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Supplement

Phytoplankton Chl-a biomass, composition, and productivity along a temperature and stratification gradient in the Northeast Atlantic Ocean

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Additional information for primary production calculations

Calculation of irradiance
The irradiance calculation were based Kirk (1994, 2010). Surface irradiance was calculated according by $E\ t\ = E_m \cdot sin\ \ \pi \cdot \frac{t}{N}$
where E_m (mol m^{-2}) is the maximum irradiance, t (h) is time, and *N* is day length (h). Irradiance at depth was calculated using the attenuation coefficient:

$E_{n,t} = E_{n-1,t} \cdot e^{-K_d \cdot dz}$
where *E* (mol m^{-2}) is irradiance, t (h) is time, K_d is the attenuation coefficient (m), and dz (m) is layer thickness.

Primary production calculations
The equation of Platt et al. (1980) was used to calculate the primary production at depth:

$$P = P_S\ \ 1 - e^{-\alpha \frac{E}{P_s}}\ \ \ \ e^{-\beta \frac{E}{P_s}}\ \ - P_0$$

where *P* is the chlorophyll *a* specific CO_2 fixation rate (µg C µg Chl-a^{-1} h^{-1}) at irradiance *E* (µmol photons m^{-2} s^{-1}), P_S is the theoretical maximum for photosynthesis in the absence of photoinhibition (µg C µg Chl-a^{-1} h^{-1}), α is the initial rate of photosynthesis (µg C µg Chl-a^{-1} h^{-1} [µmol photons m^{-2} s^{-1}]$^{-1}$), β is a measure of photoinhibition (µg C µg Chl-a^{-1} h^{-1} [µmol photons m^{-2} s^{-1}]$^{-1}$), and P_0 was used to indicate respiration or dark carbon fixation at zero irradiance.

Partitioning Chl-a between five taxonomic groups
The bio optical model calculates primary production for five taxonomic phytoplankton groups. The characteristics of these groups were determined from ^{14}C based photosynthesis versus irradiances (PE) measurements of *Prochlorococcus marinus* (group 1), *Synechococcus* sp. (group 2), *Ostreococcus* sp. (group 3), *Emiliania huxleyi* (group 4), and *Thalassiosira oceanica* (group 5). Photosynthetic characteristic of low light (50 µmol photons m^{-2} s^{-1}) and high light (125 µmol photons m^{-2} s^{-1}) acclimated phytoplankton were used (supplement table 1).

The partitioning of chlorophyll-a between the taxonomic groups was based on HPLC pigment analysis and CHEMTAX calculations (see below), resulting in eight different taxonomic

groups. The Chl-a of three taxonomic groups was assigned to other phytoplankton groups for the calculation of primary production. Chl-a of dinoflagellates was assigned to the haptophytes (group 4) and Chl-a of cryptophytes and pelagophytes was combined with that of the prasinophytes (group 3).

Relative importance of taxonomic groups
To visualize the importance of the different parameters for the respective taxonomic groups, integrated productivity was calculated for a station assuming 100 % contribution of a single group for high and low light acclimated conditions, respectively (supplement table 2). Productivity was highest for diatoms and lowest for *Prochlorococcus*. Changes in photoacclimation were most important for *Prochlorococcus* and diatoms, i.e. PE parameters for high light acclimation resulted in 55% higher productivity compared with low light acclimation.

Sensitivity of the model to changes in Chl-a, K_d, and photosynthetic parameters
The values of the photosynthetic parameters ($P_{s,}$ α, β, P_0), Chl-a and K_d were varied by 20% to assess the sensitivity of the production model to changes in photosynthetic parameters, Chl-a, and K_d. The model was most sensitive to changes in Chl-a, a 20% change resulted in a 20% change in productivity. A 20% change in P_s and K_d resulted in a 16% change in productivity. Finally a 20% change in β, and P_0 resulted in 10 and 2% change in productivity, respectively.

Supplement table 1. Photosynthetic parameters used in the production model. The theoretical maximum for photosynthesis in the absence of photoinhibition (P_s in µ µg C µg Chl-a^{-1} h^{-1}), the initial rate of photosynthesis (α in µg C µg Chl-a^{-1} h^{-1} [µmol photons m^{-2} s^{-1}]$^{-1}$), photoinhibition (β in µg C µg Chl-a^{-1} h^{-1} [µmol photons m^{-2} s^{-1}]$^{-1}$), and respiration or dark carbon fixation at zero irradiance (P_0 in µ µg C µg Chl-a^{-1} h^{-1}) are given for low light (50 µmol photons m^{-2} s^{-1}) and high light (125 µmol photons m^{-2} s^{-1}) acclimated cultures of *Prochlorococcus marinus* eMED4, *Synechococcus* sp. (RCC477 and RCC543), *Ostreococcus* sp. (clade B), *Emiliania huxleyi*, and *Thalassiosira oceanica* are given. Experiments were performed using exponentially growing cultures (12-12 h light-dark cycle) at 20°C. Values represent the mean of two cultures. Data from Kulk et al. (2011).

