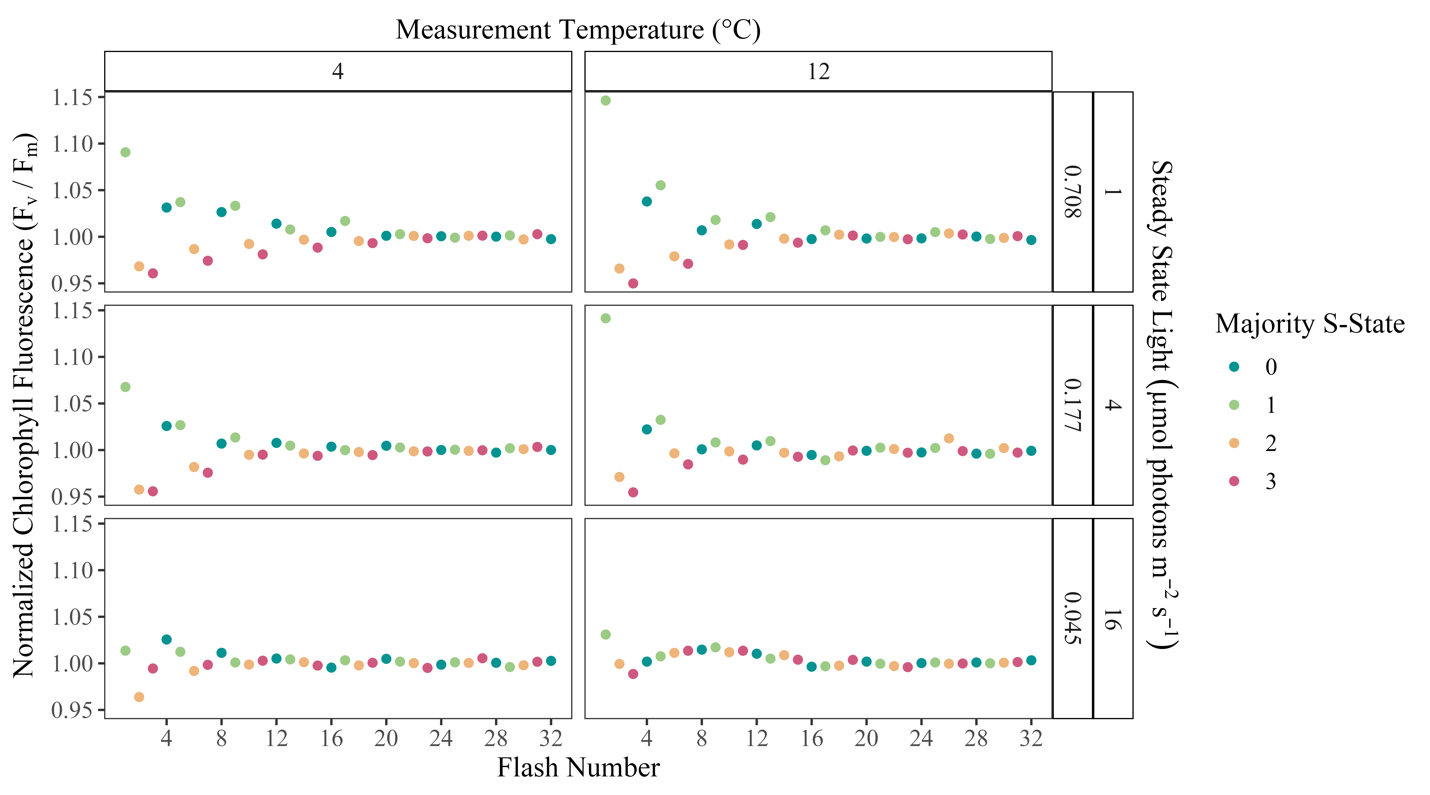
**Results**

**3.1. Single Turnover Variable Chlorophyll Fluorescence**

Exposing phytoplankton cultures to a series of 32 successive flashes produced oscillations in the maximum quantum yield of photochemistry in PSII, as estimated through the secondary chlorophyll fluorescence parameter FV/FM. As shown in Figure 1 for the polar alga *C. priscuii,* the time series of FV/FM over successive flashes reveals consistent variations in fluorescence yield as the predominant s-states follow each other across the PSII within the population, starting with S0 in the dark relaxed cells (CITATION). The amplitude of the ChlF oscillations, however, declines progressively over time, and with wider spacing of sequential flashes, equivalent to decreasing light levels.



