**2. Materials & Methods**

**2.1. Study Organisms & Culturing**

Seven phytoplankton species were studied, including polar and temperate strains of diatoms and green algae. Diatoms are represented by two taxa, *Fragilariopsis cylindrus* and *Thalassiosira pseudonana*. *F. cylindrus*, a psychrophilic pennate diatom measuring 15-55 µm, thrives in the high salinity and subzero temperatures of Arctic and Antarctic sea-ice systems[1,2]. Forming large blooms in the bottom layer of sea ice and across the wider sea ice zone, *F. cylindrus* acts as a keystone and indicator species for polar ecosystems[1,3]. Conversely, *T. pseudonana* is a small (2.5-15 μm) centric diatom found worldwide in diverse freshwater, coastal, brackish, and marine habitats[4]. *T. pseudonana* can tolerate a wide range of salinities (0.5%–37%) and temperatures (4–25°C), contributing to its frequent use as a model diatom species[4].

The remaining five taxa comprise three polar and two temperate species of green algae. *Chlamydomonas ICEMDV* and *Chlamydomonas priscuii* are marine algae isolated from the perennially ice-covered hypersaline Lake Bonney in McMurdo Dry Valleys, Antarctica[5,6]. With large (15 to 20 μm) biflagellate cells, *C. ICEMDV* dominates the shallow photic zone, where it experiences higher irradiance, extreme nutrient limitation, and low salinity[5,7]. The smaller *C. priscuii* dominates the deep photic zone, characterized by permanent low temperatures and high salinity[8,9]. The final psychrophile species is *Chlamydomonas malina,* amarine microalga isolated from the Arctic Ocean's Beaufort Sea, measuring around 10 μm in length and 5 μm in width, and growing optimally at 4°C[10,11]. The temperate green algae is comprised of *Chlamydomonas reinhardtii* and *Chlorella vulgaris*. *C. reinhardtii* is a model alga approximately 10 μm in size and found in soil and aquatic environments with an optimal temperature range of 20-32°C[12,13]. Meanwhile, *C. vulgaris*, ranging from 2 μm to 10 μm in size, is primarily found in freshwater environments and grows optimally at 27°C[14,15].

*Culturing Protocols*

A total of twelve cultures comprising seven species were used, as summarized in Table 1. Cultures of *T. pseudonana* and *C. vulgaris* were prepared by Naaman Omar (Mount Allison University), *Chlamydomonas* cultures were prepared by MacKenzie Poirier (Cvetskova Lab, University of Ottawa), and *F. cylindrus* cultures were prepared by Sébastien Guérin (Takuvik International Research Laboratory, Université Laval).

Table 1: Culturing conditions for phytoplankton strains

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Culture ID | Strain | Experimental Dilution | Par\_ue | Photoperiod | Growth Temp (°C) | Wavelength | Media |
| SeGu1001 | *F. cylindrus* | 1 | 10 | 24 | 0 | White | F2 |
| SeGu1006 | *F. cylindrus* | 1 | 10 | 24 | 6 | White | F2 |
| MaPo1001 | *C. priscuii* | 0.2 | 10 | 24 | 4 | White | BBM |
| MaPo1002 | *C. ICEMDV* | 0.2 | 10 | 24 | 4 | White | BBM |
| MaPo1003 | *C. malina* | 0.2 | 10 | 24 | 4 | White | BBM |
| MaPo1004 | *C. priscuii* | 1 | 10 | 24 | 4 | Blue | BBM |
| MaPo1005 | *C. reinhardtii* | 1 | 10 | 24 | 24 |  | BBM |
| MaPo1006 | *C. reinhardtii* | 1 | 10 | 24 | 24 |  | BBM |
| NaOm1663 | *T. pseudonana* | 1 |  |  | 18 |  |  |
| NaOm1293 | *T. pseudonana* | 1 |  |  | 18 |  |  |
| NaOm1305 | *T. pseudonana* | 1 |  |  | 18 |  |  |
| NaOm1671 | *C. vulgaris* | 1 |  |  | 18 |  |  |

**2.2. Single Turnover Variable Chlorophyll Fluorescence**

A single turnover variable chlorophyll fluorescence (St-ChlF) approach was employed to evaluate the desynchronization of the s-state cycle across a range of phytoplankton species, temperatures, and light levels.

