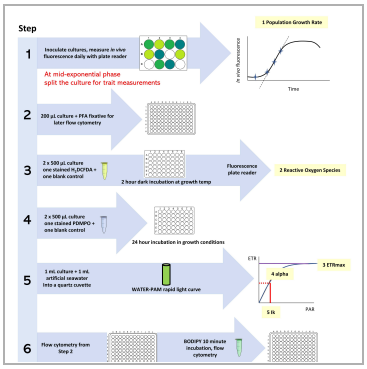

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MANUSCRIPT CITATION:
Argyle, P.A., Hinners, J., Walworth, N.G., Collins, S., Levine, N.M., Doblin, M.A., 2021. A High-Throughput Assay for Quantifying Phenotypic Traits of Microalgae. Frontiers in Microbiology 12(2910).

Protocol status: Working
We use this protocol and it's working

Created: Aug 25, 2021

A High-Throughput Assay for Quantifying Phenotypic Traits of Microalgae

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Phoebe Argyle

ABSTRACT

This outlines a workflow for measuring 10 phenotypic traits of centric diatoms using a variety of methodologies. This method is described in:

Argyle, P. A., Hinners, J., Walworth, N. G., Collins, S., Levine, N. M., & Doblin, M. A. (2021). A high-throughput assay for quantifying phenotypic traits of microalgae. *Frontiers in microbiology*, *12*, 706235.

IMAGE ATTRIBUTION

Argyle, P.A., Hinners, J., Walworth, N.G., Collins, S., Levine, N.M., Doblin, M.A., 2021. A High-Throughput Assay for Quantifying Phenotypic Traits of Microalgae. Frontiers in Microbiology 12(2910).

Last Modified: Feb 25, 2023

PROTOCOL integer ID:
52691

Set up experimental cultures

1 Experimental cultures are grown in 12-well tissue culture plates. Triplicate cultures per treatment are recommended. 5m

The initial cell concentration should be 2000 cells/mL, but may be altered depending on the anticipated growth.

During developing, 400 µL of stock culture at a concentration of 11000 cells/mL was added to 4 mL of growth media in each well, resulting in a final volume of 4.4 mL at a concentration of 2000 cells/mL.

The concentration of the initial stock culture was measured using flow cytometry as outlined in:

Protocol

NAME
Measuring growth rates of diatom cells in culture

CREATED BY
Phoebe Argyle

PREVIEW

The stock was then diluted or concentrated using centrifugation 1000 x g, 20°C, 00:05:00 to achieve the final concentration of 11000 cells/mL.

NB: The initial concentration of the cultures may be altered depending on anticipated growth, or the species of microalgae being used.

Cultures should be randomised within growth plates. Analysis during method development showed negligible variance due to 'plate' effects, however we recommend position plates randomly within growth incubators and changing their positions daily to minimize potential effects.

2 Seal plates with breathable seal. These can act in place of the plastic lids of desired.

Equipment

Breathe-Easy® sealing membrane	NAME
Plate seal	TYPE
Breathe-Easy®	BRAND
Z380059-1PAK	SKU
https://www.sigmaaldrich.com	LINK

Track growth

3 After inoculation, take an initial in vivo fluorescence measurement of each plate using a microplate reader as outlined in :

Protocol

NAME

Measuring growth rates of diatom cells in culture

CREATED BY

Phoebe Argyle

PREVIEW

Return plates to their experimental incubators.

4 Each day, track the in vivo fluorescence at least one hour after the onset of the photoperiod. During development this would be at 9am, after 'lights on' at 6am, following a 12:12 light cycle.

