

Supplemental Information

Evaluation of diagnostic pigments to estimate phytoplankton size classes

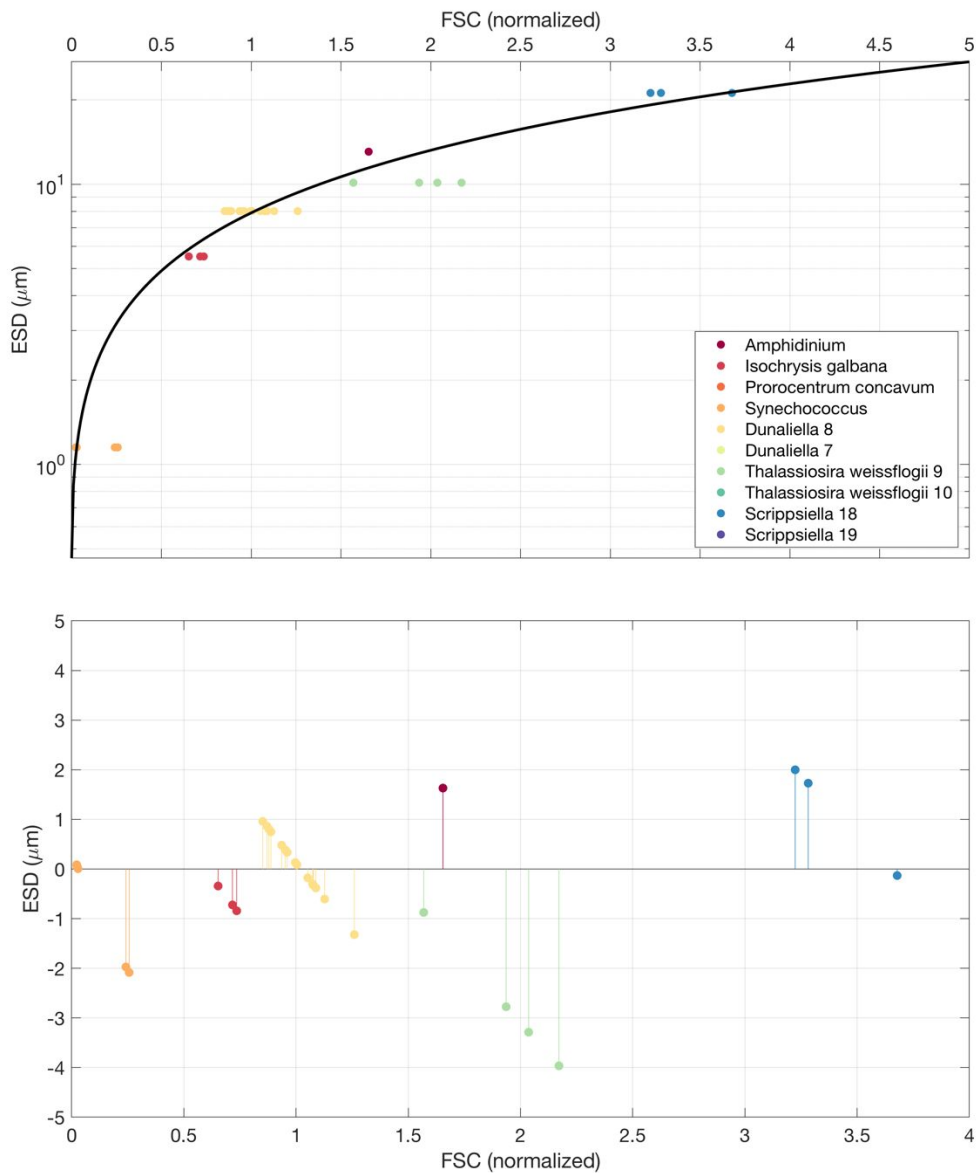
Alison P. Chase, Sasha J. Kramer, Nils Haëntjens, Emmanuel S. Boss, Lee Karp-Boss, Mimi Edmondson, Jason Graff

Table S1. Date, time, latitude, longitude, and chlorophyll *a* concentration for each sample used in the present study. NAAMES cruises 1-4 took place chronologically over the four years 2015-2018. Chl *a* was determined from HPLC analysis performed on discrete surface water samples. Date, time, latitude, and longitude are associated with the nearest IFCB sample to a given ICS sample. Apparent duplicate samples are places where the same IFCB data was compared with multiple ICS and/or HPLC water samples collected in the same water mass.