Figure 1: Experimental setup

Cultures were loaded into a temperature-controlled cuvette (PolyScience) within a Soliense LIFT-REM fluorometer (Version LIFT-REM 1.0, Soliense Inc). The apparatus was covered to block out incident light and cells were acclimated to the dark for a minimum of 30 seconds. In a dark regulated state, non-photochemical quenching processes have been fully reversed, and electrons have been passed downstream, leaving all reaction centres open for photochemistry. Therefore, when the photosystem receives a photon, the maximum proportion of energy will be partitioned to photochemistry, corresponding to minimum ChlF (Fo) [16].

The sample is then exposed to a series of short, high-intensity, evenly-spaced flashes of 445 nm light. Each flash consists of a rapid series of sub-saturating flashlets occurring on microsecond timescales [16]. These flashlets induce the absorption of light by PSII, which then passes electrons downstream to QA-, transiently reducing the pool of electron acceptors and effectively closing PSII for photochemistry[18]. Closing the photochemistry pathway redirects a greater proportion of energy to ChlF, resulting in maximum ChlF (Fm). This induction is known as the saturation phase, where the fluorescence yield increases from a minimum (Fo) to a maximum (Fm) [17]. For each flash, the ChlF minima and maxima can be used to derive the maximum quantum yield of photochemistry in PSII, a secondary ChlF parameter calculated as follows [16]:

The maximum quantum yield represents the efficiency with which PSII can convert absorbed light energy into chemical energy. Chlorophyll fluorescence parameters were fitted using LIFT software version 22.11.11 (Soliense Inc).

Each flash delivers one photon to a PSII, initiating a transition between S-states. Thus, as sequential flashes are applied to the culture, each individual PSII is driven through the four S-states [18]. In an idealized culture, the population of PSII cycles synchronously, reflected by an ongoing oscillation in chlorophyll fluorescence with a period of four [19]. However, recombination reactions, represent a loss of charge separation, causing a backward slip in the S-state cycling of an individual PSII. As more recombination events occur, desynchronization of S-state cycling among the PSII of the population will scramble the periodic changes in ChlF, dampening the observed oscillation [19]. Prolonged synchronous cycling indicates fewer wasteful recombination reactions and, thus, more efficient photosynthetic energy conversion.

Figure 2: Repeated single-turnover excitation of variable chlorophyll fluorescence for monitoring the S-state cycling in PSII during photosynthesis

*Measurement Conditions*

By evaluating the cycling of polar and temperate taxa of diatoms and green algae under a range of light and temperature conditions (Table 2), we can determine if polar taxa have evolved to increase photosynthetic energy conversion efficiency by minimizing inefficient recombination reactions.

Varying light conditions can be simulated by altering the spacing between flashes. At higher light intensities, more photons are emitted per unit of time, corresponding to shorter spacing between flashes. Cultures were evaluated at flash spacings of 1, 2, 4, 8, and 16 seconds, analogous to light levels of (do conversions). Measurement temperatures ranged from 0 to 28°C, depending on the taxa (Table 2).

Table 2: Measurement conditions by strain

|  |  |  |
| --- | --- | --- |
| Strain | Flash Spacings (s) | Temperatures (°C) |
| *Fragilariopsis cylindrus* | 1, 2, 4, 8, 16 | 0, 2, 6, 10 |
| *Thalassiosira pseudonana* | 1, 2, 4, 8, 16 | 10, 14, 18, 20, 24, 28 |
| *Chlamydomonas ICEMDV* | 1, 2, 4, 8, 16 | 4, 8, 12 |
| *Chlamydomonas priscuii* | 1, 2, 4, 8, 16 | 4, 8, 12 |
| *Chlamydomonas malina* | 1, 2, 4, 8, 16 | 4, 8, 12 |
| *Chlamydomonas reinhardtii* | 1, 2, 4, 8, 16 | 12, 16, 20, 24 |
| *Chlorella vulgaris* | 1, 2, 4, 8, 16 | 10, 14, 18, 22, 26 |

**2.3. Analytical Methods**

Data was processed using R version 4.3.2 along with RStudio version 2023.12.0+369 using the x86\_64-apple-darwin20 (64-bit) platform and running under macOS Sonoma 14.3.1. Fluorescence data generated by LIFT software was imported and tidied using the tidyverse, lubridate, and googlesheets4 packages. The tidyverse, doBy, and WaveletComp packages were used for wavelet analyses. Lastly, ggplot2 and viridis packages were used for data visualization.

