

## Supplementary Information

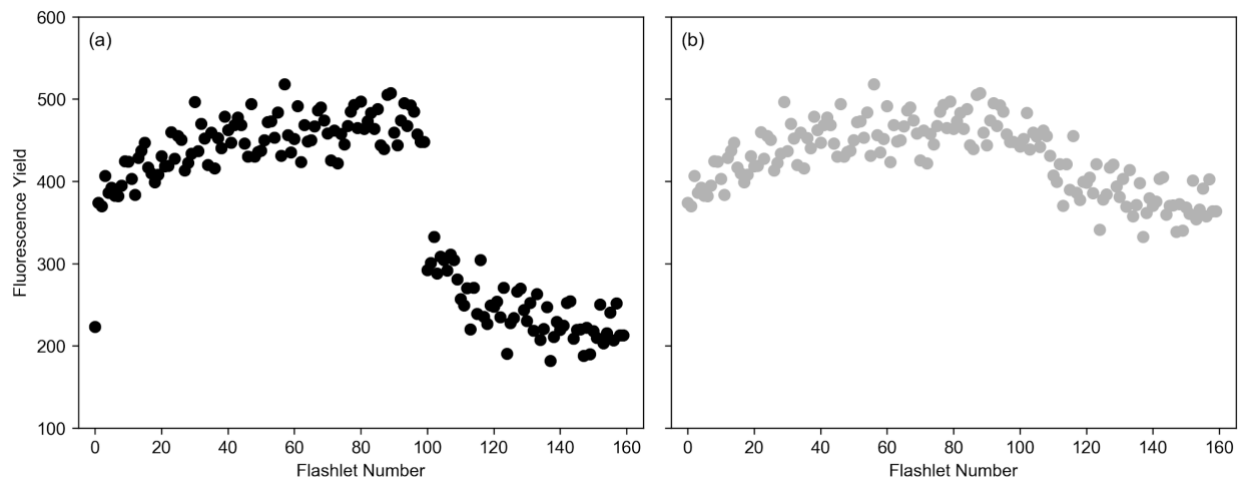


Figure S1: (a) Example induction curves from the FIRE instrument collected during the ACE voyage demonstrating the bias in the saturation and relaxation phases as a result of mismatch between the LED excitation power and reference excitation file. Figure (b) demonstrates ‘bias’ corrected induction curve after application of the skip option in *saturation.fit\_saturation* and *tools.correct\_fire\_instrument\_bias..*

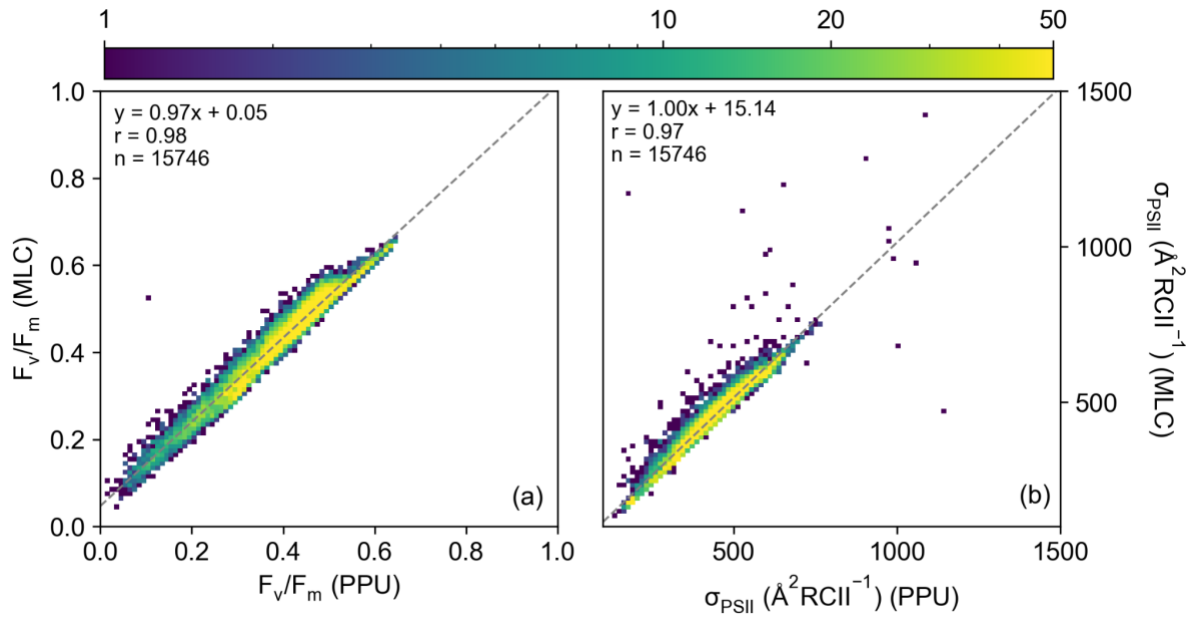


Figure S2: 2D histograms of  $F_v/F_m$  (a) and  $\sigma_{PSII}$  (b) from the modified Laney code (y-axis; Moore et al., 2007) versus PPU (x-axis) derived from the FastTracka I D350 (North Atlantic) dataset fit with the ‘ $\rho$ ’ model fitting routine.

