Light scattering to Biomass calibration experiment

1. List of cultures (21 strains)

Axenic strains

0.5 - 1.5 micron diameter

- Prochlorococcus MED4 (HLI) - **smallest**
- Prochlorococcus AS9601 (HLII)
- Prochlorococcus 1314 (HLII)
- Prochlorococcus NatI2A (LLI)
- Prochlorococcus 9303 (LLIV)
- Prochlorococcus 9313 (LLIV) - **largest**
- Synechococcus WH8102
- Synechococcus 7803
- Synechococcus 8012
- Synechococcus 8109

2-5 micron diameter

- Crocosphaera watsonii 8501

Non Axenic:

>3 micron diameter

- TAPS CCMP1335 - **smallest**
- Emiliania hux CCMP1742
- TAPS CCMP3367
- Phaeodactylum tricornutum CCMP632
- Licmophora paradoxa
- Micromonas pusilla
- Minutocellus sp. CCMP3330
- Navicula transitans
- Thalassiosira weissflogii CCMP3365 - **largest**

2. Experimental setup

Culture will be grown under continuous light (100 µmol photon m^{-2} s^{-1}). Cultures will be harvested in exponential phase.

- Triplicate samples of 50 ml will be filtered on pre-combusted GFF filter for CHN analysis. 150 ml
- Triplicate 1 ml will be used for counting (Influx). 3 ml
- Triplicate *10X diluted* 100 ml will be used for FSC measurement on SeaFlow (3 instruments). 90 ml.
- Triplicate *10X diluted* 100 ml will be used for FSC measurement on LISST (1 instrument). Will only analyze cells > 2 micron in diameter. 30 ml
- Triplicate 50 ml will be used for Volume measurement (Coulter Counter). All but Prochlorococcus cultures. 150 ml
- 100 ml will be aliquoted into 2 ml cryovials, fixed with Glutaraldehyde + Pluronic F68 and kept at -80 degC. These aliquots will be sent to other instruments for FSC calibration. 100ml

Total volume needed < 500 mL

To be more manageable, the calibration experiment will be divided in two parts:

- Cells >3 µm (11 strains) FEBRUARY 21st-24th - **NON AXENIC**
- Cells 0.5-1.5 µm (10 strains) MARCH 14th - 16th - **AXENIC**

3. Preparation - LARGE CELLS

>3 micron diameter

- TAPS CCMP1335 - **smallest**
- Emiliania hux CCMP1742
- TAPS CCMP3367
- Phaeodactylum tricornutum CCMP632
- Licmophora paradoxa
- Micromonas pusilla
- Minutocellus sp. CCMP3330
- Navicula transitans
- Thalassiosira weissflogii CCMP3365 - **largest**

February 8

2.5 mL from stock diluted 10X in 25 mL of F/2 medium for non-axenic cultures - Duplicate cultures

February 10

1 ml collected from duplicate A for counting (Influx) using 2 µm beads:

- TAPS CCMP3367 = **1,242,440** cells mL^{-1}
- TAPS CCMP1335 = **651,800** cells mL^{-1}
- Navicula transitans = **616,690** cells mL^{-1}
- Thalassiosira weissflogii CCMP3365 = **305,660** cells mL^{-1}
- Licmophora paradoxa = **87** cells mL^{-1}
- Minutocellus sp. CCMP3330 = **40,350** cells mL^{-1}
- Phaeodactylum tricornutum CCMP632 = **634,200** cells mL^{-1}
- Emiliania hux CCMP1742 = **300,340** cells mL^{-1}
- Micromonas = **2,787,500** cells mL^{-1}

Dilution of duplicate A with F/2 medium as follow (duplicate B as backup):

- Dilution 100 for CCMP3367, Micromonas
- Dilution 50 for CCMP1335, Navicula, CCMP3365, CCMP632, CCMP1742
- Dilution 10 for CCMP3330

February 15

1 ml collected from Diluted cultures for counting (Influx) using 1 µm beads:

- TAPS CCMP3367 = **2,355,180** cells mL^{-1}
- TAPS CCMP1335 = **1,439,900** cells mL^{-1}
- Navicula transitans = **1,597,640** cells mL^{-1}
- Thalassiosira weissflogii CCMP3365 = **282,450** cells mL^{-1}
- Licmophora paradoxa = **1,410** cells mL^{-1}
- Minutocellus sp. CCMP3330 = **863,380** cells mL^{-1}
- Phaeodactylum tricornutum CCMP632 = **2,290,940** cells mL^{-1}
- Emiliania hux CCMP1742 = **23,820** cells mL^{-1}
- Micromonas = ** 181,400** cells mL^{-1}