*Wavelet Transformations*

Raw fluorescence data yields a time series of Fv/Fm over 32 flashes, which was analyzed for each combination of strain, temperature, and flash spacing using wavelet transformations. Unlike traditional methods, wavelet analysis does not assume that the statistical properties of a time series are constant. Instead, wavelet transformations locally decompose the signal across different time scales and estimate spectral characteristics as a function of time [20]. By examining the frequency and wavelet power spectra, we can uncover the dominant patterns in the data [21].

The core of the wavelet transformation involves computing the wavelet power spectrum of the standardized time series using the Morlet wavelet [22]. Further, the significance of the periodic components in the time series was calculated using a simulation algorithm. Surrogate time series are generated based on a white noise model, consisting of uncorrelated random values with constant mean and variance. Comparing the wavelet of the original data with the white noise model, p-values are calculated to determine whether the observed periodic components are statistically significant [22].

The statistical significance of the wavelet power at a periodicity of four signifies if the culture is exhibiting the periodic oscillations in chlorophyll fluorescence that indicate synchronous S-state cycling across the population of PSII. For wavelets exhibiting S-state cycling, we generated a reconstruction limited to areas with a statistically significant signal. Damping of the reconstructed wavelet represents the significance of the signal dropping below the threshold of p=0.05. The damping index represents the number of flashes applied before this damping occurs, indicating how many successive photons are received before sufficient recombination reactions fully desynchronize the S-state cycle.

References

1. Otte A, Winder JC, Deng L, Schmutz J, Jenkins J, Grigoriev IV, et al. The diatom Fragilariopsis cylindrus: A model alga to understand cold-adapted life. Journal of Phycology. 2023;59: 301–306. doi:10.1111/jpy.13325

2. Cefarelli AO, Ferrario ME, Almandoz GO, Atencio AG, Akselman R, Vernet M. Diversity of the diatom genus Fragilariopsis in the Argentine Sea and Antarctic waters: morphology, distribution and abundance. Polar Biology. 2010;33. doi:10.1007/s00300-010-0794-z

3. Kang S-H, Fryxell GA. Fragilariopsis cylindrus (Grunow) Krieger: The most abundant diatom in water column assemblages of Antarctic marginal ice-edge zones. Polar Biol. 1992;12: 609–627. doi:10.1007/BF00236984

4. Poulsen N, Kröger N. Thalassiosira pseudonana (Cyclotella nana) (Hustedt) Hasle et Heimdal (Bacillariophyceae): A genetically tractable model organism for studying diatom biology, including biological silica formation. Journal of Phycology. 2023;59: 809–817. doi:10.1111/jpy.13362

5. Cook G, Teufel A, Kalra I, Li W, Wang X, Priscu J, et al. The Antarctic psychrophiles Chlamydomonas spp. UWO241 and ICE-MDV exhibit differential restructuring of photosystem I in response to iron. Photosynth Res. 2019;141: 209–228. doi:10.1007/s11120-019-00621-0

6. Stahl-Rommel S, Kalra I, D’Silva S, Hahn MM, Popson D, Cvetkovska M, et al. Cyclic electron flow (CEF) and ascorbate pathway activity provide constitutive photoprotection for the photopsychrophile, Chlamydomonas sp. UWO 241 (renamed Chlamydomonas priscuii). Photosynth Res. 2022;151: 235–250. doi:10.1007/s11120-021-00877-5

7. Li W, Podar M, Morgan-Kiss RM. Ultrastructural and Single-Cell-Level Characterization Reveals Metabolic Versatility in a Microbial Eukaryote Community from an Ice-Covered Antarctic Lake. Applied and Environmental Microbiology. 2016;82: 3659–3670. doi:10.1128/AEM.00478-16

