Vol. 78: 93–106, 2017
doi: 10.3354/ame01805

AQUATIC MICROBIAL ECOLOGY
Aquat Microb Ecol

Published online January 12

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

David L. Kirchman[1,*], Matthew T. Cottrel[1], Giacomo R. DiTullio[2]

[1]School of Marine Science and Policy, University of Delaware, Lewes, DE 19958, USA
[2]College of Charleston, Hollings Marine Laboratory, Charleston, SC 29412, USA

ABSTRACT: Our understanding of the impact of phytoplankton on bacterial communities is largely based on studies showing that a few bacteria have interactions with the phytoplankton class, which is often diatoms, dominating phytoplankton communities. To determine the effect of the complete phytoplankton community on the entire bacterial community, we used tag pyrosequences of the 16S rRNA gene and phytoplankton pigments measured by high performance liquid chromatography along with Chemtax analyses to examine bacterial and phytoplankton communities along the salinity gradient of the Delaware Estuary, USA, in August and November of 3 years (2011–2013). Salinity had a large effect on the composition, taxon richness, and evenness of bacterial communities in the estuary, but so too did the composition and biomass of the phytoplankton community. Phytoplankton classes had a larger effect in shaping the composition of bacterial communities than did total chlorophyll *a*. Although diatoms and cryptophytes dominated the phytoplankton communities in both August and November, less common phytoplankton classes, such as dinoflagellates, haptophytes, and prasinophytes, had more significant relationships with the entire bacterial community and with individual bacterial taxa. In contrast, the 2 most abundant bacterial subclades in the estuary, SAR11 IIIa and SAR 11 IIIb, had few significant relationships with chlorophyll *a* or with phytoplankton classes. These data on bacterial and phytoplankton community composition help to explain the weak coupling between bacteria and phytoplankton communities often observed in estuarine and other aquatic systems.

KEY WORDS: 16S rRNA · Chemtax · SAR11 · Diatoms · *Bacteroidetes* · *Planctomycetes*

INTRODUCTION

Phytoplankton contribute substantially to setting the growth, biomass, and taxonomic composition of bacterial communities in aquatic systems (Ducklow 2000, Andersson et al. 2010, Yilmaz et al. 2012, Walsh et al. 2015). Among many possible interactions among these microbes (Amin et al. 2012), phytoplankton directly or indirectly provide organic carbon for bacteria, which explains why bacterial abundance, production, and community composition are usually coupled with phytoplankton production and biomass, although often only over large spatial and temporal scales (Ducklow 2000, Yilmaz et al. 2012). In addition to positive effects, phytoplankton may also have negative effects on some bacteria via the excretion of anti-bacterial compounds (Lebeau & Robert 2003, Amin et al. 2012), competition for limiting nutrients (Wheeler & Kirchman 1986), or grazing by mixotrophic members of the phytoplankton community (Unrein et al. 2014). Whether positive or negative, bacteria–phytoplankton interactions likely help to explain the composition of both communities and carbon fluxes in aquatic environments.

*Corresponding author: kirchman@udel.edu

The taxonomic composition of the phytoplankton community is thought to shape the composition of bacterial communities, a conclusion largely based on studies conducted during phytoplankton blooms (Buchan et al. 2014). The implication of these bloom studies is that bacterial communities are affected by the dominant phytoplankton class making up the bloom, although often only the most abundant phytoplankton class, usually diatoms, is identified. Among the few studies reporting sufficient information about both bacteria and phytoplankton, Liu et al. (2014) found significant correlations between many abundant bacterial taxa and some phytoplankton classes, most notably diatoms but also cyanobacteria, which dominated the phytoplankton communities in a subtropical drinking water reservoir. The dissolved organic material (DOM) from diatoms can select for a more diverse bacterial community than the DOM from cyanobacteria (Landa et al. 2014). It is still unclear whether phytoplankton other than the dominant class affect bacterial communities and, more generally, whether all phytoplankton classes have similar relationships with all members of bacterial communities.

Bacteria–phytoplankton relationships in estuaries are complicated by the input of dissolved compounds and organisms from land, rivers, and neighboring coastal oceans. This input from allochthonous sources is one mechanism to explain why correlations between bacterial and phytoplankton properties, such as biomass and production, are often low in estuaries such as the Delaware Estuary, USA (Hoch & Kirchman 1993), but correlations for these bulk properties are also often low in other aquatic environments (Fouilland & Mostajir 2011). The relationship between the composition of bacterial and phytoplankton communities has not been examined extensively in estuaries. Lindh et al. (2015) found significant relationships between bacterial community composition and dinoflagellates in the Baltic Sea, but not with diatoms or other phytoplankton classes. Another study in the Baltic Sea, however, found significant correlations between a few *Bacteroidetes* taxa and genetic clusters within the diatom *Skeletonema marinoi* dominating at the time (Bunse et al. 2016).

Variation in salinity caused by mixing of fresh and oceanic waters also leads to large changes in the composition of bacterial communities in estuaries, although perhaps not their diversity. Salinity is one of the most important properties determining the taxonomic composition of bacterial communities in estuaries and other environments (Bouvier & del Giorgio 2002, Kirchman et al. 2005, Lozupone & Knight 2007). Mixing of river and marine communities in estuaries is thought to lead to a diverse bacterial community, depending on the resident time of the estuary (Crump et al. 2004), and may have larger impacts on bacterial communities than other estuarine properties that vary seasonally (Fortunato et al. 2012, 2013). However, one of the few estuarine studies using high throughput sequencing found that taxon richness did not vary with salinity in the Baltic Sea (Herlemann et al. 2011). Other estuaries need to be examined to determine if in fact microbial diversity does not vary with salinity in estuaries, in contrast to larger organisms (Hu et al. 2016).

Here we explored bacteria–phytoplankton interactions in the Delaware Estuary, where microbial communities and many biogeochemical properties have been examined extensively (Kirchman et al. 2005, Sharp et al. 2009, Campbell & Kirchman 2013). We used tag sequencing of 16S rRNA genes to examine bacterial communities along the full salinity gradient of the estuary in August and November of 2011 to 2013. Unlike other bacteria–phytoplankton studies (see studies cited above), we examined phytoplankton composition via their accessory pigments assayed by high performance liquid chromatography (HPLC) and calculated the abundance of phytoplankton classes using the Chemtax approach (Mackey et al. 1996), which has been used extensively in estuaries (Lionard et al. 2008, Keller et al. 2014, Schlüter et al. 2014). In this study, cyanobacteria were included in both the bacterial community as well as the phytoplankton community because of their potential for photoheterotrophy (Béjà & Suzuki 2008). Our 2 goals were to explore bacteria–phytoplankton interactions when total phytoplankton biomass was either high (August) or relatively low (November) and to examine the diversity of bacterial and phytoplankton communities in estuarine waters that differed in many biogeochemical properties. We hypothesized that the diatoms, often the most abundant phytoplankton group in the Delaware (Watling et al. 1979), have the largest impact on structuring bacterial communities. We found that diatoms were the most abundant phytoplankton in the Delaware Estuary but that other phytoplankton classes had many more significant relationships with bacterial communities. Based on previous studies (Herlemann et al. 2011, Ladau et al. 2013), we expected to see differences in diversity between the 2 months but no systematic change with salinity. We found that bacterial diversity varied much more with salinity than it did between the 2 months.

MATERIALS AND METHODS

Sample collection and biogeochemical properties

Surface water samples were taken at 0.5 m depth along the entire salinity gradient of the Delaware Estuary, from about 39.8° N, 75.42° W to just outside the estuary at 38.8° N, 75.11° W. The cruises were conducted in August and November 2011 to 2013 (6 cruises in total), each sampling about 25 stations separated by about 20 km along the full salinity gradient; a map showing the locations of the standard stations has been published (Sharp et al. 2009). Light attenuation was estimated from the intensity of photosynthetically active radiance (PAR) measured with a Biospherical PNF-210 radiometer over a depth profile. Ammonium, nitrate, phosphate, and silicate concentrations were measured with a SEAL Analytical AA3 Continuous Segmented Flow Analyzer, while concentrations of dissolved organic carbon (DOC) were measured by the high-temperature combustion technique using standard procedures (Sharp et al. 2009). The concentration of total chlorophyll *a* (chl *a*) was estimated in acetone extracts by fluorometry. Bacterial abundance was determined by the direct count method after staining with 4′,6-diamidino-2-phenylindole. The microcentrifuge approach was used to estimate leucine incorporation (added concentration of 20 nM) (Kirchman 2001).