Sample	Date	Time	Latitude (°N)	Longitude (°W)	Chl <i>a</i> (mg m ⁻³)
1	7-Nov-2015	17:42:05	41.7676	-65.0642	1.711
2	8-Nov-2015	13:34:14	43.0627	-60.6202	0.353
3	8-Nov-2015	18:10:04	43.3491	-59.5644	0.476
4	9-Nov-2015	5:54:15	44.0557	-56.9948	0.469
5	9-Nov-2015	22:56:43	45.1192	-53.0952	0.627
6	11-Nov-2015	10:59:20	48.9796	-46.4214	0.646
7	12-Nov-2015	8:18:59	51.048	-43.622	0.363
8	12-Nov-2015	21:22:18	51.7803	-42.8155	0.552
9	13-Nov-2015	21:51:24	54.071	-40.171	0.415
10	14-Nov-2015	20:22:11	54.086	-40.139	0.458
11	16-Nov-2015	6:59:07	51.122	-40.116	1.069
12	17-Nov-2015	13:11:47	48.3683	-38.8388	0.312
13	18-Nov-2015	21:37:47	46.0915	-37.751	0.267
14	19-Nov-2015	8:40:30	46.381	-37.717	0.386
15	21-Nov-2015	8:41:09	43.379	-40.36	0.361
16	23-Nov-2015	8:52:23	42.981	-40.187	0.451
17	23-Nov-2015	22:37:40	41.5796	-40.37	0.319
18	24-Nov-2015	8:53:03	40.51	-40.477	0.412
19	24-Nov-2015	21:32:21	40.631	-40.477	0.289
20	25-Nov-2015	8:11:25	40.686	-40.447	0.434
21	26-Nov-2015	21:02:44	42.906	-46.9847	0.494
22	27-Nov-2015	11:58:32	42.532	-50.8559	0.849
23	27-Nov-2015	16:28:48	42.4187	-52.009	0.528
24	28-Nov-2015	20:44:23	41.675	-59.2652	0.317
25	12-May-2016	14:13:05	41.2121	-66.7269	1.042
26	12-May-2016	18:56:38	41.5726	-65.4887	1.785
27	13-May-2016	1:38:10	42.1061	-63.6734	2.827

