The following supplement accompanies the article

Shaping of bacterial community composition and diversity by phytoplankton and salinity in the Delaware Estuary, USA

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Table S1. Initial pigment ratios used in Chemtax analyses, normalized to chlorophyll a, i.e. chl a =1 for all. The main source for the ratios is Schlüter et al. (2014) with additional data from estuarine studies (Lewitus et al. 2005, Adolf et al. 2006, Gameiro et al. 2007, Lionard et al. 2008).

	Pigments											
Class	chc2	perid	b_fuco	fuco	h_fuco	neo	viola	pras	allo	lut	zea	chl_b
Cyanobacteria	0	0	0	0	0	0	0	0	0	0	0.443	0
Chlorophytes	0	0	0	0	0	0.040	0.030	0	0	0.211	0.024	0.298
Prasinophytes	0	0	0	0	0	0.110	0	0.460	0	0.018	0.080	0.493
Dinoflagellates	0.220	0.750	0	0	0	0	0	0	0	0	0	0
Haptophytes 8	0.179	0	0.129	0.315	0.422	0	0	0	0	0	0	0
Haptophytes 6	0.143	0	0	0.425	0.404	0	0	0	0	0	0	0
Cryptophytes	0	0	0	0	0	0	0	0	0.317	0	0	0
Diatoms	0.22	0	0	0.69075	0	0	0	0	0	0	0	0
Pelagophytes	0	0	0.658	0.779	0	0	0	0	0	0	0	0

Abbreviation	Explanation
chc2	chlorophyll c2
perid	peridinin
b_fuco	19'-butanoyloxyfucoxanthin
Fuco	fucoxanthin
h_fuco	19'-hexanoyloxyfucoxanthin
Neo	neoxanthin
Viola	violaxanthin
pras	prasinoxanthin
allo	alloxanthin
lut	lutein
zea	zeaxanthin
chl_b	chlorophyll b

Table S2. Regression analyses of microbial diversity versus salinity in the Delaware Estuary. Here phytoplankton diversity was calculated with 16S rRNA gene sequences from cyanobacteria and chloroplasts genes of eukaryotic phytoplankton. Evenness is the Simpson index. N=55 for August; n=58 for November.

Community	Index	Month	Slope	Error	p value	Intercept	Error	p value
Bacteria	Richness	Aug	-1.98	0.24	3.2E-11	128	4.30	< 2e-16
Bacteria	Richness	Nov	-2.41	0.32	3.8E-10	134	6.20	< 2e-16
Bacteria	Evenness	Aug	-0.0008855	0.0002702	1.9E-03	0.0755	0.0049	< 2e-16
Bacteria	Evenness	Nov	-0.0020078	0.0003372	1.8E-07	0.0822	0.0066	< 2e-16
Bacteria	Shannon	Aug	-0.0356	0.0052	9.8E-09	3.07	0.10	< 2e-16
Bacteria	Shannon	Nov	3.24	0.13	<2.00E-16	3.24	0.13	< 2e-16
Phyto	Richness	Aug	0.625	0.2644	2.2E-02	52.7	4.8	2.7E-15
Phyto	Richness	Nov	24.6	3.0	4.6E-11	24.6	3.0	4.6E-11
Phyto	Evenness	Aug	-0.00120	0.00055	3.5E-02	0.132	0.010	<2e-16
Phyto	Evenness	Nov	-0.00216	0.00089	1.8E-02	0.162	0.017	4.9E-13
Phyto	Shannon	Aug	-0.00147	0.00601	8.1E-01	2.66	0.11	<2e-16
Phyto	Shannon	Nov	0.00581	0.00443	2.0E-01	1.92	0.09	<2e-16

Phytoplankton community composition revealed by Chemtax versus 16S rRNA gene sequences

Nearly all of our analyses of the phytoplankton communities in the Delaware estuary were based on the Chemtax approach using 12 phytoplankton pigments. However, the species richness and evenness of phytoplankton communities were examined using 16S rRNA gene sequences from cyanobacteria and from chloroplasts of eukaryotic phytoplankton. The 16S approach has a higher phylogenetic resolution than the Chemtax approach and is directly comparable with bacterial communities determined by the same approach. An obvious question is whether or not the two approaches, Chemtax and 16S rRNA gene sequences, yield the same estimates for the relative abundance of the major phytoplankton classes.