Sequencing and initial analysis of 16S rRNA genes

The composition of the bacterial community was explored by tag sequencing of 16S rRNA genes extracted from DNA collected by passing water samples (1–2 l, depending on location and suspended material load) through 0.22 µm pore size Durapore filters using previously described methods (Campbell & Kirchman 2013). The filters were placed in buffer and stored at −80°C until DNA extraction by a modified protocol using 2 chloroform extractions (Dempster et al. 1999). DNA was quantified via a picogreen assay following the manufacturer's instructions (Invitrogen). The V1-V3 region of the 16S rRNA genes was amplified by PCR using previously described primers and barcodes (Cottrell et al. 2015) and sequenced on a Roche 454 platform with titanium chemistry by Molecular Research LP (www.MrDNAlab.com). Sequences were deposited in the NCBI Short Read Archive, under accession PRJNA 338249.

Sequences were analyzed using the QIIME pipeline (Caporaso et al. 2010). In brief, sequences were trimmed, de-noised, and assigned to operational taxonomic units (OTUs) having >97% similarity using default settings in QIIME. ChimeraSlayer was used to identify chimeras which were then removed. The taxonomy of the OTUs was determined in QIIME using uclust and the Greengenes database. Any OTUs represented by less than 5 reads among all samples were removed from further analyses to minimize the impact of sequencing errors. Fourteen samples were removed because of low numbers of reads (<1034 reads). The sequences in the remaining 115 samples were rarified in QIIME so that each sample had 1034 reads. The rarified samples were used in all subsequent analyses.

We examined bacterial communities either at the OTU level or in groups organized at different phylogenetic levels, ranging from phylum (e.g. *Actinobacteria* and *Bacteroidetes*) to subclades of the SAR11 clade (see 'Results'). The taxonomy was taken from the QIIME analysis except for the SAR11 subclades. The SAR11 clade was divided into previously defined subclades (Vergin et al. 2013). All sequences identified as belonging to the *Pelagibacteraceae* by QIIME were compared to the SAR11 subclade database from Vergin et al. (2013) using a local BLAST analysis with an e-value cutoff of 10^{-3}.

The diversity of phytoplankton communities was examined in more detail using 16S rRNA gene sequences (Milici et al. 2016, Needham & Fuhrman 2016). All OTUs identified as from chloroplasts by QIIME were analyzed separately by local BLASTn against the PhytoREF database (Decelle et al. 2015), downloaded in March 2016. These algal sequences were combined with the cyanobacterial sequences and used to calculate diversity indices of the entire phytoplankton community.

Phytoplankton pigment analysis and community composition

Samples for phytoplankton pigments were collected in Niskin bottles using the same method as for total chl *a*. After filtration of 1 to 2 l (depending on location and suspended material loads), the GF/F filters were stored at −80°C until analysis. The pigments were extracted in 90% acetone at −20°C and analyzed using a Hewlett-Packard 1100 HPLC system equipped with an autosampler, photodiode array, and fluorescence detection. The HPLC conditions and method are described elsewhere in detail (DiTullio et al. 2005).

The abundance of phytoplankton classes was estimated by the Chemtax iterative matrix-factorization approach using the 12 phytoplankton pigments we measured (Mackey et al. 1996). The initial pigment ratio matrix (Table S1 in the Supplement at www.int-res.com/articles/suppl/a078p093_supp.pdf) was based mainly on data from Schlüter et al. (2000), augmented with data from other estuaries, including the nearby Chesapeake Bay (see Table S1 for references). The pigment ratios were randomly varied 80 times within a 35% window, and the 8 ratios with the lowest residual root mean square were averaged and used for the final calculation (Higgins et al. 2011). This procedure was applied to samples binned according to the attenuation coefficient because of the known effect of light on algal pigment ratios (Schlüter et al. 2000). The 6 attenuation coefficient bins were selected to yield about 30 samples per bin through the salinity gradient. Attenuation correlated with salinity ($r = -0.56$, $p < 0.001$, $n = 141$), nutrients (for example, for nitrate: $r = 0.72$, $p < 0.001$, $n = 140$), and other properties likely to affect pigment ratios and the Chemtax analyses.

Statistical analyses

Variation in bulk bacterial and biogeochemical properties with salinity, month, and year was examined by multivariate analysis of variance (ANOVA). The data for chl *a*, DOC, leucine incorporation, and bacterial abundance were log-transformed before the ANOVA whereas a $\log(X + 0.05)$ transformation was used for the nitrate data. ANOVA was also used to examine variation in the phytoplankton classes after a $\log(X + 0.01)$ transformation and bacterial OTU abundance after a $\log(X + 1)$ transformation. These analyses were done in R (https://www.r-project.org) as were 2 nonparametric tests, Spearman rank correlation and the Mann-Whitney test. The p-values for all correlations were adjusted for multiple comparisons using the Benjamini-Hochberg false discovery rate approach.

Several multivariate statistical approaches were used to explore relationships among bacterial and phytoplankton communities and biogeochemical properties. Nonmetric multidimensional scaling (NMDS) plots were constructed using Bray-Curtis distances, and vectors for salinity and temperature were fit to the NMDS plot using the envfit function in the vegan package (version 2.4-0) in R (Oksanen et al. 2016). Variation in bacterial community composition as a function of salinity, month, and year was examined using a permutational multivariate analysis of variance (PERMANOVA, the adonis function in vegan) with Bray-Curtis distance matrices. We used redundancy analysis with forward selection of select environmental parameters (see 'Results') and the 9 phytoplankton classes via the ordistep function (999 permutations) to explore the contribution of these properties to explaining the composition of bacterial communities in the estuary. The best model was picked by maximizing the adjusted R^2 as each parameter was added.

Richness (number of OTUs) and the Shannon diversity index were calculated with the vegan package. Two indices of evenness, Simpson and Pielou (Smith & Wilson 1996), were calculated using properties generated by the vegan package. Variation in the Shannon index as a function of several parameters was examined by multivariate regression analysis using the leaps package (version 2.9) in R (James et al. 2013). The variables were chosen by exhaustive search (both forward and backward inclusion of the variables), and the best model was selected based on the adjusted R^2. The best model was then examined to ensure the assumptions (e.g. normality and homogeneity) of the regression analysis were met. The variables remaining in the model were judged not to be co-linear based on variance inflation factors calculated using the car package (2.1-3) in R (Fox & Weisberg 2011). Standardized beta coefficients for the best model were calculated using the QuantPsyc package (1.5) (Fletcher 2012).

RESULTS

We examined the community composition of phytoplankton and bacteria along with several biogeochemical properties of the Delaware Estuary in August and November of 2011 to 2013. Nearly all biogeochemical properties varied significantly between the 2 months and with salinity, whereas only a few varied significantly among the 3 years (Table 1). In general, properties differed more clearly between the 2 months than with salinity. For example, chl *a*, leucine incorporation, and bacterial abundance were significantly higher by approximately 2- to 7-fold in August than in November, whereas these properties varied with salinity but not in any clear pattern. The differences between the 2 months are likely due to temperature (over 2-fold decrease from August to November) and the light environment; PAR decreased by 2-fold while the attenuation coefficient increased by nearly 50% from August to November (Table 1).