	Low light acclimated				**High light acclimated**			
	P_s	α	β	P_0	P_s	α	β	P_0
Prochlorococcus marinus	2.17	0.032	0.002	0.036	5.05	0.031	0.002	-.2187
Synechococcus sp.	5.45	0.121	0.003	0.205	4.72	0.062	0.003	0.154
Ostreococcus sp.	7.96	0.097	0.006	0.229	10.13	0.097	0.004	0.424
Emiliania huxleyi	50.83	0.091	0.176	0.398	13.39	0.785	0.008	0.461
Thalassiosira oceanica	18.61	0.071	0.012	0.306	229.2	0.153	0.350	1.461

Supplement table 2. Daily depth integrated productivity (mg C m^{-2} day^{-1}) calculated for a random station assuming 100 % contribution to chlorophyll *a* of one taxonomic phytoplankton group, for low light (LL, 50 µmol photons m^{-2} s^{-1}) and high light (HL, 125 µmol photons m^{-2} s^{-1}) acclimated conditions.

	LL	**HL**
Group 1 Prochlorococcus marinus	479	1064
Group 2 Synechococcus sp.	972	1302
Group 3 Ostreococcus sp.	1544	1912
Group 4 Emiliania huxleyi	1500	1859
Group 5 Thalassiosira oceanica	2200	4904

Supplement table 6. Irradiance conditions during PAR and PAR+UVR used in the excess light experiments (W m^{-2}).

	PAR (400-600 nm)	**UVA (315-400 nm)**	**UVB (280-315 nm)**
PAR	355	16	0.05
PAR+UVR	323	26	1.62

Comparison of CHEMTAX with light microscopy and flow cytometry

The taxonomic information obtained by CHEMTAX was compared with light microscopy observations on fixed sampled and with flow cytometry data (Mojica et al. submitted). For light microscopy, 100 ml seawater was fixed by 1 ml of Lugol iodine solution, supplemented with 0.5% glutarealdehyde in dark bottles. Based on CHEMTAX, 18 samples (7 and 11 from spring and summer, respectively) were selected for light microscopic analysis and compared with the taxonomic data obtained with CHEMTAX. Fifty ml of fixed sample was concentrated by sedimentation (24 h) and observations were made on an Olympus IMT-2 inverted microscope, using 20 and 40 times magnification for phytoplankton larger and smaller than 20 µm, respectively. The microscopy observations are briefly discussed below.

In summer, the haptophytes *Phaeocystis sp* (free cells, on bladders and in colonies) increased in concentration from low to high latitude (38,000 up to 2,208,000 cells l^{-1}, dominating the phytoplankton biomass at higher latitudes). Diatom concentrations in summer were low and increased from low to higher latitudes (0-2,000 up to 25,000 cells l^{-1}, *Pseudo-Nitzschia delicatessima*, *Nitzschia longissima*). Larger diatoms were found at low concentrations at high latitudes (*Rhizosolenia*, *Proboscia* sp <1,000 cells l^{-1}). Small dinoflagellates (< 15 µm) appeared mostly heterotrophic (concentrations 22,000-100,000 cells l^{-1}). Larger dinoflagellates were observed at higher latitudes in low concentrations (*Ceratium* sp, < 2,000 cells l^{-1}). In spring, *Phaeocystis* was not abundant, but small *Emiliania huxleyi* like cells were abundant at mid-latitudes (7,074,887 cells l^{-1}). However, the presence of this species was not confirmed by flow cytometry. Furthermore, unidentified pico-eukaryotes were abundant (584,000-4,162,433 cells l^{-1}) at low and mid latitudes in spring. Small diatoms (*Chaetoceros* sp and *Nitszchia longissima*) concentrations were around 3,760 cells l^{-1} at stratified stations, whereas small (presumably heterotrophic) dinoflagellates were around 4,000 cells ml^{-1}. Large dinoflagellates (*Ceratium* sp.) were found in concentrations of 40 cells l^{-1}. At non-stratified stations, large diatoms (*Chaetoceros* sp., *Thalassiosira* sp., *Proboscia* sp., *Rhizosolenia* sp., dominated the phytoplankton community at latitude 25 °N. At higher latitudes, large (>20 µm) Prasinophytes (5,600 cells l^{-1}) and Cryptophytes (6,000 cells l^{-1}) were observed, whereas diatom concentrations were lower.