28	13-May-2016	13:27:13	43.0643	-60.4052	1.611
29	13-May-2016	16:59:55	43.3529	-59.4045	0.427
30	13-May-2016	23:42:55	43.8889	-57.5117	0.655
31	15-May-2016	19:59:18	49.0875	-47.9165	2.122
32	18-May-2016	2:37:51	56.341	-46.046	1.951
33	19-May-2016	3:13:13	56.264	-45.908	1.859
34	19-May-2016	18:08:39	54.2772	-43.4296	2.438
35	20-May-2016	4:01:20	53.552	-42.259	3.480
36	21-May-2016	4:11:28	53.478	-42.076	3.622
37	23-May-2016	3:27:22	49.9451	-43.9558	5.141
38	23-May-2016	1:52:49	49.971	-43.939	5.141
39	23-May-2016	11:45:04	48.884	-41.729	0.875
40	25-May-2016	3:17:11	47.579	-38.849	0.637
41	25-May-2016	15:04:30	47.515	-38.798	0.686
42	25-May-2016	15:04:30	47.515	-38.798	0.686
43	25-May-2016	19:52:30	47.4686	-38.777	0.686
44	25-May-2016	20:17:53	47.4602	-38.776	0.686
45	26-May-2016	6:12:25	47.392	-38.707	0.726
46	26-May-2016	16:36:23	47.36	-38.5567	0.987
47	26-May-2016	17:00:00	47.358	-38.551	0.987
48	27-May-2016	3:11:18	47.365	-38.32	1.346
49	27-May-2016	5:59:38	47.335	-38.287	1.276
50	27-May-2016	5:59:38	47.335	-38.287	1.276
51	30-Aug-2017	23:32:45	40.436	-70.18	0.469
52	2-Sep-2017	17:43:42	41.387	-53.54	0.183
53	3-Sep-2017	3:27:20	41.622	-51.214	0.204
54	3-Sep-2017	15:04:28	41.91	-48.18	0.219
55	11-Sep-2017	15:39:34	50.15	-39.259	0.392
56	12-Sep-2017	3:16:07	51.65	-39.483	0.397
57	13-Sep-2017	3:15:19	51.816	-39.727	0.324
58	13-Sep-2017	19:55:39	53.274	-39.517	0.792
59	14-Sep-2017	3:07:55	53.307	-39.498	0.853
60	14-Sep-2017	10:13:49	53.35	-39.522	0.926
61	14-Sep-2017	18:15:16	53.37	-39.489	0.848
62	14-Sep-2017	19:05:37	53.372	-39.485	0.848
63	15-Sep-2017	2:53:23	53.401	-39.499	0.711
64	16-Sep-2017	2:58:14	53.387	-39.562	0.777
65	16-Sep-2017	9:46:32	53.387	-39.581	0.822
66	16-Sep-2017	17:53:16	53.384	-39.59	0.875
67	16-Sep-2017	17:53:16	53.384	-39.59	0.875
68	17-Sep-2017	4:37:58	53.387	-39.613	0.849
69	17-Sep-2017	12:08:59	53.436	-39.599	0.669
70	17-Sep-2017	15:39:05	53.395	-39.625	0.859
71	18-Sep-2017	18:43:01	50.638	-45.115	0.429
72	19-Sep-2017	11:05:05	48.651	-48.807	0.411
73	20-Sep-2017	11:53:30	46.087	-54.443	0.304
74	25-Mar-2018	13:59:14	35.534	-48.029	0.611
75	26-Mar-2018	8:06:54	37.53	-45.574	0.693
76	26-Mar-2018	9:40:58	37.678	-45.403	1.069
77	27-Mar-2018	8:54:25	39.407	-43.446	0.570
78	27-Mar-2018	6:10:37	39.407	-43.437	0.704
79	27-Mar-2018	15:21:23	39.409	-43.536	0.722
80	27-Mar-2018	16:54:58	39.419	-43.537	0.722
81	28-Mar-2018	4:14:16	39.275	-41.215	1.172
82	28-Mar-2018	7:44:55	39.278	-41.212	0.990
83	28-Mar-2018	19:19:49	39.305	-41.211	0.947

84	28-Mar-2018	19:19:49	39.305	-41.211	0.947
85	30-Mar-2018	8:57:14	43.47	-42.123	0.458
86	29-Mar-2018	10:35:18	41.853	-42.133	0.425
87	30-Mar-2018	15:25:12	43.737	-41.072	0.965
88	31-Mar-2018	8:19:51	44.655	-38.331	0.854
89	31-Mar-2018	11:26:57	44.58	-38.373	0.973
90	31-Mar-2018	14:32:06	44.501	-38.329	0.989



12 **Figure S1.** Size calibration of cells measured by the ICS. Forward scattering (FSC) vs.
13 equivalent spherical diameter (ESD) calculated from particle cross sectional area (top panel) and

deviation from the fit line (bottom panel). Points are colored according to the different phytoplankton cultures that were used for the calibration. The 95% confidence interval for the coefficients of the best fit line in the top panel are used in a Monte Carlo simulation with 1000 iterations to calculate a distribution of sizes for each cell observed. This information is used to propagate the uncertainty in the size calibration to the estimates of cell size and phytoplankton size class estimates.

