S2. Spectral reconstruction of phytoplankton absorption spectra

Chlorophyll a-specific spectral light absorption by phytoplankton ($a^*_{phy}(\lambda)$, m^2 mg $chla^{-1}$) is most commonly measured from in situ samples using the quantitative filter-pad technique (QFT) (e.g. Mitchell 1989). It can also be derived using spectral reconstruction of photosynthetic pigments, measured using HPLC analysis. This latter approach allows $a^*_{phy}(\lambda)$ to be separated into two components representing absorption by photosynthetic and photoprotective pigments ($a^*_{psp}(\lambda)$ and $a^*_{ppc}(\lambda)$, respectively) (e.g. Bidigare et al. 1990; Babin et al. 1996; Allali et al. 1997; Moore et al. 2006; Letelier et al. 2017).

In the present study we utilized a spectral reconstruction approach, using HPLC derived pigment concentration data and weight specific in vivo absorption (wsa) spectra for each pigment group as provided in Bidigare et al. (1990):

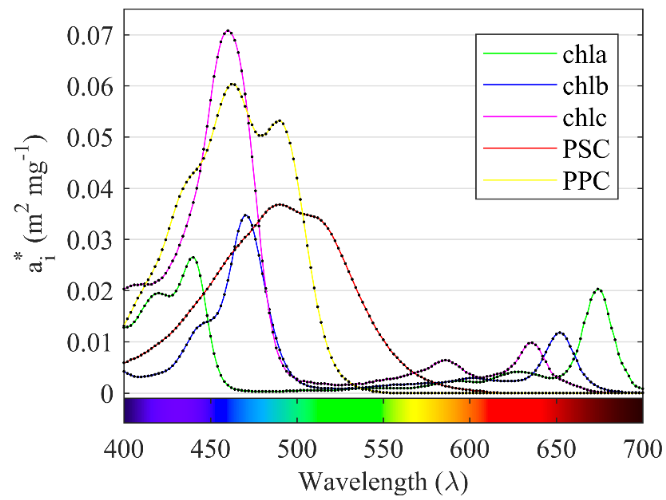

Figure S2.1: Weight-specific absorption data for different pigments and pigment groups in vivo (i.e. peak wavelengths are shifted to in vivo values, but absorption values are still for dissolved pigments in vitro without correction for packaging effects). Pigment groups are defined as: chla = total chlorophyll a; chlc = chlorophyll c1c2 + c3; photosynthetic carotenoids (PSC) = fuco + 19BF + 19HF + peri + prasino; photoprotective carotenoids (PPC) = ddx + allo + zeax + caro. Pigments for which no data were available are dtx + lut + viola + neo, which comprise ~2% of total pigment in our HPLC data.

Pigment group concentration (c_i) and weight-specific absorption ($a^*i(\lambda)$) were used to construct phytoplankton absorption spectra as:

$$a_{phy}(\lambda) = \sum a_i^*(\lambda) \cdot c_i \quad \text{(Eq. S2.1)}$$

Absorption spectra reconstructed from pigment concentrations in this way do not include the flattening of the spectrum due to pigment packaging. The amount of pigment packaging can be estimated as described by Le et al. (2009) and Letelier et al. (2017), where packaging is estimated following Morel and Bricaud (1981) as:

$$Q_a^*(\lambda) = \frac{3}{2\rho'(\lambda)} \left(1 + \frac{2e^{-\rho'(\lambda)}}{\rho'(\lambda)} + 2\,\frac{e^{-\rho'(\lambda)} - 1}{\rho'^2(\lambda)}\right) \quad \text{(Eq. S2.2)}$$

$$\rho' = a^{*}_{phy-hplc}(\lambda) \cdot size\_parameter \quad \text{(Eq. S2.3)}$$

And the size parameter is taken from the empirical relation to [chla] established by Wozniak (1999):

$$size\_parameter = 24.68\,[chla]^{0.7515} \quad \text{(Eq. S2.4)}$$

Figure S2.2 shows the reconstructed absorption spectrum, with and without correction for pigment packaging, for one example time-point of the OCE17 dataset.

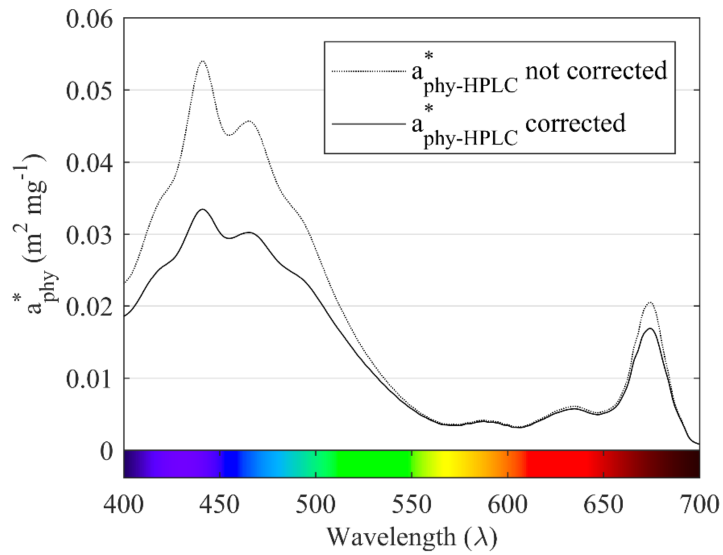

Figure S2.2: Chlorophyll a-specific light absorption by phytoplankton ($a^{*}_{phy}(\lambda)$, m^2 mg $chla^{-1}$) estimated from HPLC derived pigment concentration and weight-specific absorption spectra before and after correction for pigment packaging.