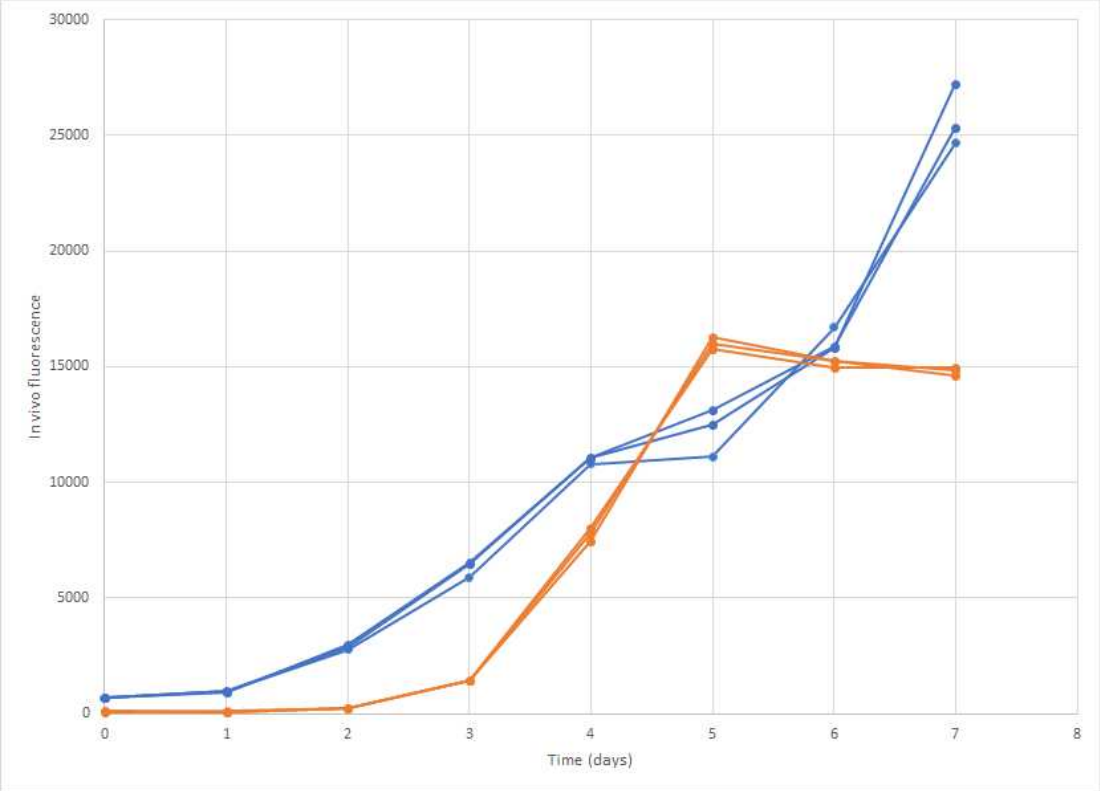
Light settings may vary depending on the nature of the experiment being conducted.

5 Track the in vivo fluorescence over time, and note the growth phase of the cultures.

Trait measurements are conducted during mid-exponential growth, so some discernment is required to estimate this stage.

For example, in this experiment, the cultures shown in blue are in exponential growth between days 2 and 7, whereas the orange cultures have a short exponential phase between days 2 and 5.

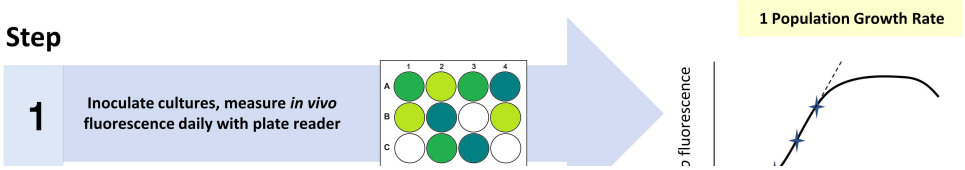
Once the experiment is harvested growth measures can no longer be taken, so if in doubt of the growth phase, an experiment of just the growth may be prudent to anticipate the best time to harvest for trait measurements.