8. Cvetkovska M, Vakulenko G, Smith DR, Zhang X, Hüner NPA. Temperature stress in psychrophilic green microalgae: Minireview. Physiologia Plantarum. 2022;174: e13811. doi:10.1111/ppl.13811

9. Hüner NPA, Szyszka-Mroz B, Ivanov AG, Kata V, Lye H, Smith DR. Photosynthetic adaptation and multicellularity in the Antarctic psychrophile, Chlamydomonas priscuii. Algal Research. 2023;74: 103220. doi:10.1016/j.algal.2023.103220

10. Balzano S, Gourvil P, Siano R, Chanoine M, Marie D, Lessard S, et al. Diversity of cultured photosynthetic flagellates in the northeast Pacific and Arctic Oceans in summer. Biogeosciences. 2012;9: 4553–4571. doi:10.5194/bg-9-4553-2012

11. Morales-Sánchez D, Schulze PSC, Kiron V, Wijffels RH. Temperature-Dependent Lipid Accumulation in the Polar Marine Microalga Chlamydomonas malina RCC2488. Frontiers in Plant Science. 2020;11. doi:10.3389/fpls.2020.619064

12. Sasso S, Stibor H, Mittag M, Grossman AR. From molecular manipulation of domesticated Chlamydomonas reinhardtii to survival in nature. King SR, Rodgers PA, editors. eLife. 2018;7: e39233. doi:10.7554/eLife.39233

13. Xie B, Bishop S, Stessman D, Wright D, Spalding MH, Halverson LJ. Chlamydomonas reinhardtii thermal tolerance enhancement mediated by a mutualistic interaction with vitamin B12-producing bacteria. The ISME Journal. 2013;7: 1544–1555. doi:10.1038/ismej.2013.43

14. Wiel JBV, D. Mikulicz J, R. Boysen M, Hashemi N, Kalgren P, M. Nauman L, et al. Characterization of Chlorella vulgaris and Chlorella protothecoides using multi-pixel photon counters in a 3D focusing optofluidic system. RSC Advances. 2017;7: 4402–4408. doi:10.1039/C6RA25837A

15. Leyva LA, Bashan Y, Mendoza A, de-Bashan LE. Accumulation fatty acids of in Chlorella vulgaris under heterotrophic conditions in relation to activity of acetyl-CoA carboxylase, temperature, and co-immobilization with Azospirillum brasilense. Naturwissenschaften. 2014;101: 819–830. doi:10.1007/s00114-014-1223-x

16. Schuback N, Tortell PD, Berman-Frank I, Campbell DA, Ciotti A, Courtecuisse E, et al. Single-Turnover Variable Chlorophyll Fluorescence as a Tool for Assessing Phytoplankton Photosynthesis and Primary Productivity: Opportunities, Caveats and Recommendations. Frontiers in Marine Science. 2021;8. doi:10.3389/fmars.2021.690607

17. Berman-Frank I, Campbell D, Ciotti A, Erickson Z, Fujiki T, Halsey K, et al. Application of Single Turnover Active Chlorophyll Fluorescence for Phytoplankton Productivity Measurements. Version 2.0. Scientific Committee on Oceanic Research (SCOR) Working Group 156; 2023 Jun. doi:10.25607/OBP-1084

18. Dau H, Haumann M. Time-resolved X-ray spectroscopy leads to an extension of the classical S-state cycle model of photosynthetic oxygen evolution. Photosynth Res. 2007;92: 327–343. doi:10.1007/s11120-007-9141-9

19. de Wijn R, van Gorkom HJ. S-state dependence of the miss probability in Photosystem II. Photosynthesis Research. 2002;72: 217–222. doi:10.1023/A:1016128632704

20. Cazelles B, Chavez M, Berteaux D, Ménard F, Vik JO, Jenouvrier S, et al. Wavelet analysis of ecological time series. Oecologia. 2008;156: 287–304. doi:10.1007/s00442-008-0993-2

21. Theis FJ, Meyer-Bäse A. Spectral Transformations. 1st ed. Biomedical Signal Analysis : Contemporary Methods and Applications. 1st ed. MIT Press; 2010. p. 42.

22. Roesch A, Schmidbauer H. WaveletComp: Computational wavelet analysis. 2018. Available: https://CRAN.R-project.org/package=WaveletComp