**Figure 1:** Oscillations in the maximum quantum yield of the photochemistry in *Chlamydomonas priscuii* cultures over a series of 32 flashes, as measured through the secondary chlorophyll fluorescence parameter FV/FM. Representative conditions include cultures measured at 4 or 12 °C, with flash spacings of 8 – 1 seconds, equivalent to steady-state light levels of 0.088 – 0.708 µmol photons m-2s-1.

**3.2. Wavelet Analysis**

Wavelet transformations were computed for the fluorescence time series of each unique combination of measurement temperature, flash spacing (equivalent to steady-state light level), and strain. Assessing the wavelet power of a 4-step periodicity across conditions, key trends emerge. As exemplified by the polar alga *C. priscuii* (Figure 2A), the average wavelet power declines with increasing measurement temperature and decreasing measurement light. While this trend remains in the temperate taxa (Figure 2B), the wavelet power is consistently lower, suggesting weaker 4-step periodicity in temperate taxa, and a sharper decrease in periodicity with increasing measurement temperature and with decreasing measurement temperature.

**A**

A diagram of a temperature measurement

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**B**

A chart of a temperature measurement

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**Figure 2:** Sample plot of wavelet power by period of oscillations in the maximum quantum yield of photochemistry in A. Polar *Chlamydomonas priscuii* and B. Temperate *Chlamydomonas reinhardtii* cultures across a range of measurement temperatures and flash spacings, with the equivalent steady-state light levels.

Polar taxa maintained significant 4-step oscillations in Fv/Fm , and thus stronger synchronization of PSII S-State cycling across a broader range of conditions, than did their temperate counterparts (Figure 3). The temperate diatom *T. pseudonana* showed significant S-State cycling across 20.57 % of measured conditions, compared to 85% of measured conditions in its polar counterpart, *F. cylindrus*. Similarly, the temperate green algae *C. vulgaris* and *C. reinhardtii* displayed significant cycling across 32% and 40 % of conditions. Meanwhile, significant cycling occurred in 60, 73, and 80% of measurement conditions for the polar algae *C. ICEMDV*, *C. malina*, and *C. priscuii*.

A graph of different temperature levels

Description automatically generated with medium confidence

**Figure 3:** Statistical significance of 4-step oscillations in the maximum quantum yield of PSII photochemistry across polar and temperate phytoplankton taxa, as measured through variable chlorophyll fluorescence.

**3.3. Generalized Additive Modelling** *By Deviation from Growth Temperature*

Predictions from generalized additive modelling were generated for the damping of S-state-induced chlorophyll fluorescence oscillations, as predicted by the tensor product smooth of the deviation from growth temperature (°C) and the equivalent steady-state measurement light level (µmol photons m-2s-1; Table 1) for each taxa.

Table 1: Summary statistics by phytoplankton strain of GAM models using the restricted maximum likelihood method to model the response of the damping of S-state-induced chlorophyll fluorescence oscillations to the predictors of deviation from growth temperature (°C) and the steady-state light level (µmol photons m-2s-1).

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Strain | Significance of smooth term | | | Adjusted R2 | Deviance Explained |
| EDF | F-ratio | P-value |
| *F. cylindrus* | 7.23 | 6.82 | 2.8e-05 | 0.584 | 66.1% |
| *T. pseudonana* | 6.08 | 9.01 | 5.38e-06 | 0.674 | 73.2% |
| *C. ICEMDV* | 4.51 | 6.59 | 0.00687 | 0.687 | 78.8% |
| *C. priscuii* | 4.31 | 2.17 | 0.115 | 0.389 | 57.7% |
| *C. malina* | 4.34 | 2.56 | 0.0843 | 0.423 | 60.2% |
| *C. reinhardtii* | 6.05 | 9.49 | 0.000274 | 0.786 | 85.4% |
| *C. vulgaris* | 5.39 | 13.83 | 4.08e-06 | 0.772 | 82.3% |

The smoothing term was significant for the polar diatom *F. cylindrus* (F = 6.835, p = 2.8e-05), explaining 66.1% (adjusted R2 = 0.584) of the deviance in the damping index. Similarly, the model of the temperate diatom, *T. pseudonana*, produced a significant smoothing term (F = 9.01, p = 5.38e-06), which accounted for 73.2% of the deviance in the damping index (adjusted R2 = 0.674). These models were used to predict the number of consecutive flashes before the damping of ChlF oscillations for each strain at each combination of temperature deviation and light level (Figure 4).