It has been suggested by Letelier et al. (2017) that CDOM or other organic material in the GFF filter used for QFT spectra can contribute to absorption at short wavelengths. This would lead to an overestimation of $a^{*}_{phy}(\lambda)$ derived by QFT relative to $a^{*}_{phy}(\lambda)$ derived by pigment reconstruction in the blue part of the spectrum; a trend which was clearly visible in our data. Letelier et al. (2017) suggest correcting $a^{*}_{phy}(\lambda)$ derived from the QFT approach for $a_{CDOM}(\lambda)$ in the filter using values of $a^{*}_{phy}(400)$, taken from the spectral reconstruction approach.

$$a(\lambda)_{CDOM} = \left(a^{*}_{phy-QFT}(400) - a^{*}_{phy-HPLC}(\lambda)\right)^{-0.014(\lambda-400)} \quad \text{(Eq. S2.5)}$$

Figure S2.3 shows the quenching corrected absorption spectrum calculated using the spectral reconstruction approach as described above, relative to the absorption spectrum measured at the same time-point using the QFT approach, before and after correction for CDOM absorption using Eq. S2.5.

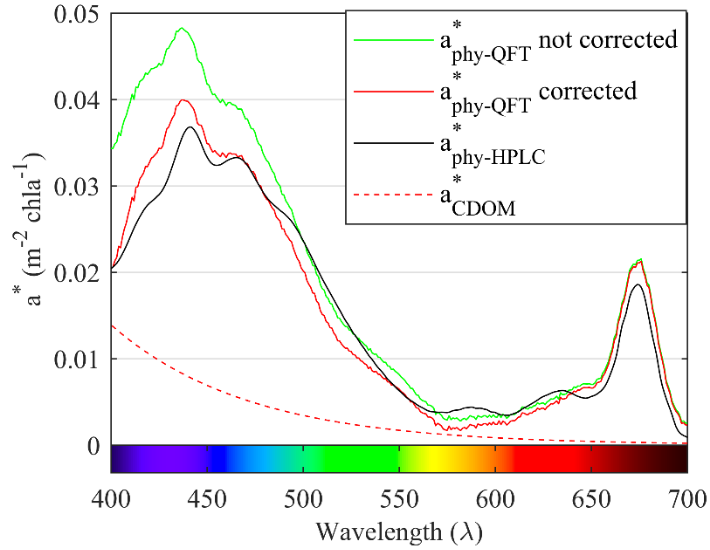

Figure S2.3: Chlorophyll a-specific light absorption by phytoplankton ($a^*_{phy}(\lambda)$, m^2 mg $chla^{-1}$) estimated from HPLC-derived pigment concentration ($a^*_{phy\text{-}HPLC}$), and the QFT approach ($a^*_{phy\text{-}QFT}$). Spectra of $a^*_{phy\text{-}QFT}$ are shown before and after correction for CDOM or other organic material in the filter (a^*_{CDOM}).

After correction for packaging, values of $\hat{a}^*_{phy}$ (mean 400-700nm) from the spectral reconstruction approach correlated strongly to values of CDOM-corrected $\hat{a}^*_{phy}$ from the QFT approach (R^2 = 0.95, n= 20).

The spectral reconstruction approach allows the separation of absorption spectra into photosynthetic pigments (psp) and photoprotective carotenoids (ppc), enabling quantification of changes in $\hat{a}^*_{psp}$ relative to $\hat{a}^*_{ppc}$ in response to environmental variability, and improved estimation of the fraction of light absorbed at a specific wavelengths used for photosynthesis.

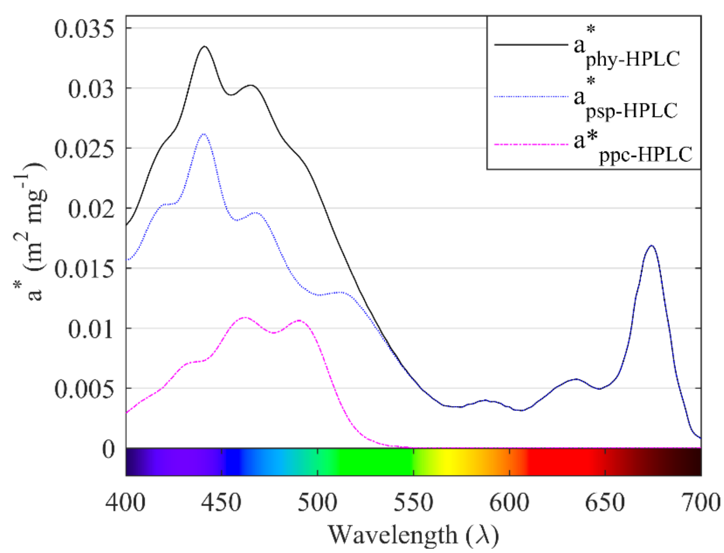

Figure S2.4: Chlorophyll a-specific light absorption by all phytoplankton pigments ($a^*_{phy}(\lambda)$, m^2 mg $chla^{-1}$), by photosynthetic pigments $a^*_{psp}(\lambda)$, and by photoprotective carotenoids $a^*_{ppc}(\lambda)$, all estimated from HPLC derived pigment concentration

The accuracy of the described approach can be limited by a number of factors, including differences in pigment absorption (magnitude and spectral shifts) *in vitro* vs. *in vivo*, incomplete information on weight specific absorption for a for a number of pigments, lack of data on phycobiliprotein concentrations (Johnsen and Sakshaug, 2007; Lutz et al., 2001; Sosik and Mitchell, 1991, 1995), uncertainty in the pigment packaging correction, and uncertainty in the definition of photosynthetic and photoprotective pigments and the energy transfer potential between them.

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