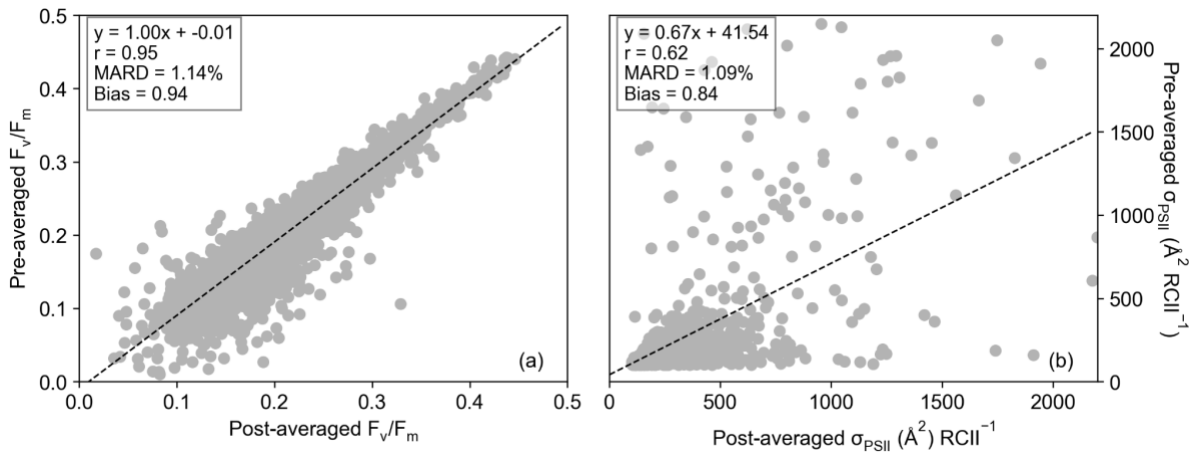


Figure S3: Plot of post-averaged (x-axis) vs pre-averaged (y-axis)  $F_v/F_m$  (a) and  $\sigma_{PSII}$  (b) from the FIRE instrument with the ‘ $\rho$ ’ model fitting routine.

Table S1: Options available for different fitting options and provided statistical metrics on the fit.

|                 | $R^2$ | p-value | $\chi^2$ | RMSE | nRMSE | Bias | Parameter Error (i.e. $F_0$ error) |
|-----------------|-------|---------|----------|------|-------|------|------------------------------------|
| <i>PPU</i>      |       |         |          | ✓    | ✓     | ✓    | ✓                                  |
| <i>Laney</i>    |       |         | ✓        |      |       |      |                                    |
| <i>FIREPro</i>  |       |         |          |      |       |      |                                    |
| <i>FIRECom</i>  |       |         |          | ✓    |       |      |                                    |
| <i>FIREWORX</i> | ✓     | ✓       |          |      |       |      | ✓                                  |
| <i>FastPro</i>  |       |         |          |      |       |      |                                    |
| <i>FastPro8</i> |       |         |          |      |       |      |                                    |
| <i>LIFT-FRR</i> |       |         | ✓        |      |       |      |                                    |
| <i>RunSTAF</i>  |       |         |          |      |       |      |                                    |

Table 2: Functions available in *Phytoplankton Photophysiology Utilities*.

| Function                            | Description                                                                                                                                                                        |
|-------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| calculate_blank_FIRe                | Calculates the blank from a discrete SATlantic FIRe file                                                                                                                           |
| calculate_blank_FastOcean           | Calculates the blank value from a CTG FASTast <sup>Ocean</sup> data output. This function can also calculate blanks from the CTG FAST <sup>Tracka</sup> II files as well.          |
| calculate_chl_specific_absorption   | Calculates either the chlorophyll specific or phytoplankton specific from raw absorbance                                                                                           |
| calculate_amplitude_etr             | Calculates irradiance dependent and independent electron transport rates                                                                                                           |
| calculate_instrument_led_correction | Calculates the instrument spectral correction factor                                                                                                                               |
| calculate_npq                       | Calculates non-photochemical quenching using either the Stern-Volner or normalised Stern-Volner derivation                                                                         |
| correct_fire_instrument_bias        | Corrects the large gap in the fluorescence yield between the end of the saturation phase and start of the relaxation phase                                                         |
| fit_saturation                      | Fits the saturation model of Kolber et al. (1998) with either no connectivity coefficient, a fixed value of the connectivity coefficient or an estimated connectivity coefficient. |
| fit_relaxation                      | Fits either the single or triple decay relaxation model of Kolber et al. (1998)                                                                                                    |
| load_FASTTrackaI_files              | Loads files from a CTG FAST <sup>Tracka</sup> I that have                                                                                                                          |