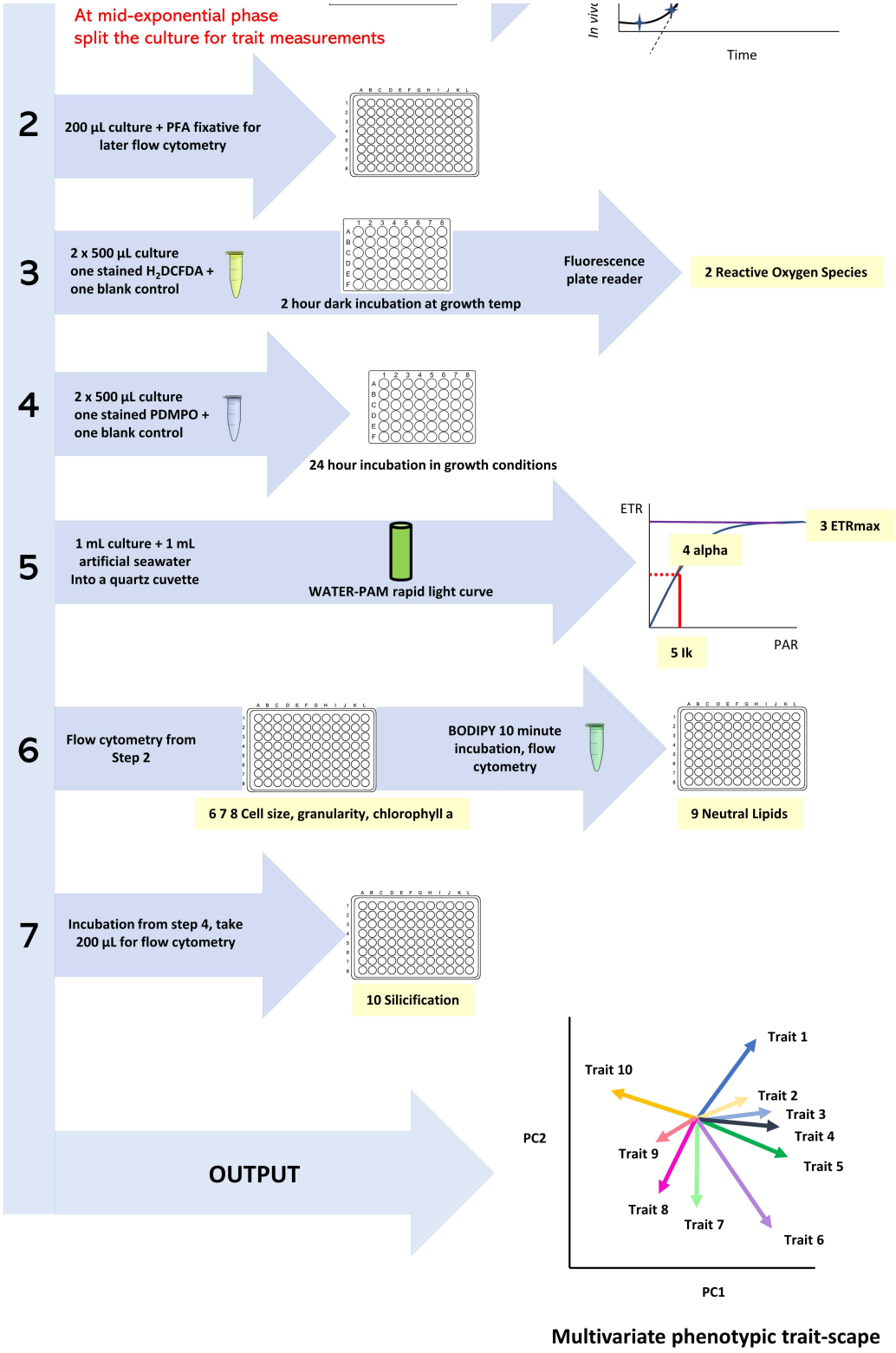


Growth of *Thalassiosira* spp. cultures as measured by in vivo fluorescence over time. The blue represent 3 biological replicates of *T. rotula* and the orange are three biological replicates of *T. pseudonana*, all grown at 30°C in f/2 media.

Trait measurements

6 Once a culture has reached mid-exponential phase, trait measurements begin according to the workflow. Note not all culture wells will be ready to harvest on any one day, creating a staggered approach.





The workflow of the Quantitative Phenotyping Assay (QPA) outlining the sequence of actions, measurements, and data outcomes. (from Argyle et al. 2021).

Flow cytometry

7 Taken an aliquot and fix for flow cytometry, according to the protocol:

Protocol

NAME

Flow cytometry trait measurements (size, granularity and chlorophyll-a) of diatoms

CREATED BY

Phoebe Argyle

PREVIEW

Reactive oxygen species

8 Initiate the Reactive oxygen species assay according to the protocol:

Protocol

NAME

Quantifying Reactive Oxygen Species in diatoms

CREATED BY

Phoebe Argyle

PREVIEW

Silicification via PDMPO

9 Initiate the silicification assay according to the protocol:

LINK TO PDMPO assay to insert after publication

9.1 On the following day, when harvesting the next days' cultures, take the aliquots from the previous days' incubation and analyse via flow cytometry (according to the protocol). As this is

done in plate-mode, these samples can be analysed while step 10 is completed.