Both diatom taxa exhibited the longest predicted periodic oscillations in ChlF at higher light levels and lower temperatures. Notably, the polar *F. cylindrus* sustained cycling longer than its temperate counterpart, *T. pseudonana*, under comparable conditions. This disparity was particularly prevalent above the growth temperature and under low steady-state light (Figure 4). Under these conditions, *T. pseudonana* cultures did not retain the significant 4-step oscillation in ChlF indicative of synchronized S-state cycling.

A diagram of a heat wave

Description automatically generated

**Figure 4**: GAM model predictions of the consecutive flashes before the damping of S-state-induced chlorophyll fluorescence oscillations as predicted by the deviation from growth temperature (°C) during measurements, and the steady-state measurement light level (µmol photons m-2s-1), experienced by polar and temperate diatoms.

The GAM outputs varied more among algal strains (Table 1). The smoothing term was significant for the temperate strains *C. reinhardtii* (F = 9.49, p = 0.000274) and *C. vulgaris* (F = 13.83, p = 4.08e-06), predicting 85.4% (adjusted R2 = 0.786) and 82.3% (adjusted R2 = 0.772) of the deviance in the damping index, respectively. However, while the smoothing term was significant (F = 6.59, p = 0.00687), predicting 78.8% (adjusted R2 = 0.687) of the deviance for the polar *C. ICEMDV*, it was not significant for the other two polar taxa. For *C. priscuii*, the statistical evaluation of the smoothing term produced an F value of 2.17 and a corresponding p-value of 0.115, explaining only 57.7% (adjusted R2 = 0.389) of the deviance in the response variable. Similarly, the non-significant smoothing term for *C. malina* (F = 2.56, p = 0.0843) only explained 60.2% of the deviance in the damping index (adjusted R2 = 0.423).

Overall, model predictions for algal strains exhibited a similar pattern to the diatoms, with the longest predicted periodic oscillations in ChlF at higher measurement light levels and lower measurement temperatures relative to growth temperature (Figure 5). The trends shown by the three strains of polar algae are remarkably consistent, displaying virtually identical patterns and differing by only 1-2 flashes before S-state damping occurred, under identical conditions (Figure 5). Further, much like the temperate diatoms, the temperate algae *C. reinhardtii* and *C. vulgaris* did not exhibit significant periodic oscillations in ChlF around their growth temperature, under low steady-state measurement light (Figure 5).

A diagram of a light level

Description automatically generated with medium confidence

**Figure 5**: GAM model predictions of the consecutive flashes before the damping of S-State-induced chlorophyll fluorescence oscillations as predicted by the deviation from growth temperature (°C) and the equivalent measurement light level (µmol photons m-2s-1) experienced by polar and temperate green algae.

**3.3. Generalized Additive Modelling** *By Measurement Temperature*

To facilitate comparison across actual measurement temperatures, additional generalized additive models were fit to the data. For *T. pseudonana, C. ICEMDV, C. priscuii, C. malina, C. reinhardtii,* and *C. vulgaris,* replicate cultures of the strain were grown at the same temperatures. Therefore, the GAMs generated for these strains encompass a different temperature range but are represented by the same summary statistics (Table 2). Conversely, the polar diatom *F. cylindrus* was cultured at both 0 and 6°C. Thus, separate models were fit for each *F. cylindrus* culture to account for possible physiological differences resulting from the distinct growth conditions.

The smoothing term was significant for both *F. cylindrus* cultures (0°C F = 4.46, p = 0.00827; 6°C F = 3.42, p = 0.0279). However, while the model accounted for 73.4% of the deviance in the damping response of *F. cylindrus* grown at 0°C (adjusted R2 = 0.609), it only accounted for 65.1% (adjusted R2 = 0.509) in *F. cylindrus* grown at 6°C (Table 2).

Table 2: Summary statistics by phytoplankton strain of GAM models using the restricted maximum likelihood method to model the response of the damping of S-state-induced chlorophyll fluorescence oscillations to the predictors of measurement temperature (°C) and the steady-state light level (µmol photons m-2s-1).