Table 1. Biogeochemical properties of the Delaware Estuary in 2011–2013. Means and SD were calculated for August and November using data from all locations in the estuary during the 3 years (n = 143). Significance was determined by ANOVA (*p < 0.05; **p < 0.01; ***p < 0.001; NS: not significant). The relationship between a property and salinity either varied inconsistently ('Varied'), was negatively correlated, or was not significant. Two properties were highest at salinity 23 ('Max at 23'). PAR: photosynthetically active radiance, integrated over a day; Atten: attenuation coefficient; Leu incorp: leucine incorporation; Abundance: bacterial abundance

Property	August		November		Variation due to:		
	Mean	SD	Mean	SD	Month	Salinity	Year
Temperature (°C)	26.1	2.3	10.9	1.8	***	Varied***	NS
PAR (mol m^{-2})	34.7	12.1	15.5	5.7	***	NS	NS
Nitrate (µM)	52	53	63	51	***	Negative***	*
Atten (m^{-1})	1.6	1.1	2.4	2.1	***	Negative***	NS
Chlorophyll *a* (µg l^{-1})	8.3	4.1	3.5	4.0	**	Max at 23**	NS
Leu incorp (nM h^{-1})	377	160	52	22	**	Max at 23**	NS
Abundance (10^6 cells ml^{-1})	3.8	1.9	1.6	0.8	***	NS	NS

Variation in bacterial and phytoplankton communities in the estuary

Bacterial community structure varied significantly and substantially with salinity (R^2 = 0.19, p < 0.001, n = 115, PERMANOVA). Communities from low salinity waters separated from the high-salinity communities along the first dimension of the NMDS plot (Fig. 1A). The abundance of bacterial groups also varied with salinity as expected based on previous studies in the Delaware and other estuaries. For example, *Actinobacteria*, *Burkholderiales*, and other *Betaproteobacteria* were most abundant in low-salinity waters while most of the alphaproteobacterial clades were most abundant in high-salinity waters (Table 2).

The SAR11 clade was quite abundant in the estuary and was dominated by 2 subclades which varied differently along the salinity gradient (Fig. 2). The relative abundance of subclade SAR11 IIIa increased with salinity from detection limits to about 40 and 80% of the community in August and November, respectively. In contrast, the relative abundance of SAR11 IIIb, the 'freshwater clade' (Vergin et al. 2013), was low in low-salinity waters (<5 salinity) and highest in 10 salinity waters, where it was as much as 70% of the total community, and then it decreased to as low as 5% in high-salinity waters near the mouth of the estuary (Fig. 2). Other SAR11 subclades abundant in oceanic waters, such as the 1a subclade (Vergin et al. 2013), were barely detected (data not shown).

Bacterial communities also differed between the 2 months, but this temporal effect was less than that observed for salinity, according to PERMANOVA (R^2 = 0.10, p < 0.001 for the 2 months, nearly half the

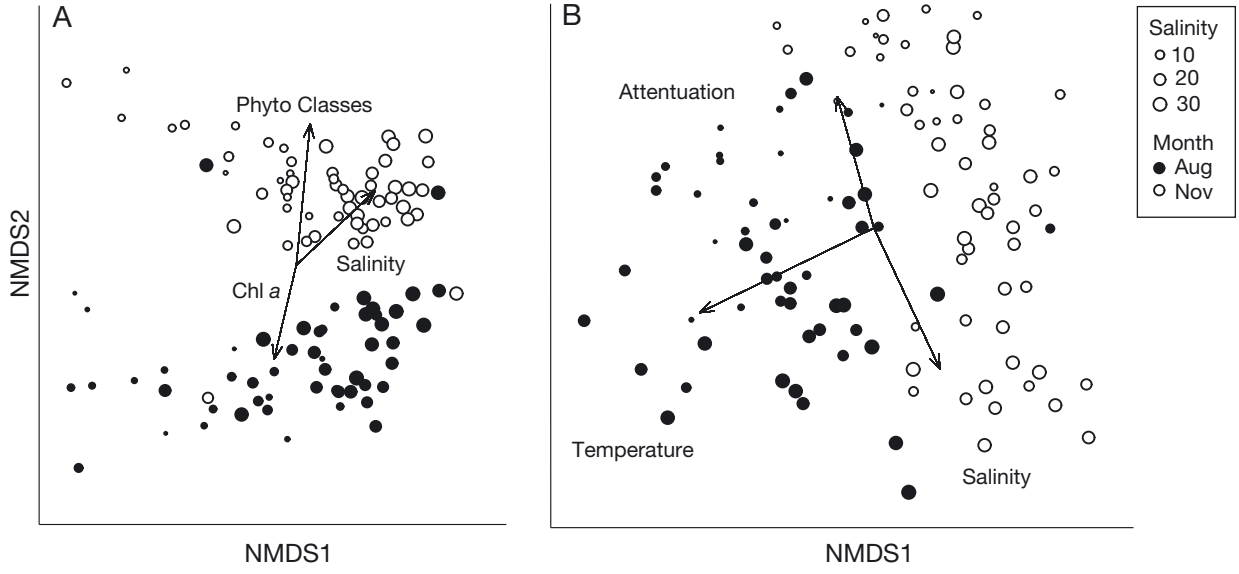

Fig. 1. Nonmetric multidimensional scaling (NMDS) of bacterial and phytoplankton communities in the Delaware Estuary. (A) Bacterial community. The vector for 'Phyto Classes' is the first principal component score from a principal component analysis of the 9 phytoplankton classes; N = 113, stress = 0.125. (B) Phytoplankton communities, based on pigment-defined classes; N = 113, stress = 0.214

Table 2. Relative abundance (% of total) of bacterial taxa in the Delaware Estuary 2011–2013. Means and SD were calculated for August and November using data from all locations in the estuary during the 3 years (n = 115). Significance was determined by ANOVA (*p < 0.05; **p < 0.01; ***p < 0.001; NS: not significant). The relationship with salinity was either positively or negatively correlated ('Pos,' 'Neg'), was maximal ('Max') at 10 or 24 salinity, or was not significant. Other *Beta*: *Betaproteobacteria* other than *Burkholderiales*; Other *Gamma*: *Gammaproteobacteria* other than *Oceanospirillales*; Other *Alpha*: *Alphaproteobacteria* other than *Rhodobacterales* and the SAR11 subclades. SAR11 other: SAR11 subclades other than IIIa and IIIb

Group	August		November		Significance		
	Mean	SD	Mean	SD	Month	Salinity	Year
Actinobacteria	10.1	12.9	6.5	10.9	**	Neg***	***
Bacteroidetes	0.8	0.7	1.5	1.3	**	Neg***	NS
Burkholderiales	1.1	1.8	2.1	4.0	**	Neg***	***
Other *Beta*	1.1	0.9	2.2	1.6	***	Neg***	***
Cyanobacteria	10.7	8.6	0.5	0.6	***	Max at 24*	**
Deltaproteobacteria	0.6	0.6	0.9	3.7	**	NS	***
Oceanospirillales	1.6	1.2	1.9	1.8	NS	Pos***	**
Other *Gamma*	1.1	1.0	1.3	0.9	NS	Neg***	***
Planctomycetes	3.4	3.9	0.5	0.7	***	Neg***	***
Rhodobacterales	4.5	8.3	3.2	2.6	***	Pos***	NS
SAR11 IIIa	27.9	20.9	43.2	25.2	**	Pos***	NS
SAR11 IIIb	28.8	18.3	25.9	20.9	NS	Max at 10***	NS
SAR11 other	0.8	0.7	0.7	0.5	NS	Pos***	NS
Other *Alpha*	2.8	1.8	4.0	2.3	***	NS	**
Other known	1.8	2.4	1.4	3.4	***	Neg***	*
Unknown *Bacteria*	2.8	3.6	4.0	2.7	**	Varied*	NS

value for salinity) and NMDS analysis. Communities from August formed a cluster separate from November communities along the second dimension of the NMDS plot (Fig. 1A). There was a small but significant difference in overall composition among the 3 years ($R^2 = 0.03$; $p < 0.001$). The relative abundance of nearly all bacterial groups also varied significantly between the 2 months, and several groups also varied significantly among the 3 years (Table 2).

The 9 phytoplankton classes defined by the Chemtax analyses varied substantially in the estuary, but this variation differed greatly from that of the bacterial communities. In contrast to the bacteria, phytoplankton communities separated by month rather than by salinity along the first NMDS axis; separation along the second axis was associated with salinity and the light environment, evident from the attenuation coefficient vector (Fig. 1B). Six of the 9 phytoplankton classes were significantly more abundant in August than in November while the other 3 did not differ significantly between the 2 months (Table 3). In both months and all salinities, diatoms were most abundant, making up about half of the total phytoplankton community. The 2 least abundant phytoplankton classes were usually prasinophytes and dinoflagellates (Table 3).

