

was lower than for nighttime, f -independent underway CF measurements (0.74 vs. 0.90 in Figs. 5A and 3A, respectively).

This may also explain the only marginal improvement in the $CF(PY^{-1}-1)$ correlation with HPLC CC as compared to the CF correlation for the dark-adapted samples ($R^2 = 0.83$ vs. 0.80 in Figs. 5B and 3B, respectively). If our interpretation is correct, the PQ variability in CF was minimized by using PSII saturating excitation, but the CF magnitudes in the morning samples were still moderately NPQ -affected because of their incomplete recovery from NPQ (empty dots in Fig. 3B). This “leftover” NPQ effect was not manifested in the $CF(PY^{-1}-1)$ parameter, but the overall transect variability in the PSII photochemical functionality might have affected the $CF(PY^{-1}-1)/CC$ relationship.

Similar comparisons for the CF/PY parameter revealed significant improvements in correlations with HPLC CC retrievals for both the underway and sample measurements: $R^2 = 0.93$ vs. -0.22 in Figs. 5C and 3A; $R^2 = 0.96$ vs. 0.80 in Figs. 5D and 3B. Though the use of CF/PY parameter was not justified by the simplified biophysical model and needs further consideration, the CF/PY ratio may be advantageous vs. the $CF(PY^{-1}-1)$ parameter for minimizing the effects of both NPQ and PQ variability on the accuracy of CC fluorescence assessments. For evaluation, the linear regression relationship $CC = 1.88CF_U/PY_U$ between CC and underway fluorescence measurements at the sampling locations (Fig. 5C) was used to calculate the CC transect distribution (light green line Fig. 2). As evident from comparison with the independent CC sample measurements, the concurrent CF and PY measurements provided accurate high-resolution CC data despite the significant NPQ and PSII physiological variability in the water masses.

7. Conclusion

The biophysical analysis and field measurements show that significant (up to 15-fold) photo-physiological variability in fluorescence yield is one of the major factors contributing to the overall variability in *in vivo* chlorophyll fluorescence per unit of chlorophyll concentration. The fluorescence yield and PSII photochemical efficiency are controlled by PQ and NPQ mechanisms and depend on incident light intensity, phytoplankton “light history”, PSII photochemical functionality, and other physiological factors. Minimizing the PQ and NPQ magnitudes can help to reduce the variability and improve the accuracy of CC fluorescence assessments. This can be achieved via isolation of the measurement volume from ambient light, PSII saturating fluorescence excitation, optimization of phytoplankton exposure to the excitation, and phytoplankton dark adaptation before the measurements. If the measurement conditions do not allow for dark adaptation (e.g., *in situ* or flow-through underway measurements from a moving platform), concurrent measurements of variable fluorescence can be used to adjust fluorescence intensity for non-photochemical quenching developed due to prior exposure to ambient light. The field evaluation in estuarine waters of the Chesapeake and Delaware Bays showed significant potential of this approach for improved fluorescence assessments of chlorophyll concentration. Nonetheless, it needs evaluation in more diverse coastal and offshore oceanic waters. An improved biophysical model needs to be developed to account for the complexity of the photo-physiological mechanisms involved.