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Strain | Significance of smooth term | | | Adjusted R2 | Deviance Explained |
| EDF | F-ratio | P-value |
| *F. cylindrus* (grown at 0°C) | 6.06 | 4.46 | 0.00827 | 0.609 | 73.4% |
| *F. cylindrus* (grown at 6°C) | 5.49 | 3.42 | 0.0279 | 0.509 | 65.1% |
| *T. pseudonana* | 6.08 | 9.01 | 5.38e-06 | 0.674 | 73.2% |
| *C. ICEMDV* | 4.51 | 6.59 | 0.00687 | 0.687 | 78.8% |
| *C. priscuii* | 4.31 | 2.17 | 0.115 | 0.389 | 57.7% |
| *C. malina* | 4.34 | 2.56 | 0.0843 | 0.423 | 60.2% |
| *C. reinhardtii* | 6.05 | 9.49 | 0.000274 | 0.786 | 85.4% |
| *C. vulgaris* | 5.39 | 13.83 | 4.08e-06 | 0.772 | 82.3% |

Although measured at the same range of temperatures, the two *F. cylindrus* cultures from different growth temperatures exhibited different trends in ChlF oscillations. First, the culture grown at 0°C exhibited significant S-State cycling across only 75% of the evaluated conditions, compared to significant S-State cycling across 95% of evaluated conditions for the culture grown at 6°C (Figure 6). Further, at comparable measurement temperatures under wide flash spacing, equivalent to low light, the culture grown at 6°C maintained significant cycling for longer (Figure 7). At flash spacings equivalent to light levels above 0.2 µmol photons m-2s-1, cultures from both 0 and 6°C growth temperatures exhibited similar, stable damping indices (Figure 7).

A graph of temperature and growth

Description automatically generated with medium confidence

**Figure 6:** Statistical significance of 4-step oscillations in the maximum quantum yield of PSII photochemistry across measurement conditions in the polar diatom *F. cylindrus*, as measured through variable chlorophyll fluorescence.

A diagram of different types of heat

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**Figure 7**: GAM model predictions of the consecutive flashes before the damping of S-state-induced chlorophyll fluorescence oscillations as predicted by the measurement temperature (°C) and the steady-state light level (µmol photons m-2s-1) experienced by polar and temperate diatoms. White dashed lines represent the growth temperatures of the individual cultures.

At a measurement temperature of 10°C, the model predicts that the duration of S-State cycling for *F. cylindrus* will sharply increase with steady-state light before plateauing at approximately 11 flashes around 0.2 µmol photons m-2s-1 (Figure 8). At light levels below 0.5 µmol photons m-2 s-1, this corresponds to *F. cylindrus* exhibiting more sustained S-State cycling than does *T. pseudonana* also measured at 10°C. However, at higher equivalent measurement light levels, at this shared measurement temperature, *T. pseudonana* sustains S-state cycling for durations comparable to its polar counterpart *F. cylindrus* (Figure 8).

A graph of light level

Description automatically generated**Figure 8:** GAM model predictions of the consecutive flashes before the damping of S-state-induced chlorophyll fluorescence oscillations in polar and temperate diatom taxa at the common measurement temperature of 10°C, as predicted by the equivalent light level (µmol photons m-2s-1) predicted from flash spacing.

For the green algae, the models using measurement temperature as a predictor depicted the same trends between and within strains as the models using the deviation from growth temperature as a predictor. (Figure 9).

However, the model predictions at a shared measurement temperature of 12°C demonstrate limited variation among taxa (Figure 10). For all strains, the duration of S-State cycling increased with the equivalent measurement light level. Two polar strains, *C. priscuii* and *C. malina*, and the temperate *C. reinhardtii* showed near-identical trajectories. While the temperate *C. vulgaris* exhibits less sustained S-State cycling at low equivalent measurement light, it achieves similar cycling durations to the *Chlamydomas* strains by a measurement equivalent light of approximately 0.3 µmol photons m-2s-1. Unexpectedly, the polar alga *C. ICEMDV* consistently exhibits the least sustained S-state cycling across all measurement equivalent light levels (Figure 10).

A diagram of different colors

Description automatically generated

**Figure 9**: GAM model predictions of the consecutive flashes before the damping of S-state-induced chlorophyll fluorescence oscillations (Damping Index; colour), as predicted by the measurement temperature (°C) and the steady-state light level (µmol photons m-2s-1) experienced by polar and temperate green algae. White dashed lines represent the growth temperatures of the individual cultures.

A graph of different colored lines

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**Figure 10:** GAM model predictions of the consecutive flashes before the damping of S-state-induced chlorophyll fluorescence oscillations in polar and temperate green algae strains at the shared measurement temperature of 12°C, as predicted by the steady-state light level (µmol photons m-2s-1).