Data obtained by flow cytometry will be presented in detail by Mojica et al. (submitted). Patterns obtained by flow cytometry of *Synechococcus* spp. and *Prochlorococcus* spp. were comparable with those obtained by pigment composition. However, flow cytometry abundance of *Prochlorococcus* spp. in the upper 50 m of oligotrophic stations in summer was higher than the contribution to Chl-a suggested from pigment composition.

Direct comparison of phytoplankton composition between these methods is complicated by the differences in units and by the specific limitations of each method. Flow cytometry provides abundance data of phytoplankton groups that are smaller than 20 µm, including some groups that are difficult to identify using light microscopy (e.g. small eukaryotes, *Prochlorococcus* spp., and *Synechococcus* spp.). Light microscopy gives detailed information on larger phytoplankton species. In contrast, CHEMTAX provides taxonomic information relative to Chl-a for phytoplankton with a size range > 0.7 µm. In this respect, all methods are complementary to each other. Overall, patterns in phytoplankton composition obtained by light microscopy and flow cytometry were in agreement with CHEMTAX. The dominance of phytoplankton with a haptophytes pigment signature, and the low contribution of diatoms in summer to the phytoplankton community were revealed by light microscopy and CHEMTAX. The dominance of diatoms at higher latitudes in spring was observed by both CHEMTAX and light microscopy. Also the overall low contribution of (photosynthetic) dinoflagellates was shown by CHEMTAX and light microscopy. In oligotrophic waters, increasing dominance of *Prochlorococcus* spp., *Synechococcus* spp. was shown by CHEMTAX and flow cytometry with decreasing latitude.

Supplement table 3. Starting pigment ratios for CHEMTAX (relative to chlorophyll *a*) for high light acclimated phytoplankton. Prasinophytes, dinophytes, cryptophytes, haptophytes, pelagophytes, *Synechococcus* spp., *Prochlorococcus* spp., and diatoms were distinguished. Note that two haptophytes pigment profiles were used to account for the variability in pigment ratios in this group, pooled afterwards to one haptophytes group. Chl-c_3: Chlorophyll c_3; Chl-c_2: Chlorophyll c_2; Perid: Peridinin; 19-BF:19 butanoloxy fucoxanthin; Fuco: fucoxanthin; 19-HF 19 hexanoloxy fucoxanthin; Neox: neoxanthin; Prasinox: prasinoxanthin; Allox: alloxanthin; Zeax: Zeaxanthin; Chl-b: chlorophyll-b; DV Chl-a: divinyl chlorophyll-a; Chl-a: Chlorophyll-a.

Pigment	Chl-c_3	Chl-c_2	Perid	19-BF	Fuco	19-HF	Neox	Prasinox	Allox	Zeax	Chl-b	DV Chl_a	Chl-a
Prasinophytes							0.055	0.2		0.036	0.45		1
Dinophytes		0.17	0.32										1
Cryptophytes		0.078							0.34				1
Haptophytes_1	0.2	0.19		0.001	0.25	0.44							1
Haptophytes_2	0.2	0.17		0.022	0.66	0.08							1
Pelagophytes	0.25	0.25		0.8	0.19	0.01							1
Synechococcus										1			1
Prochlorococcus										1		1	
Diatoms		0.1			0.44								1

Supplement table 4. Starting pigment ratios for CHEMTAX (relative to chlorophyll *a*) for low light acclimated phytoplankton taxonomic groups. Prasinophytes, dinophytes, cryptophytes, haptophytes, pelagophytes, *Synechococcus*, *Prochlorococcus*, and diatoms were distinguished. Note that two haptophytes pigment profiles were used to account for the variability in pigment ratios this group, pooled afterwards to one haptophytes group. Chl-c_3: Chlorophyll c_3; Chl-c_2: Chlorophyll c_2; Perid: Peridinin; 19-BF:19 butanoloxy fucoxanthin; Fuco: fucoxanthin; 19-HF 19 hexanoloxy fucoxanthin; Neox: neoxanthin; Prasinox: prasinoxanthin; Allox: alloxanthin; Zeax: Zeaxanthin; Chl-b: chlorophyll-b; DV Chl-a: divinyl chlorophyll-a; Chl-a: Chlorophyll-a.

Pigment	Chl-c_3	Chl-c_2	Perid	19-BF	Fuco	19-HF	Neox	Prasinox	Allox	Zeax	Chl-b	DV Chl_a	Chl-a
Prasinophytes							0.055	0.2		0.005	0.70		1
Dinophytes		0.17	0.4										1
Cryptophytes		0.078							0.229				1
Haptophytes_1	0.2	0.19		0.001	0.25	0.44							1
Haptophytes_2	0.2	0.17		0.022	0.66	0.08							1
Pelagophytes	0.25	0.25		0.8	0.19	0.01							1
Synechococcus										0.6			1
Prochlorococcus										0.2		1	
Diatoms		0.1			0.44								1