February 16

Dilution with F/2 medium, final volume 100 mL

- TAPS CCMP3367 = D100 (2 ml in 200 ml)
- TAPS CCMP1335 = D100 (2 ml in 200 ml)
- Navicula transitans = D100 (2 ml in 200 ml)
- Thalassiosira weissflogii CCMP3365 = D20 (10 ml in 200 ml)
- Licmophora paradoxa = merge all cultures together (50 ml) and add 150 ml F/2
- Minutocellus sp. CCMP3330 = D100 (2 ml in 200 ml)

- Phaeodactylum tricornutum CCMP632 = D100 (2 ml in 200 ml)
- Emiliania hux CCMP1742 = D8 (50 ml in 200 ml)
- Micromonas = D20 (10 ml in 200 ml)

February 17

1 ml collected from 200-ml cultures for counting (Influx) using 2 µm beads:

- TAPS CCMP3367 = **35,130** cells mL^{-1}
- TAPS CCMP1335 = **38,680** cells mL^{-1}
- Navicula transitans = **33,250** cells mL^{-1}
- Thalassiosira weissflogii CCMP3365 = **75,330** cells mL^{-1}
- Licmophora paradoxa = **145** cells mL^{-1}
- Minutocellus sp. CCMP3330 = **3,680** cells mL^{-1}
- Phaeodactylum tricornutum CCMP632 = **72,720** cells mL^{-1}
- Emiliania hux CCMP1742 = **1,453** cells mL^{-1}
- Micromonas = **8,390** cells mL^{-1}

D2 for TW CCMP3365 (remove 100 ml of culture and replaced by F/2 medium)

February 21

1 ml collected from 200-ml cultures for counting (Influx) using 2 µm beads:

- TAPS CCMP3367 = **1,264,600** cells mL^{-1}
- TAPS CCMP1335 = **1,033,920** cells mL^{-1}
- Navicula transitans = **1,372,740** cells mL^{-1}
- Thalassiosira weissflogii CCMP3365 = **335,260** cells mL^{-1}
- Licmophora paradoxa = **700** cells mL^{-1}
- Minutocellus sp. CCMP3330 = **339,120** cells mL^{-1}
- Phaeodactylum tricornutum CCMP632 = **2,524,320** cells mL^{-1}
- Emiliania hux CCMP1742 = **1,64** cells mL^{-1}
- Micromonas = **15,260** cells mL^{-1}

Dilutions (F/2 medium)

- D3 for TAPS-1335, TAPS-3367, Navicula, TW3365, Minutocellus (Add 400 ml F/2)
- D5 PT-632 (remove 100 ml, add 400 ml F/2)
- No dilution for Ehux, Micromonas, Licmophora

February 22

- D3 of Navicula and Minutocellus (collected 200 ml of surface bottle and added 400 ml F/2)

February 23

1 ml collected from 600-ml cultures for counting (Influx) using 2 µm beads:

- TAPS CCMP3367 = **828,060** cells mL^{-1}
- TAPS CCMP1335 = **651,16** cells mL^{-1}
- Navicula transitans - D3= **277,280** cells mL^{-1}
- Navicula transitans = **651,140** cells mL^{-1}
- Thalassiosira weissflogii CCMP3365 = **164,180** cells mL^{-1}
- Licmophora paradoxa = **660** cells mL^{-1}
- Minutocellus sp. CCMP3330 = **27,320** cells mL^{-1}
- Minutocellus sp. CCMP3330 - D3 = **28,520** cells mL^{-1}
- Phaeodactylum tricornutum CCMP632 = **651,140** cells mL^{-1}
- Emiliania hux CCMP1742 = **1,840** cells mL^{-1}
- Micromonas = **21,980** cells mL^{-1}

Dilutions (filtered seawater)

- D50 for TAPS3367, TAPS1335, PT632
- D10 for Navicula - D3 and TW3365

HARVEST 1

1) Counting 100-300 µL with Influx using 1 µm beads

2) filtration on pre-combusted GFF, triplicate:

- Phaeodactylum tricornutum CCMP632 = **50 ml**
- TAPS CCMP3367 = **50 ml**
- Blank = **75 ml**
- TAPS CCMP1335 = **100 ml** (a fourth filter for 1335 is either 100ml or 75ml, not sure, but written on the box)
- Navicula transitans = **75 ml**
- Thalassiosira weissflogii CCMP3365 = **50 ml**