|                                  |                                                                                                                                                                                                                                                                                                                                                                                        |
|----------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
|                                  | been converted from binary format to txt format                                                                                                                                                                                                                                                                                                                                        |
| load_FIRe_files                  | Loads files from a SAtlantic benchtop FIRe with file format .000                                                                                                                                                                                                                                                                                                                       |
| load_FastOcean_files             | Loads files from a CTG FAST <sup>Ocean</sup> that have been copied (CTRL+U) into a .csv file. Additional options allow the user to load in a single acquisition and calculate the fits on each transient as opposed to the instrument averaged transient. This function can also handle FAST <sup>Tracka</sup> II files that have undergone conversion within FastPro8 <sup>TM</sup> . |
| load_LIFT_FRR_files              | Loads excel files from a Soliense LIFT FRR                                                                                                                                                                                                                                                                                                                                             |
| plot_fluorescence_light_curve    | Plots the fluorescence light curve data                                                                                                                                                                                                                                                                                                                                                |
| plot_relaxation_data             | Plots the relaxation phase data                                                                                                                                                                                                                                                                                                                                                        |
| plot_saturation                  | Plots the saturation phase data                                                                                                                                                                                                                                                                                                                                                        |
| remove_outlier_from_time_average | Averages acquisitions together using time windows whilst removing outliers                                                                                                                                                                                                                                                                                                             |

Table S3: Suggested upper and lower bounds for input into PhytoPhotoUtils saturation and relaxation fitting routines.

| Parameter                                           | Definition                                                                                          | Lower Bound | Upper Bound | Reference                                                                                                                                                                                    |
|-----------------------------------------------------|-----------------------------------------------------------------------------------------------------|-------------|-------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| $\sigma_{PSII}$ ( $\text{\AA}^2 \text{RCII}^{-1}$ ) | Functional absorption cross section of PSII                                                         | 100         | 2000        | Kolber et al. 1998, Behrenfeld & Kolber 1999, Moore et al. 2003, Suggett et al. 2004, Moore et al. 2005, Moore et al. 2006b, Suggett et al. 2006a, Suggett et al. 2006b, Suggett et al. 2009 |
| $\rho$                                              | Connectivity coefficient; probability of energy transfer between individual photosystems            | 0.001       | 1           | Babin et al. 2008, Suggett et al. 2001, Suggett et al. 2004                                                                                                                                  |
| $\tau_1$ ( $\mu\text{s}$ )                          | Time constants of the re-oxidation of PSII, equivalent to electron transport between $Q_A$ to $Q_B$ | 100         | 800         | Kolber & Falkowski 1993, Kolber et al. 1998, Suggett et al. 2009                                                                                                                             |

|                       |                                                                                                     |     |      |                                                                     |
|-----------------------|-----------------------------------------------------------------------------------------------------|-----|------|---------------------------------------------------------------------|
| $\tau_2(\mu\text{s})$ | Time constant of the re-oxidation of PSII equivalent to electron transport between $Q_B$ to PQ pool | 800 | 2000 | Kolber et al. 1998                                                  |
| $\tau_3(\text{ms})$   | Time constant of the re-oxidation of PSII equivalent to electron transport between PQ pool to PSI   | 2   | 50   | Kolber & Falkowski 1993, Kolber et al. 1998, Behrenfeld et al. 2006 |
| $\alpha$              | Amplitude of the decay phase of the fluorescence transient                                          | 0   | 1    | Kolber et al. 1998                                                  |

Table S4: Upper and lower limits and initial estimates (where set) for derived parameters as preset in Laney V6, modified Laney V6 and FastPro8 v1.0.55. See Table S3 for parameter definitions.

| Parameter                                       | Laney V6                                                                            | Modified Laney V6                                          | FastPro8 v1.0.55                                                                                 |
|-------------------------------------------------|-------------------------------------------------------------------------------------|------------------------------------------------------------|--------------------------------------------------------------------------------------------------|
| $F_o$                                           | Initial: Mean first 3 saturation flashlets<br>Bounds: $\pm 50\%$<br>If $< 0 = 0.01$ | Initial: Mean first 3 saturation flashlets<br>Bounds: None | Initial: Intercept of the linear regression through first 8 saturation flashlets<br>Bounds: None |
| $F_m$                                           | Initial: Mean last 9 saturation flashlets<br>Bounds: $\pm 50\%$                     | Initial: Mean last 9 saturation flashlets<br>Bounds: None  | Initial: Linear regression through last 24 saturation flashlets<br>Bounds: None                  |
| $\sigma_{PSII}$ ( $\text{\AA}$ quanta $^{-1}$ ) | Bounds: 0-3000<br>Initial: 1                                                        | Initial: 500<br>Bounds: None                               |                                                                                                  |
| $\rho$                                          | Initial: 0.0001<br>Bounds: None                                                     | Initial: 0.2<br>Bounds: None                               | Bounds: 0-0.4                                                                                    |

## References

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