Photophysiolgical traits

10 Measure the photophysiological traits according to the protocol:

Protocol

NAME

Measuring photophysiological traits of diatoms from Rapid Light Curves using a Water-PAM

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PREVIEW

Not that during this time, it may be necessary to return to the ROS assay and take the final measurement.

Flow cytometry traits

11 Conduct flow cytometry analysis of the fixed samples collected in the morning according to the protocol:

Protocol

NAME

Flow cytometry trait measurements (size, granularity and chlorophyll-a) of diatoms

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Phoebe Argyle

PREVIEW

12 Measure neutral lipids according to the protocol:

Protocol

NAME

Measuring neutral lipids in fixed diatom cells using BODIPY 505/515

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PREVIEW

Statistical analysis

13 Multivariate trait data can be analysed using Principal Component Analysis to generate a multivariate trait-scape, in which differences between strains or species, as well as relationships between traits, can be visualised.

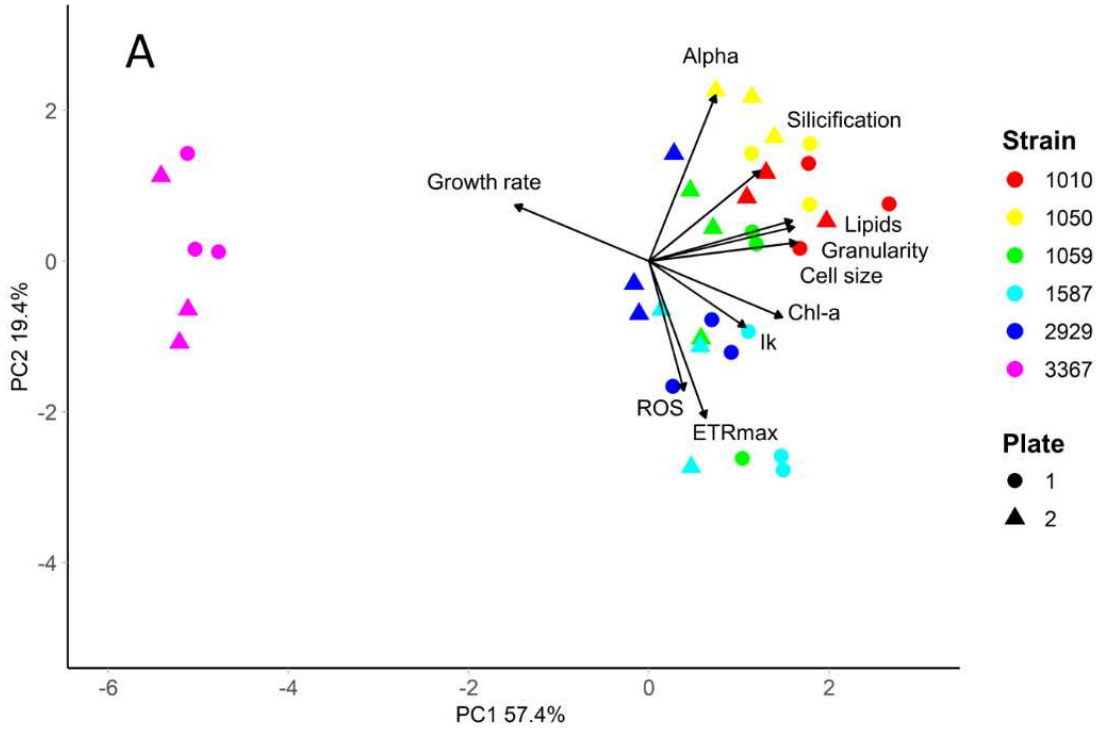

Figure 2A from Argyle et al. 2021. A trait-scape of *Thalassiosira* spp. strains grown in multi-well plates and assayed using the QPA. Shapes represent different growth plates, each point is a biological replicate.