Relationship between bacterial taxon abundance and phytoplankton

To explore relationships between the abundance of the bacterial taxa and the phytoplankton, we did partial correlation analysis of the 2 parameters controlling for salinity. We used the relative abundance

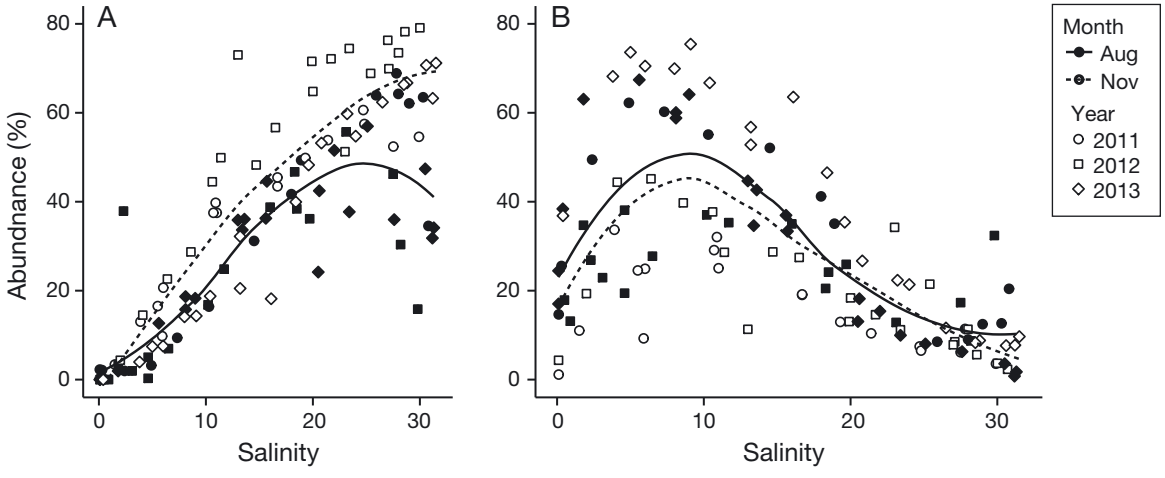

Fig. 2. Relative abundance (% of total sequences for each sample) of (A) SAR11 IIIa and (B) SAR11 IIIb in the Delaware Estuary. The lines are from locally weighted scatterplot smoothing

Table 3. Abundance of phytoplankton classes determined by Chemtax in the Delaware Estuary in 2011–2013 (n = 128). Abundance was expressed as the concentration (ng l^{-1}) of chlorophyll *a* allocated to that class. Examples of haptophytes-6 and -8 include coccolithophorids and *Phaeocystis* spp., respectively. Mean and SD were calculated for August and November using data from all locations in the estuary during the 3 years. Significance was determined by ANOVA (*p < 0.05; **p < 0.01; ***p < 0.001; NS: not significant)

Group	August		November		% of total		Variation due to:		
	Mean	SD	Mean	SD	Aug	Nov	Month	Salinity	Year
Chlorophytes	477	691	78	81	6.0	2.9	***	***	NS
Cryptophytes	1030	1009	732	288	13.0	26.7	NS	NS	**
Cyanobacteria	521	382	14	28	6.6	0.5	***	***	NS
Diatoms	4637	3023	1330	693	58.5	48.6	***	*	NS
Dinoflagellates	257	573	16	43	3.2	0.6	***	***	NS
Haptophytes-6	459	449	229	208	5.8	8.3	NS	NS	***
Haptophytes-8	344	1017	159	145	4.3	5.8	***	*	***
Pelagophytes	123	184	152	155	1.6	5.6	NS	***	NS
Prasinophytes	74	88	29	37	0.9	1.1	*	***	NS

(normalized sequence numbers) to focus on effects on the taxonomic composition of the bacterial communities rather than on total abundance. The 2 months were analyzed separately to remove the effect of temperature; since temperature varied by only a few degrees along the transects within a month, it is unlikely to have an effect. In August, none of the bacterial taxa significantly correlated with chl *a*, whereas in November, there were 5 positive significant relationships and 2 negative ones, controlling for salinity (Fig. 3). The phyla with the highest positive partial correlations with chl *a* were *Bacteroidetes* and *Planctomycetes* (ρ = 0.644 and 0.545, respectively, adjusted p < 0.001, n = 113). Both SAR11 IIIa and IIIb were negatively correlated with chl *a*, controlling for salinity (ρ = −0.469 and −0.447, respectively, adjusted p < 0.001).

The same analysis was used to explore relationship between bacterial taxa and the 9 phytoplankton classes (identified by Chemtax) while minimizing salinity and temperature effects (data not shown, except for the numbers given here). In August, there were only 2 significant relationships, both positive, among the 144 possible relationships. The significant relationships were between *Rhodobacterales* and haptophytes-6 and between *Deltaproteobacteria* and diatoms (ρ = 0.458 and 0.469, respectively, adjusted p = 0.035, n = 113). In November, there were 5 significant relationships. Dinoflagellates had significant partial correlations, controlling for salinity, with *Bacteroidetes*, *Deltaproteobacteria*, and *Planctomycetes* (ρ = 0.556, 0.437, and 0.421; adjusted p = 0.001, 0.019, and 0.023, respectively). *Bacteroidetes* also correlated with pelagophytes in November (ρ = 0.459, adjusted p < 0.012). In contrast, there was a negative partial correlation between pelagophytes and SAR11 IIIa (ρ = −0.565, adjusted p = 0.001).

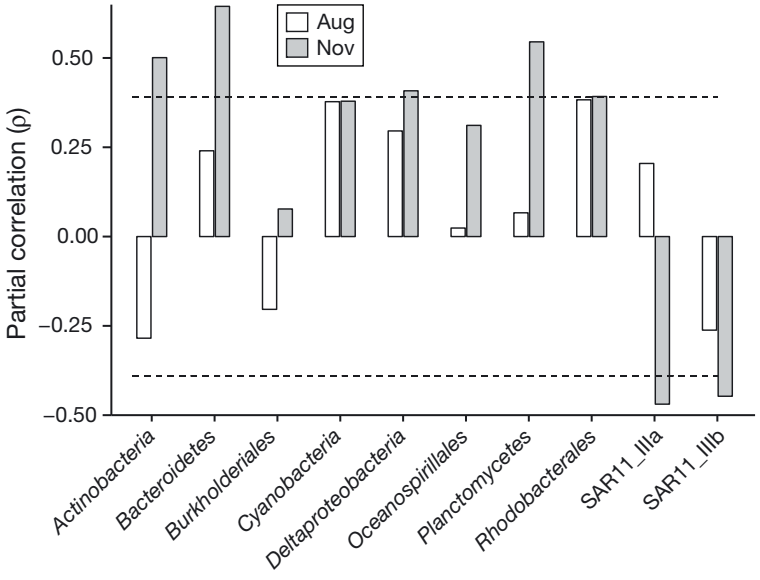

Fig. 3. Partial correlation between indicated bacterial taxa and chlorophyll *a*, controlling for salinity. The dashed lines indicate the p < 0.05 significance level

Bacterial community composition explained by biogeochemical and phytoplankton properties

We next examined the extent to which the taxonomic composition of the entire community and of individual bacterial taxa could be explained by phytoplankton properties. The difference in bacterial community composition between August and November can be attributed to phytoplankton biomass (chl *a*) and the composition of the phytoplankton community (Chemtax), along with temperature (Fig. 1A). PERMANOVA indicated that the fraction of variation in bacterial composition explained by phytoplankton community composition was higher than by salinity or temperature (r^2 = 0.269, 0.186, and 0.108 respectively), all higher

Table 4. Contribution of salinity, temperature, chlorophyll *a* (Chl *a*), and phytoplankton classes to explaining the variation in the composition of the total community ('All') and of individual bacterial taxa. The numbers are p-values from redundancy analyses of indicated bacterial taxa as a function of salinity, temperature, chl *a* and phytoplankton classes. Bacterial groups are arranged from most to least abundant on average. *Alpha*, *Beta*, *Gamma*, *Delta*: *Alpha-*, *Beta-*, *Gamma-*, *Deltaproteobacteria*. Phytoplankton names were abbreviated by deleting 'phytes', except for cyanobacteria (Cyano). Salinity was significant for all ($p < 0.005$), as was temperature, except for *Oceanospirillales* ($p > 0.05$). Diatoms were not significant for any bacterial group, while cryptophytes contributed significantly only for Other *Gammaproteobacteria*. Insignificant contributions are indicated with —

