ml (average)
Fe01: 340,468 particles/ml (average)
Fe02: 347,808 particles/ml (average)
Fe03: 360,125 particles/ml (average)

AquaPen measurement (Chlamydomonas)
see p.28 for procedure

5/12/2018

Measure cell count in culture bottles (Chlamydomonas)
count stopped after 5000 counts!

Preliminary (unsorted) results:

Crtl:	200,133	particles/ml	(average)
Fe01:	401,251	particles/ml	(average)
Fe02:	386,226	particles/ml	(average)
Fe03:	430,464	particles/ml	(average)

AquaPen measurements (Microcystis and Chlamydomonas)
see p. 28 for procedure

Microcystis cell count (microscope)
dilution: 100x

Average cell counts:

Crtl: 63.88571 cells/squ		6,388,571 cells/ml
Fe01: 63.70952 cells/squ	x1000	6,370,952 cells/ml
Fe02: 61.97143 cells/squ	x100	6,197,143 cells/ml
Fe03: 60.33571 cells/squ		6,033,571 cells/ml

Filtering for Fe, N, P, C analysis (Chlamydomonas + Microcystis)
see p. 33/34 for procedure
done by Raphael
Microcystis: 10ml filtered
Chlamydomonas: 20ml filtered

7/12/2018

Measure cell counts in culture bottles
strain 3

Preliminary (unsorted) results:

Crtl:	4509	particles/ml	(average)
Fe01:	4146	particles/ml	(average)
Fe02:	873	particles/ml	(average)
Fe03:	203	particles/ml	(average)

strain 2

Preliminary (unsorted) results

Crtl:	239	particles/ml	(average)
Fe01:	219	particles/ml	(average)
Fe02:	283	particles/ml	(average)
Fe03:	246	particles/ml	(average)

AquaPen measurements (Strain 3 + Strain 2)
see p. 28 for procedure

Filtering for Fe, N, P, C analysis (Strain 3 + Strain 2)
see p. 33/34 for procedure
done by Raphael: Strain 3 (25ml per filter)
Strain 2: 1 filter per treatment by combining 4 replicates

Karin: checked Gonyo-cells for chloroplast with microscopes
pictures taken with Nikon
→ pictures in „Karin", „Gonyo-Fe"
Note: reduction in chloroplasts only in Strain 3, not 1 and 2

~~7/12/2018~~

Sorting cells from FlowCam measurements
Strain 2 (7/12/2018)
double Gonyo: Fe02-28: 1x

7/12/2018
[...]
Strain 3 (7/12/2018): double Gonyo pictures

Fe01-41: 9x	Crtl-56(2): 6x
Fe01-42: 5x	Crtl-57: 6x
Fe01-43: 8x	Crtl-58: 7x
Fe01-44: 8x	Crtl-59: 7x
Fe01-45: 7x	Crtl-60(2): 6x

10/12/2018
checked FlowCam measurements for Chlamydomonas
- deleted clumps with >4 cells
- counted 2x, 3x, 4x cells per picture
- compared to no-cell pictures: it appears to balance out

12/12/2018
Taken subsamples of the following Strains:
~~NO00~~ NO019
PA008
BO182
JE009
transported in 7ml vials in styropore box
also taken: ready-to-use medium
buffer
medium stock
metal stock
experiment metal stocks (unfiltered + sterile filtered)
vitamin mix
selenium mix
culture flasks 70ml (2 packs à 10 bottles)

13/12/2018
In sterile bank: filled G. semen strains into culture bottles
~~stored~~ in incubator in basement: 20°C
12h light, 12h dark
high light (how much?)

20/3/2019
In sterile bank: transferred G. semen strains NO-018 and PA-008 to culture bottles (2 each)
Stored: incubator in fish lab (left, ~18°C, in light, no cycle)

27/3/2019
Filter preparation for chl-a analysis
use: vials (cleaned) from chl-a shelf (on top of spectrophotometer)
defreeze filters (Crtl and Fe01 combined filters for Gonyo 53)
clean tweezers with ethanol
add filters to vials — best in the dark
add 10 ml 95% ethanol
leave in ~~fridge~~ (4°C) for 24h to extract (dark)

28/3/2019
Chl-a measurement — best in the dark
1) filter samples into new tubes using a syringe filter
2) start the spectrophotometer (let warm up ~10 minutes)
3) Use 95% ethanol to zero ← should be done before every sample, it changes quickly
4) add sample (ca. 5ml) to cuvette.
5) put cuvette into spectrophotometer, measure at 750nm and 665nm
- same orientation ! write down manually !
- same cuvette
6) add 0.1 ml 3M/L HCl to cuvette. Wait 1 minute. Measure at 750 and 665nm again

28/3/2019
[...]
measurements

	665 nm	750 nm	after HCl addition 665 nm	after HCl addition 750 nm
ethanol (95%)	0	0	-0.009	-0.011
CrtL	0.195	-0.006	~~-0.009~~ 0.104	~~-0.011~~ -0.009
FeO1	0.186	-0.009	0.097	-0.013

11/4/2019
Making fresh medium: 1L (see p. 31)
1) Fill a 1L glass bottle with 1L milliQ-water.
2) add buffer (0.115 g)
3) add 1ml stock solution
4) autoclave at 115°C for 20 minutes
- autoclave here requires reference bottle
- remember not to close bottle, leave lid a bit open
5) put in 20°C room to ~~cold~~ cool down.

13/4/2019
Finish making medium
1) sterile-filter vitamin mix (10 ml)
2) add 1ml vitamin mix to medium bottle
— " — metal mix — " —
add 650µl Selenium — " —

Transfer cultures: ~~Fee~~ 2x NO-018 (35 ml medium, 5ml culture)
2x PO-008 (35 ml medium, 5ml culture)
kept: 1x NO-018 (20-3-2019)
1x PO-008 (20-3-2019)
} filled with 30 ml medium (10 ml culture kept)
store: incubator fish lab, 18°C

7/5/2019
ChL-α extraction (Strain 1 and 3, CrtL and FeO3 treatments)
→ see Labfolder (evatheresakarla)
↳ also in Project: Iron Experiment (10/5/2019 date)

8/5/2019
ChL-α measurement (Strain 1 and 3, CrtL and FeO3 treatment)
→ see labfolder
└ Project: Iron Experiment

17/5/2019
ChL-α extraction (Strain 1 and 3, FeO1 and FeO2 treatments, Chlamydomonas CrtL treatment)
→ see Labfolder
└ Project: Iron Experiment

18/5/2019
ChL-α extraction (Chlamydomonas FeO1, FeO2, FeO3 treatments, Microcystis CrtL and FeO3 treatments)
ChL-α measurement (Strain 1 and 3, FeO1 and FeO2 treatments, Chlamydomonas CrtL treatment)
→ see Labfolder
└ Project: Iron Experiment

19/5/2019
ChL-α extraction (Microcystis FeO1 and FeO2 treatments)
ChL-α measurement (Chlamydomonas FeO1, FeO2, FeO3 treatments, Microcystis CrtL and FeO3 treatments)
→ see Labfolder
└ Project: Iron experiment

20/5/2019
ChL-α measurement (Microcystis CrtL and FeO3 treatments)
→ see Labfolder
└ Project: Iron Experiment