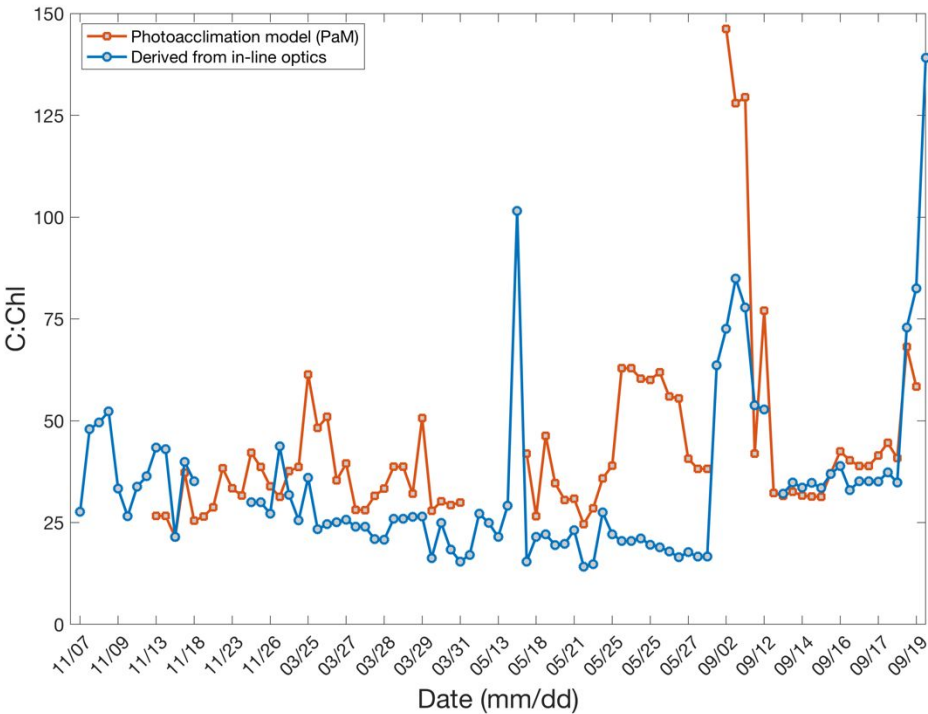


Figure S2. Carbon-to-Chlorophyll (C:Chl) values estimated for each merged cytometry sample as a function of sample collection date (NAAMES 1 in November 2015, NAAMES 2 in May-June 2016, NAAMES 3 in August-September 2017, and NAAMES 4 in March-April 2018). Values derived from in-line optics (blue line, circle symbols) were estimated using spectral backscattering ($b_{bp}(\lambda)$) converted to phytoplankton carbon following Graff et al. (2015) and divided by Chl *a* concentrations, where Chl *a* is derived from particulate absorption ($a_p(\lambda)$) red peak line height as in Boss et al. (2013). All $a_p(\lambda)$ and $b_{bp}(\lambda)$ data are available at the NASA

SeaBASS data repository (<https://seabass.gsfc.nasa.gov/>). Red lines and square symbols show values derived from the photoacclimation model of Behrenfeld et al. (2016); see Fox et al. (2020) for details of the calculation. Missing data points in both lines represent locations where one or more of the parameters needed for the C:Chl calculation was not available.

Statistics used in DPA – cytometry comparison

Contributions of the three phytoplankton size fractions to Chl *a* as derived from the DPA and cytometry approaches were compared using Pearson's linear correlation coefficient (*r*; calculated using MATLAB *corr* function), and four additional metrics, defined as:

$$RMSE = \sqrt{\frac{1}{n} \sum_{i=1}^n (X_i^D - X_i^C)^2}$$

$$rRMSE = 100 \left(\sqrt{\frac{1}{n} \sum_{i=1}^n \left(\frac{X_i^D - X_i^C}{0.5(X_i^D - X_i^C)} \right)^2} \right)$$

$$Bias = \frac{1}{n} \sum_{i=1}^n (X_i^D - X_i^C)$$

$$rBias = 100 \left(\frac{1}{n} \sum_{i=1}^n \left(\frac{X_i^D - X_i^C}{0.5(X_i^D - X_i^C)} \right) \right)$$

where *n* is the number of samples, *X* is a given PSC (either the unitless fraction or the Chl *a* concentration), and the superscripts *D* and *C* denote the PSCs determined by either the DPA or from cytometry, respectively. As both the pigment and cytometry-based PSC estimates have uncertainties and outliers, we assume neither method is the “truth,” thus we normalized by a mean of the two for calculating the relative *RMSE* (*rRMSE*) and relative bias (*rBias*).