Taxon	Chl *a*	Cyano	Chloro	Prasino	Dino	Hapto-8	Hapto-6	Pelago
All	0.005	0.045	—	0.01	0.005	0.025	—	0.025
SAR11 IIIa	—	—	—	—	—	0.02	—	—
SAR11 IIIb	0.01	—	—	—	—	—	—	—
Actinobacteria	0.03	0.045	—	0.015	0.005	0.005	—	—
Cyanobacteria	—	—	0.02	—	0.005	—	—	—
Rhodobacterales	—	—	—	—	0.005	—	0.005	0.005
Other *Alpha*	0.035	—	0.02	0.03	0.01	—	—	0.005
Planctomycetes	0.01	—	—	0.01	0.05	—	0.03	—
Oceanospirillales	—	0.03	—	—	—	0.01	—	—
Other *Beta*	0.005	—	—	0.05	0.015	—	—	—
Burkholderia	0.015	—	—	—	—	—	—	—
Other *Gamma*	0.005	—	—	0.01	0.025	0.005	—	—
Bacteroidetes	0.025	0.015	—	0.02	0.01	—	—	0.025
Delta	—	—	—	—	0.005	—	—	—
Other SAR11	—	0.01	0.03	—	—	0.035	—	0.005

than that for total chl *a* (0.072; all significant at the $p < 0.001$ level, $n = 113$).

We then used redundancy analysis (RDA) to find the smallest set of parameters that could best explain variation in the composition of the total bacterial community and of individual bacterial taxa (Table 4). This approach was used to identify significant relationships between a phytoplankton class and bacterial community composition while also including temperature and salinity effects. Adding DOC concentrations and leucine incorporation did not substantially improve (1%) models of variation in community composition, so they were not included in further analyses. Salinity and temperature were significant in nearly all analyses for both the total community and individual bacterial taxa (Table 4). Along with total chl *a*, 5 of the 9 phytoplankton classes significantly contributed to explaining the composition of the total bacterial community (Table 4). The insignificant classes included the most abundant ones, diatoms and cryptophytes, as well as chlorophytes and haptophytes-6.

Several phytoplankton classes contributed significantly to explaining the variation in the composition of several bacterial taxa in the estuary (Table 4). The 2 phytoplankton classes with the lowest abundance overall in the estuary had significant relationships with the highest number of bacterial taxa. Dinoflagellates, which made up about 2% of the phytoplankton community overall, had significant relationships with 9 bacterial taxa. Prasinophytes, another relatively minor phytoplankton class in the estuary (about 1% of the total), had significant relationships with 6 bacterial taxa. In contrast, the 2 most abundant phytoplankton classes, diatoms (about 50%) and cryptophytes (nearly 20%), had no and 1 significant relationship with a bacterial group, respectively.

Bacterial and phytoplankton diversity along the salinity gradient

The diversity of bacterial and phytoplankton communities in the estuary varied substantially with salinity and month. Richness of the bacterial communities significantly decreased with salinity in both months (Fig. 4A, ANOVA, $p < 0.02$), but there was no significant difference in richness between the 2 months ($p > 0.05$). Two indices of evenness, Simpson (Fig. 4B) and Pielou (data not shown), decreased significantly with salinity and were significantly higher in August than in November (ANOVA, $p < 0.01$) by 23 and 11%, respectively. The Shannon index also decreased substantially along the freshwater to saltwater gradient (Fig. 4C), more so in November than in August. The Shannon index for bacterial communities was slightly but significantly higher in August than in November (2.5 vs. 2.2, Mann-Whitney test, $p < 0.001$, $n = 58$ and 55, respectively); the difference was greatest in waters with salinities >20 (Fig. 4C).

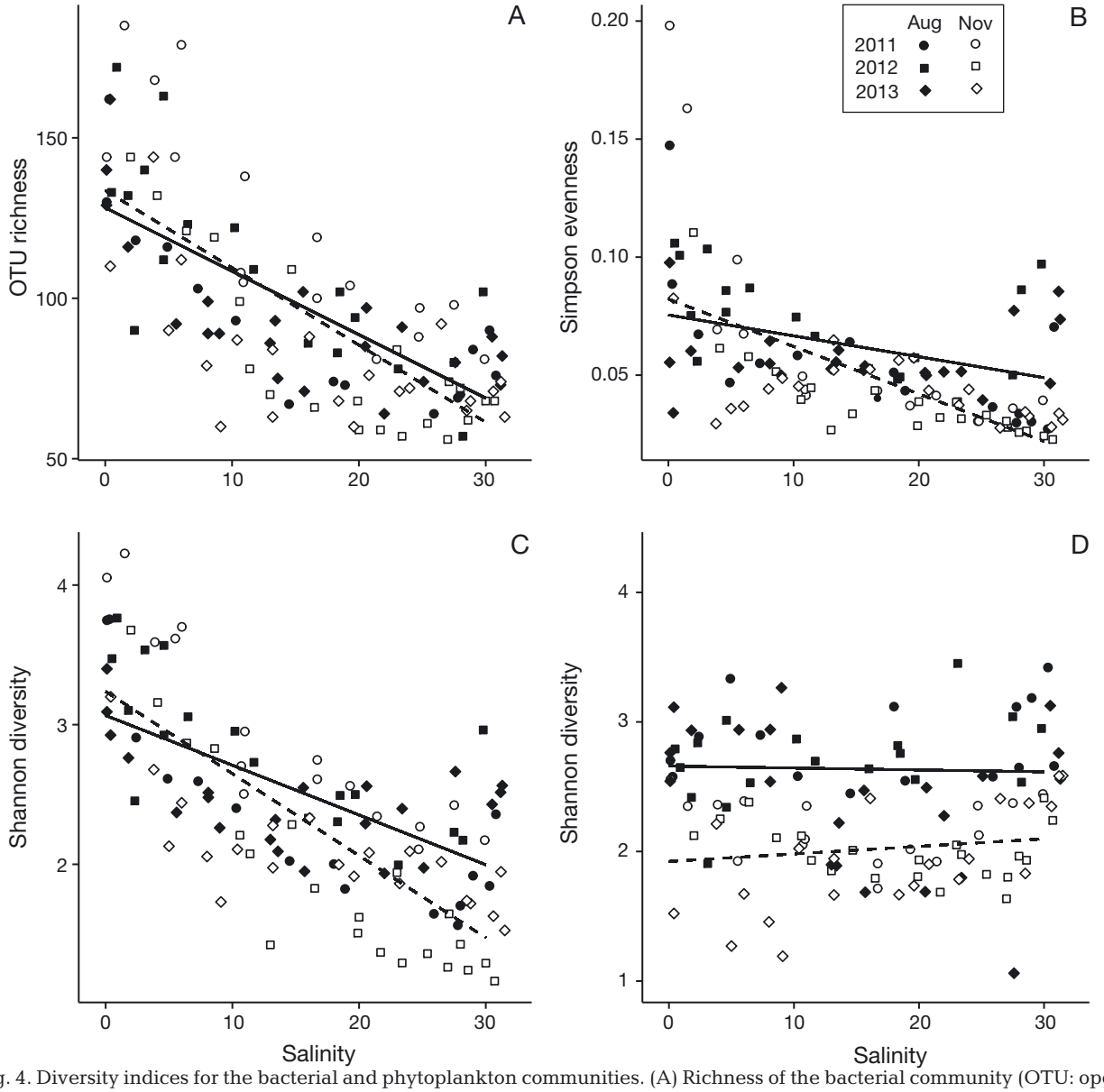

Fig. 4. Diversity indices for the bacterial and phytoplankton communities. (A) Richness of the bacterial community (OTU: operational taxonomic unit); (B) Simpson evenness index for the bacterial community; (C) Shannon diversity index for the bacterial community; and (D) Shannon diversity index for the phytoplankton community, based on 16S rRNA gene sequences from cyanobacteria and chloroplasts from eukaryotic phytoplankton. The solid and dashed lines are from linear regression analyses for August and November, respectively. Results from the regression analyses are summarized in Table S2 in the Supplement at www.int-res.com/articles/suppl/a078p093_supp.pdf

Thus far, the Chemtax data have been used to explore phytoplankton communities, but to examine the diversity of those communities at a finer taxonomic level, we used 16S rRNA gene sequences. In contrast to the bacteria, the Shannon index for phytoplankton diversity did not vary significantly with salinity in August, whereas it increased slightly with salinity in November (Fig 4D). Phytoplankton communities were more diverse in August than in November, in terms of OTU richness (by 2-fold) and the Shannon index (23%; Mann-Whitney test, $p < 0.001$). The Pielou index indicated that the phytoplankton community was significantly, albeit only slightly (9%), more even in August than in November (Mann-Whitney test, $p < 0.001$), whereas the Simpson evenness index did not differ significantly between the 2 months (data not shown).