3) Counting for 6 minutes (2 files) with SeaFlow #740 and #751 using 1 µm beads

February 24

HARVEST 2

1) Counting 100 µL with Influx using 1 µm beads

2) filtration on pre-combusted 0.7 µm GFF, triplicate:

- Micromonas = **50 ml**
- Blank = **75 ml**
- Phaeodactylum tricornutum CCMP632 = **50 ml**
- Emiliania hux CCMP1742 = **26 ml** (only 1 filter for Ehux)
- Licmophora paradoxa = **50 ml**

3) Counting for 6 minutes (2 files) with SeaFlow #740 and #751 using 1 µm beads

4. Preparation - SMALL CELLS

0.5 - 1.5 micron diameter

- Prochlorococcus MED4 (HLI) - **smallest**
- Prochlorococcus AS9601 (HLII)
- Prochlorococcus 1314 (HLII)
- Prochlorococcus NatI2A (LLI) - **largest**
- Synechococcus WH8102
- Synechococcus 7803

April 25

1 mL from stock grown in Pro99 medium for axenic cultures under continuous light
D100 before counting

- MED4 = **676 x 10^6** cells mL^{-1}
- AS9601 = **199 x 10^6** cells mL^{-1} -- 2 populations (contamination?)
- 1314 = **291 x 10^6** cells mL^{-1}
- NatI2A = **32 x 10^6** cells mL^{-1}
- WH8102 = **38 x 10^6** cells mL^{-1} -- unhealthy
- 7803 = **48 x 10^6** cells mL^{-1}

D5 for all stocks with Pro99

April 27

1 mL from stock, D100 before counting

- MED4 = **302 x 10^6** cells mL^{-1}
- AS9601 = **181 x 10^6** cells mL^{-1} -- 2 populations (contamination?)
- 1314 = **115 x 10^6** cells mL^{-1}
- NatI2A = **32 x 10^6** cells mL^{-1}
- WH8102 = **24 x 10^6** cells mL^{-1} -- unhealthy
- 7803 = **35 x 10^6** cells mL^{-1}

May 1st

D5 of all cultures

May 2nd

1 mL from stock, D100 before counting

- MED4 = **140 x 10^6** cells mL^{-1} -- Low FSC pop
- AS9601 = **227 x 10^6** cells mL^{-1} =
- 1314 = **34 x 10^6** cells mL^{-1}
- NatI2A = **7 x 10^6** cells mL^{-1} -- small pop
- WH8102 = **24 x 10^6** cells mL^{-1} -- Low CHL pop
- 7803 = **15 x 10^6** cells mL^{-1} -- Ok, but low CHL pop

Washing 6 - 500 ml glass Erlen with 10% HCL

May 3

1 mL from stock, D100 before counting

- MED4 = **263 x 10^6** cells mL^{-1}
- AS9601 = **556 x 10^6** cells mL^{-1}
- 1314 = **45 x 10^6** cells mL^{-1}
- NatI2A = **11 x 10^6** cells mL^{-1}
- WH8102 = **46 x 10^6** cells mL^{-1} -- Ok, but low CHL pop
- 7803 = **41 x 10^6** cells mL^{-1} -- Ok, but low CHL pop

May 4

1 mL from stock, D100 before counting

- MED4 = **377 x 10^6** cells mL^{-1}
- AS9601 = **469 x 10^6** cells mL^{-1}
- 1314 = **79 x 10^6** cells mL^{-1}
- NatI2A = **15 x 10^6** cells mL^{-1}
- WH8102 = **70 x 10^6** cells mL^{-1}
- 7803 = **48 x 10^6** cells mL^{-1}

Take 30 ml stock into 120 ml of Pro99 medium in flask (flask was washed with 10%HCl, rinsed with MQ and autoclaved)

May 5

HARVEST 3

1) Influx count: 985 µL media , 5 µL beads + 10 µL cultures
Counting 100 µL with Influx using 1 µm beads

2) filtration on pre-combusted 0.3 µm GF-75 (Advantec), triplicate:

- 30 ml for cultures
- 30 ml for Blank (Pro99)
  3) Counting for 6 minutes (2 files) with SeaFlow #740 and #751 using 1 µm beads

NB: 100-300 dilution before counting cells on SeaFlow; Trigger on FSC (Red noisy on #751)

NB2: Pressure sensor not working on #751