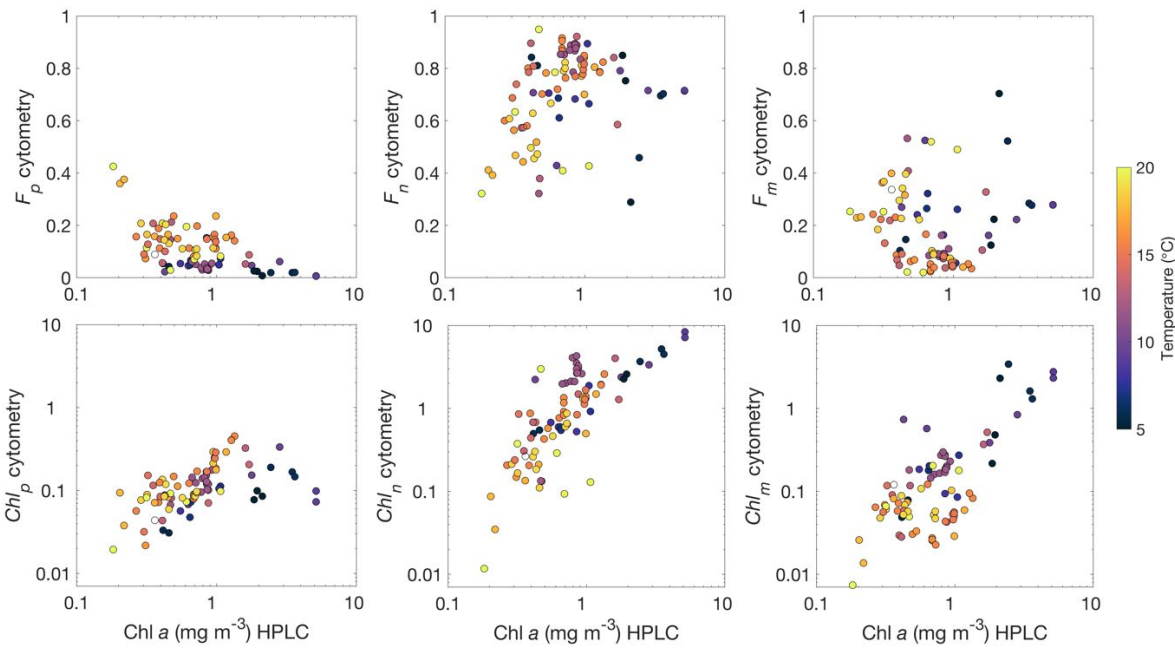


Figure S3. Total Chl *a* vs the fractions of pico-, nano-, and microplankton (top row), and Chl *a* concentrations of pico-, nano-, and microplankton (bottom row) calculated from cytometry data. Points are colored by surface water temperature (°C).

Table S2. Size metrics of seven taxonomic phytoplankton groups and all unidentifiable cells. Equivalent spherical diameter is calculated from cell biovolume (ESD_V), where biovolume is estimated following Moberg et al. (2012). The mean, median, standard deviation, and the number of cells observed (n) are shown for each group. Values have been converted from pixels to micrometers (μm) using a conversion factor determined with bead calibration of the Imaging FlowCytobot.

ESD _V (μm)			
Mean	Median	Standard Dev.	n

Diatoms	11.5	9.5	7.0	19,952
Dinoflagellates	11.1	10.0	4.2	23,806
Silicoflagellates	14.5	13.6	3.9	1,864
Prymnesiophytes	10.9	9.2	4.8	3,578
Cryptophytes	7.8	7.8	1.2	8,638
Chlorophytes	8.3	7.3	4.1	3,010
Euglenoids	9.4	8.9	1.9	3,380
Unidentifiable	8.7	8.0	2.9	122,766

References

- Behrenfeld, M. J., R. T. O'Malley, E. S. Boss and others. 2016. Revaluating Ocean Warming Impacts on Global Phytoplankton. *Nat. Clim. Chang.* 6(3): 323–30, doi.org/10.1038/nclimate2838.
- Boss, E., M. Picheral, T. Leeuw and others. 2013. The Characteristics of Particulate Absorption, Scattering and Attenuation Coefficients in the Surface Ocean; Contribution of the Tara Oceans Expedition. *Methods in Oceanography* 7 (September): 52–62, doi.org/10.1016/j.mio.2013.11.002.
- Fox, J., M. J. Behrenfeld, N. Haëntjens and others. 2020. Phytoplankton Growth and Productivity in the Western North Atlantic: Observations of Regional Variability From the NAAMES Field Campaigns. *Front. Mar. Sci.* 7(24): 1–15, doi.org/10.3389/fmars.2020.00024.

74 Graff, J. R., T. K. Westberry, A. J. Milligan and others. 2015. Analytical Phytoplankton Carbon
75 Measurements Spanning Diverse Ecosystems. Deep Sea Res., Part I. 102: 16–25,
76 doi.org/10.1016/j.dsr.2015.04.006.
77
78 Moberg, E. A., and H. M. Sosik. 2012. Distance maps to estimate cell volume from two-
79 dimensional plankton images, Limnol. Oceanogr.: Methods. 10: 278–288,
80 doi:10.4319/lom.2012.10.278.
81

For Review Only