To explore relationships between bacterial diversity and the phytoplankton classes, we used multivariate regression analysis to determine the best

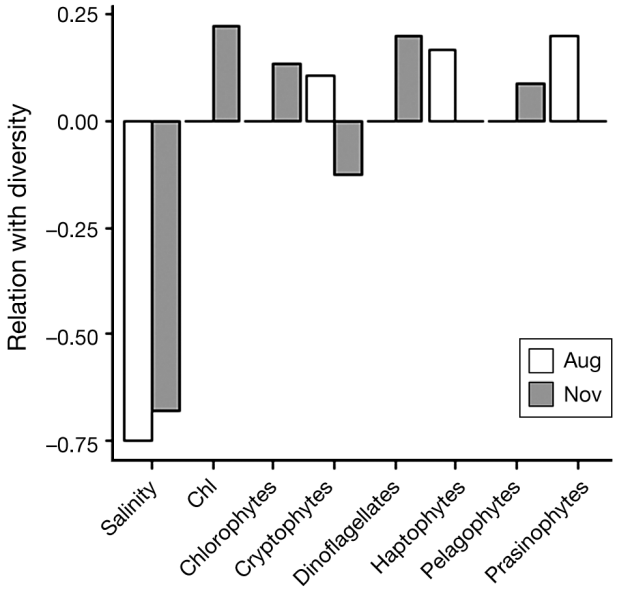

Fig. 5. Standardized coefficients (β) from regression analyses of the Shannon diversity index for bacterial communities as a function of salinity, chlorophyll *a* (Chl), and the phytoplankton classes. The phytoplankton classes not shown had no significant relationship with the index

model (measured by the adjusted R^2) that explains bacterial diversity as a function of the phytoplankton classes (the Chemtax data), chl *a*, and salinity. The 2 months were analyzed separately to minimize the contribution of temperature. In both months, salinity contributed the most to explaining variation in the Shannon index for bacterial diversity (standardized coefficients [β] equal to −0.748 and −0.680 for August and November, respectively) while only a few phytoplankton classes had significant roles (β = 0.10 to 0.197; Fig. 5). The significant phytoplankton classes were cryptophytes, haptophytes-6, and prasinophytes in August and chlorophytes, dinoflagellates, and pelagophytes in November. Cryptophytes had a significant but negative relationship with bacterial diversity (β = −0.125). The relationship between bacterial diversity and chl *a* was significant only in November (β = 0.221). Diatoms did not have a significant role in explaining bacterial diversity in either month.

DISCUSSION

The growth, biomass, and taxonomic composition of bacterial communities partially depend on phytoplankton even in estuaries with extensive terrestrial inputs and mixing of freshwater and oceanic waters. Even accounting for strong salinity effects, we found that the abundance and composition within some bacterial taxa and overall bacterial community composition had significant statistical relationships with chl *a* and with the composition of the phytoplankton community, suggesting substantial connections between the 2 microbial communities. Surprisingly, the most abundant phytoplankton classes (diatoms and cryptophytes) had few significant relationships with overall bacterial community composition and with the abundance and composition of individual bacterial taxa.

The paucity of significant relationships between diatoms and bacteria may indicate that coupling between these microbes is looser than previously hypothesized (Amin et al. 2012). However, caution is warranted here because of the usual problems using correlations to infer causation. Also, perhaps more relationships would have been detected if the active bacterial community had been examined (Campbell & Kirchman 2013). In addition, the lack of a relationship may simply reflect the lack of samples needed to detect lags in the response of bacteria to phytoplankton over relatively short time scales (days to weeks). The abundance of *Flavobacteria*, for example, has been observed to increase days after a diatom bloom (Fandino et al. 2005), a relationship that would have been missed in our study. Rather than focusing on short time-scale relationships, our study was designed to explore longer-term interactions between bacteria and phytoplankton using data from 2 months over 3 years. A final problem is that the taxonomic resolution of Chemtax is probably too coarse to detect highly specific interactions between bacteria and phytoplankton.

Even though its taxonomic resolution is low, we used the Chemtax approach for nearly all analyses of the phytoplankton community because it has been extensively tested in estuaries and other marine waters (Lionard et al. 2008, Keller et al. 2014, Schlüter et al. 2014). We are not aware of a published study using 16S rRNA gene sequence data to explore phytoplankton in estuaries, although it has been used in other systems (Milici et al. 2016, Needham & Fuhrman 2016). In any case, these sequence data and the Chemtax approach share a related problem: chloroplast numbers and thus 16S rRNA gene sequences and pigments can vary due to environmental properties independent of variation in taxon abundance. The Chemtax approach, however, was tuned to minimize variation due to the light environment and other properties co-varying with salinity. In contrast, there is no obvious way to correct for changes in the relative abundance of 16S rRNA gene

sequences within a phytoplankton taxon due to variation in the number of chloroplasts along the salinity gradient or between the 2 months. In spite of these potential problems, however, there was a significant correlation between the 2 approaches when the 16S rRNA gene data were aggregated into the phytoplankton classes defined by the Chemtax data (Spearman $\rho = 0.575$; $p < 0.001$); the comparison for each class is discussed in more detail in the Supplement at www.int-res.com/articles/suppl/a078p093_supp.pdf. In addition, both approaches indicated that phytoplankton community composition consistently varied more between the 2 months than along the salinity gradient in the estuary.

In spite of methodological limitations, we did find several significant relationships between bacteria and phytoplankton taxa. One mechanism leading to different phytoplankton–bacteria relationships is the variation in the amount and types of organic compounds produced by different phytoplankton classes (Biersmith & Benner 1998, Aluwihare & Repeta 1999). One class of organic compounds needed by some microbes are vitamins, especially the B-vitamins (Sañudo-Wilhelmy et al. 2014). It is noteworthy that the 2 most abundant taxa in the bacterial and phytoplankton communities, the SAR11 clade and diatoms (some species), require vitamin B_{12} (Sañudo-Wilhelmy et al. 2014). Perhaps competition for this vitamin accounts for the negative relationships between SAR11 and the phytoplankton community observed in the Delaware Estuary.

Some of the bacterial taxa with significant relationships with phytoplankton were expected while others were unexpected. The observed high correlation between *Bacteroidetes* and chl *a* was expected based on previous work associating this phylum with phytoplankton (Fandino et al. 2005, Buchan et al. 2014, Lucas et al. 2015, Farnelid et al. 2016). However, unlike studies of phytoplankton blooms, *Bacteroidetes* abundance did not correlate significantly with diatoms in the Delaware Estuary, but rather with dinoflagellates and pelagophytes in November when chl *a* was lower. Two SAR11 subclades, which are abundant in the Delaware Estuary and in the Baltic Sea (Herlemann et al. 2014), were negatively correlated with chl *a* in November, as previously observed for the freshwater clade of SAR11 in a lake (Heinrich et al. 2013). Unexpectedly, we found *Planctomycetes* to be highly correlated with chl *a* and with dinoflagellates. The *Planctomycetes* genus *Pirellula* was abundant in a diatom bloom in Oregon coastal waters (Morris et al. 2006), and recently, these bacteria were implicated in the degradation of complex polysaccharides in soils (Wang et al. 2015). Perhaps polymer-degrading capabilities of aquatic *Planctoymycetes* help to explain the strong relationship of this phylum with phytoplankton biomass in the Delaware Estuary.