Methods: As described in the Methods section of the main text of this paper, the 16S rRNA gene sequences from chloroplasts were classified using a local BLAST analysis against the PhytoREF database (Decelle et al. 2015) downloaded March 2016. Each sequence was then put into the appropriate phytoplankton class to compare with the Chemtax data and then added up to calculate the total number of sequences in each class. Relative abundance estimates for each sample were calculated by dividing the observed number of sequences by the total number for that sample. A similar approach was used with the Chemtax data.

The following taxa in the 16S data set had no equivalent in the Chemtax data and were not included in the analyses: Archaeplastida_X, chrysophytes, euglenophytes, ochrophyta, rhodophyta, and streptophyta. These classes, however, accounted for <1% of all phytoplankton sequences. The Chemtax approach recognized two types of haptophytes, 6 and 8, but these were combined into just “haptophytes to compare with the 16S data.

There was a significant correlation between the Chemtax and 16S approaches in estimating the relative abundance of the phytoplankton classes (Spearman $\rho=0.575$; $p<0.001$, $n=113$), but the relationship varied with the eight classes analyzed here (Figure S1). Cyanobacteria, cryptophytes and to lesser extent chlorophytes were overestimated by the 16S approach relative to Chemtax whereas the opposite was the case for diatoms and haptophytes. Dinoflagellate and pelagophyte abundance was substantially underestimated by the 16S approach relative to Chemtax.

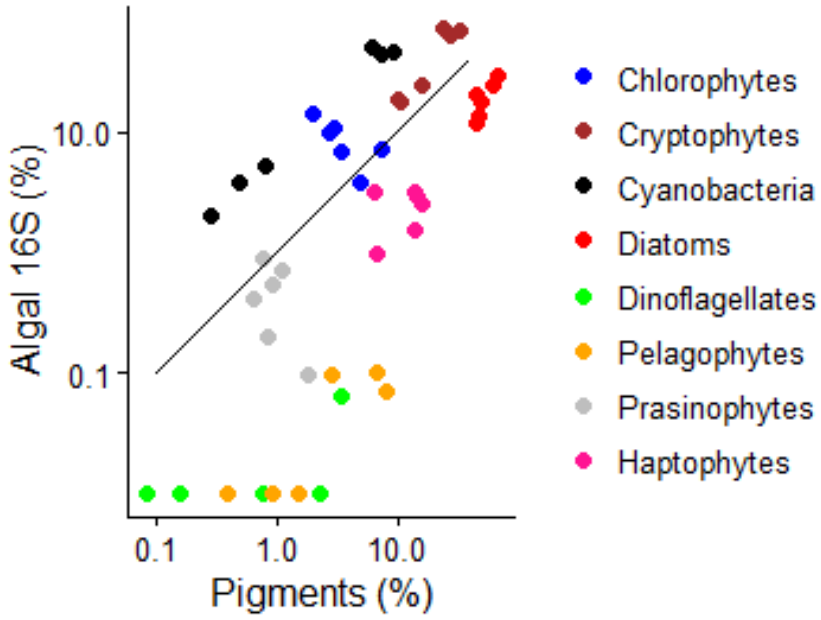

Figure S1. Relative abundance (% of total) of phytoplankton classes determined by 16S rRNA gene sequences and the Chemtax approach using phytoplankton pigments. The data were log(X+0.01) transformed. The solid line indicates a 1:1 relationship.

The relationship between Chemtax and 16S estimates within a phytoplankton class also varied from class to class (Table S3). When calculated for all samples, the two approaches yielded abundance estimates that correlated for four of the eight classes. In particular, abundance estimates calculated by 16S sequences were highly correlated with the Chemtax data for cyanobacteria and chlorophytes (Spearman $\rho>0.7$, $p<0.001$, $n=113$). In contrast, there was a significant, but negative correlation between Chemtax and 16S-based estimates for chlorophytes (Table S3).

Concluding Remarks The two approaches gave similar overall pictures of the phytoplankton community in the estuary, but there were also some big differences between the Chemtax and 16S estimates. The main text of the paper discusses some of the problems with both approaches and speculates about mechanisms to explain the differences. In spite of these problems and differences, the two approaches yield similar patterns in diversity between the two months and with salinity. We suggest that the two approaches have complementary value in exploring the structure of phytoplankton communities.

Table S3. Spearman correlation between the Chemtax and 16S approaches for estimating the relative abundance of

Class	rho	p_value
Chlorophytes	-0.380	<0.001
Cryptophytes	0.718	<0.001
Cyanobacteria	0.724	<0.001
Diatoms	0.329	<0.001
Dinoflagellates	0.139	0.14
Haptophytes	0.220	0.02
Pelagophytes	0.061	0.52
Prasinophytes	0.091	0.34

Reference

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