Salinity has a large effect on the taxonomic composition of bacterial communities in estuaries, but it also may have a large effect on the richness and evenness of those communities. Both aspects of diversity declined substantially with salinity in the Delaware Estuary. The high richness in the freshwater end of the estuary may be due to the input of bacterial taxa from the river which then die out as salinity increases. However, there is little evidence that the activity of taxa such as *Betaproteobacteria*, which are most abundant in brackish waters of the estuary (Kirchman et al. 2005), declines with salinity in the Delaware Estuary (Cottrell & Kirchman 2004). Another possibility is that the diversity of the DOM pool (Osterholz et al. 2016) or some other bottom-up factor co-varying with salinity drives the diversity of bacterial communities in the estuary. It is harder to envision top-down factors varying as consistently with salinity. Bacterial diversity is low in waters with very high salinity (Wang et al. 2011), but variation in diversity due to salinity has not been observed in estuaries. Previous studies did not detect systematic variation in taxon richness in estuaries using DNA fingerprinting methods (Troussellier et al. 2002, Hewson & Fuhrman 2004). Likewise, studies using a tag sequencing approach similar to that used here also did not observe bacterial diversity to vary with salinity in the Columbia River (calculated with data provided by Fortunato et al. 2013) nor in the Baltic Sea (Herlemann et al. 2011, Hu et al. 2016). Perhaps the diversity–salinity relationship depends on the residence time of estuaries, which is thought to be important in shaping estuarine bacterial communities (Crump et al. 2004). The residence time of the Delaware Estuary (about 90 d) differs greatly from that of the Columbia River (2 d) and the Baltic Sea (3–30 yr). Whatever the mechanism, it differs from that driving variation in phytoplankton communities with salinity. In contrast to bacteria, phytoplankton diversity did not consistently vary with salinity, and the phytoplankton community composition was affected more by differences between the 2 months than by salinity.

The diversity of both bacteria and phytoplankton varied between the 2 months as well as with salinity. The number of phytoplankton taxa, evenness of bacterial communities, and the Shannon index for both phytoplankton and bacteria were lower in November

than in August in the Delaware Estuary. The lower diversity in November may seem to contradict reports of higher diversity in the winter (Ladau et al. 2013), but the conditions of the Delaware system in November are far from winter; water temperatures, for example, are much warmer in November than in late January (11 versus 2°C). Biomass production, which was higher in August than November, is another factor that potentially affects diversity (Schabhüttl et al. 2013).

The relationships between bacteria and phytoplankton communities found here may help to explain previous studies of total biomass and production by these 2 communities in aquatic systems. While we found that phytoplankton biomass and taxonomic composition played significant roles in shaping the composition and diversity of bacterial communities in the Delaware Estuary, perhaps more noteworthy was the paucity of significant relationships between the most abundant phytoplankton classes, especially diatoms, and the bacterial community. A recent study in the Baltic Sea also noted the lack of significant relationships between abundant bacterial taxa and the phytoplankton community during a diatom-dominated bloom (Bunse et al. 2016). The weak relationships with diatoms help to explain the low correlation or coupling between bacteria and phytoplankton bulk properties frequently observed in these waters (Hoch & Kirchman 1993) and elsewhere (Fouilland & Mostajir 2011). Since total biomass and likely primary production would be dominated by the most abundant phytoplankton class, weak relationships between diatoms and the bacterial community would result in weak correlations between phytoplankton and bacteria viewed at the total community level. The correlations would be further weakened by the paucity of significant relationships between phytoplankton and the most abundant bacterial taxa in the estuary, the SAR11 subclades, even though other bacteria can have strong connections with members of the phytoplankton community. Overall, the results illustrate the use of community composition data to explore the flow of material and energy between bacteria and phytoplankton in aquatic systems.

Acknowledgements. We thank Kevin Vergin and Cameron Thrash for sending the SAR11 subclade data, and Simon Wright, Louise Schlüter, and Jay Pinckney for their help with Chemtax and initial pigment ratios. Helena Osterholz provided many useful comments and suggestions. We also thank Caroline Fortunato, Thomas Jeffries, and Marc Trousellier for sending data from their estuarine studies about the diversity–salinity relationship. The field work was only possible with the help of Monica Stegman, Thomas Lankiewicz, Katie Kalis, Raphael Lami, and Dave Kieber, while Liying Yu was essential in the laboratory analyses. This work was supported by NSF grants OCE-1030306 and OCE-1261359 to D.L.K. and M.T.C.

LITERATURE CITED

Aluwihare LI, Repeta DJ (1999) A comparison of the chemical characteristics of oceanic DOM and extracellular DOM produced by marine algae. Mar Ecol Prog Ser 186: 105–117

Amin SA, Parker MS, Armbrust EV (2012) Interactions between diatoms and bacteria. Microbiol Mol Biol Rev 76:667–684

Andersson AF, Riemann L, Bertilsson S (2010) Pyrosequencing reveals contrasting seasonal dynamics of taxa within Baltic Sea bacterioplankton communities. ISME J 4:171–181

Béjà O, Suzuki MT (2008) Photoheterotrophic marine prokaryotes. In: Kirchman DL (ed) Microbial ecology of the oceans, 2nd edn. John Wiley & Sons, New York, NY, p 131–157

Biersmith A, Benner R (1998) Carbohydrates in phytoplankton and freshly produced dissolved organic matter. Mar Chem 63:131–144

Bouvier TC, del Giorgio PA (2002) Compositional changes in free-living bacterial communities along a salinity gradient in two temperate estuaries. Limnol Oceanogr 47: 453–470

Buchan A, LeCleir GR, Gulvik CA, Gonzalez JM (2014) Master recyclers: features and functions of bacteria associated with phytoplankton blooms. Nat Rev Microbiol 12: 686–698

Bunse C, Bertos-Fortis M, Sassenhagen I, Sildever S and others (2016) Spatio-temporal interdependence of bacteria and phytoplankton during a Baltic Sea spring bloom. Front Microbiol 7:517

Campbell BJ, Kirchman DL (2013) Bacterial diversity, community structure and potential growth rates along an estuarine salinity gradient. ISME J 7:210–220

Caporaso JG, Kuczynski J, Stombaugh J, Bittinger K and others (2010) QIIME allows analysis of high-throughput community sequencing data. Nat Methods 7:335–336

Cottrell MT, Kirchman DL (2004) Single-cell analysis of bacterial growth, cell size, and community structure in the Delaware estuary. Aquat Microb Ecol 34:139–149

Cottrell MT, del Giorgio PA, Kirchman DL (2015) Biogeography of globally distributed bacteria in temperate and boreal Québec lakes as revealed by tag pyrosequencing of 16S rRNA genes. Aquat Microb Ecol 76:175–188

Crump BC, Hopkinson CS, Sogin ML, Hobbie JE (2004) Microbial biogeography along an estuarine salinity gradient: combined influences of bacterial growth and residence time. Appl Environ Microbiol 70:1494–1505

Decelle J, Romac S, Stern RF, Bendif EM and others (2015) PhytoREF: a reference database of the plastidial 16S rRNA gene of photosynthetic eukaryotes with curated taxonomy. Mol Ecol Resour 15:1435–1445

Dempster EL, Pryor KV, Francis D, Young JE, Rogers HJ (1999) Rapid DNA extraction from ferns for PCR-based analyses. Biotechniques 27:66–68

DiTullio GR, Geesey ME, Maucher JM, Alm MB, Riseman SF, Bruland KW (2005) Influence of iron on algal commu-

nity composition and physiological status in the Peru upwelling system. Limnol Oceanogr 50:1887–1907

Ducklow H (2000) Bacterial production and biomass in the oceans. In: Kirchman DL (ed) Microbial ecology of the oceans. John Wiley & Sons, New York, NY, p 85–120

Fandino LB, Riemann L, Steward GF, Azam F (2005) Population dynamics of *Cytophaga-Flavobacteria* during marine phytoplankton blooms analyzed by real-time quantitative PCR. Aquat Microb Ecol 40:251–257

Farnelid HM, Turk-Kubo KA, Zehr JP (2016) Identification of associations between bacterioplankton and photosynthetic picoeukaryotes in coastal waters. Front Microbiol 7:339

Fletcher TD (2012) QuantPsyc: quantitative psychology tools. R package version 15. https://CRAN.R-project.org/package=QuantPsyc

Fortunato CS, Herfort L, Zuber P, Baptista AM, Crump BC (2012) Spatial variability overwhelms seasonal patterns in bacterioplankton communities across a river to ocean gradient. ISME J 6:554–563

Fortunato CS, Eiler A, Herfort L, Needoba JA, Peterson TD, Crump BC (2013) Determining indicator taxa across spatial and seasonal gradients in the Columbia River coastal margin. ISME J 7:1899–1911

Fouilland E, Mostajir B (2011) Complementary support for the new ecological concept of 'bacterial independence on contemporary phytoplankton production' in oceanic waters. FEMS Microbiol Ecol 78:206–209

Fox J, Weisberg S (2011) An R companion to applied regression, 2nd edn. SAGE Publications, Los Angeles, CA

Heinrich F, Eiler A, Bertilsson S (2013) Seasonality and environmental control of freshwater SAR11 (LD12) in a temperate lake (Lake Erken, Sweden). Aquat Microb Ecol 70:33–44

Herlemann DPR, Labrenz M, Jürgens K, Bertilsson S, Waniek JJ, Andersson AF (2011) Transitions in bacterial communities along the 2000 km salinity gradient of the Baltic Sea. ISME J 5:1571–1579

Herlemann DPR, Woelk J, Labrenz M, Jürgens K (2014) Diversity and abundance of '*Pelagibacterales*' (SAR11) in the Baltic Sea salinity gradient. Syst Appl Microbiol 37: 601–604

Hewson I, Fuhrman JA (2004) Richness and diversity of bacterioplankton species along an estuarine gradient in Moreton Bay, Australia. Appl Environ Microbiol 70: 3425–3433

Higgins HW, Wright SW, Schlüter L (2011) Quantitative interpretation of chemotaxonomic pigment data. In: Roy S, Llewellyn CA, Egeland ES, Johnsen G (eds) Phytoplankton pigments: characterization, chemotaxonomy and applications in oceanography. Cambridge University Press, Cambridge, p 257–313

Hoch MP, Kirchman DL (1993) Seasonal and inter-annual variability in bacterial production and biomass in a temperate estuary. Mar Ecol Prog Ser 98:283–295

Hu YO, Karlson B, Charvet S, Andersson AF (2016) Diversity of pico- to mesoplankton along the 2000 km salinity gradient of the Baltic Sea. Front Microbiol 7:679

James G, Witten D, Hastie T, Tibshirani R (2013) An introduction to statistical learning with applications in R. Springer, New York, NY

Keller DP, Lee DY, Hood RR (2014) Turbidity maximum entrapment of phytoplankton in the Chesapeake Bay. Estuaries Coasts 37:279–298

Kirchman DL (2001) Measuring bacterial biomass production and growth rates from leucine incorporation in natural aquatic environments. In: Paul JH (ed) Marine microbiology, Vol 30. Academic Press, San Diego, CA, p 227–237

Kirchman DL, Dittel AI, Malmstrom RR, Cottrell MT (2005) Biogeography of major bacterial groups in the Delaware estuary. Limnol Oceanogr 50:1697–1706

Ladau J, Sharpton TJ, Finucane MM, Jospin G and others (2013) Global marine bacterial diversity peaks at high latitudes in winter. ISME J 7:1669–1677

Landa M, Cottrell MT, Kirchman DL, Kaiser K and others (2014) Phylogenetic and structural response of heterotrophic bacteria to dissolved organic matter of different chemical composition in a continuous culture study. Environ Microbiol 16:1668–1681

Lebeau T, Robert JM (2003) Diatom cultivation and biotechnologically relevant products. Part II: Current and putative products. Appl Microbiol Biotechnol 60: 624–632

Lindh MV, Sjöstedt J, Andersson AF, Baltar F and others (2015) Disentangling seasonal bacterioplankton population dynamics by high-frequency sampling. Environ Microbiol 17:2459–2476

Lionard M, Muylaert K, Tackx M, Vyverman W (2008) Evaluation of the performance of HPLC–CHEMTAX analysis for determining phytoplankton biomass and composition in a turbid estuary (Schelde, Belgium). Estuar Coast Shelf Sci 76:809–817

Liu L, Yang J, Lv H, Yu Z (2014) Synchronous dynamics and correlations between bacteria and phytoplankton in a subtropical drinking water reservoir. FEMS Microbiol Ecol 90:126–138

Lozupone CA, Knight R (2007) Global patterns in bacterial diversity. Proc Natl Acad Sci USA 104:11436–11440

Lucas J, Wichels A, Teeling H, Chafee M, Scharfe M, Gerdts G (2015) Annual dynamics of North Sea bacterioplankton: seasonal variability superimposes short-term variation. FEMS Microbiol Ecol 91:fiv099

Mackey MD, Mackey DJ, Higgins HW, Wright SW (1996) CHEMTAX – a program for estimating class abundances from chemical markers: application to HPLC measurements of phytoplankton. Mar Ecol Prog Ser 144:265–283

Milici M, Deng ZL, Tomasch J, Decelle J and others (2016) Co-occurrence analysis of microbial taxa in the Atlantic Ocean reveals high connectivity in the free-living bacterioplankton. Front Microbiol 7:649

Morris RM, Longnecker K, Giovannoni SJ (2006) *Pirellula* and OM43 are among the dominant lineages identified in an Oregon coast diatom bloom. Environ Microbiol 8: 1361–1370

Needham DM, Fuhrman JA (2016) Pronounced daily succession of phytoplankton, archaea and bacteria following a spring bloom. Nat Microbiol 1:16005

Oksanen J, Blanchet FG, Friendly M, Kindt R and others (2016) vegan: community ecology package. http://CRAN.R-project.org/package=vegan

Osterholz H, Kirchman DL, Niggemann J, Dittmar T (2016) Environmental drivers of dissolved organic matter molecular composition in the Delaware Estuary. Front Earth Sci 4:95

Sañudo-Wilhelmy SA, Gómez-Consarnau L, Suffridge C, Webb EA (2014) The role of B vitamins in marine biogeochemistry. Annu Rev Mar Sci 6:339–367

Schabhüttl S, Hingsamer P, Weigelhofer G, Hein T, Weigert A, Striebel M (2013) Temperature and species richness effects in phytoplankton communities. Oecologia 171: 527–536

Schlüter L, Møhlenberg F, Havskum H, Larsen S (2000) The use of phytoplankton pigments for identifying and quantifying phytoplankton groups in coastal areas: testing the influence of light and nutrients on pigment/chlorophyll *a* ratios. Mar Ecol Prog Ser 192:49–63

Schlüter L, Møhlenberg F, Kaas H (2014) Temporal and spatial variability of phytoplankton monitored by a combination of monitoring buoys, pigment analysis and fast screening microscopy in the Fehmarn Belt Estuary. Environ Monit Assess 186:5167–5184

Sharp JH, Yoshiyama K, Parker AE, Schwartz MC and others (2009) A biogeochemical view of estuarine eutrophication: seasonal and spatial trends and correlations in the Delaware Estuary. Estuaries Coasts 32:1023–1043

Smith B, Wilson JB (1996) A consumer's guide to evenness indices. Oikos 76:70–82

Troussellier M, Schäfer H, Batailler N, Bernard L and others (2002) Bacterial activity and genetic richness along an estuarine gradient (Rhône River plume, France). Aquat Microb Ecol 28:13–24

Unrein F, Gasol JM, Not F, Forn I, Massana R (2014) Mixotrophic haptophytes are key bacterial grazers in oligotrophic coastal waters. ISME J 8:164–176

Vergin KL, Beszteri B, Monier A, Thrash JC and others (2013) High-resolution SAR11 ecotype dynamics at the Bermuda Atlantic Time-series Study site by phylogenetic placement of pyrosequences. ISME J 7:1322–1332

Walsh EA, Smith DC, Sogin ML, D'Hondt S (2015) Bacterial and archaeal biogeography of the deep chlorophyll maximum in the South Pacific Gyre. Aquat Microb Ecol 75:1–13

Wang J, Yang D, Zhang Y, Shen J, van der Gast C, Hahn MW, Wu Q (2011) Do patterns of bacterial diversity along salinity gradients differ from those observed for macroorganisms? PLOS ONE 6:e27597

Wang X, Sharp CE, Jones GM, Grasby SE, Brady AL, Dunfield PF (2015) Stable-isotope probing identifies uncultured *Planctomycetes* as primary degraders of a complex heteropolysaccharide in soil. Appl Environ Microbiol 81: 4607–4615

Watling L, Bottom D, Pembroke A, Maurer D (1979) Seasonal variations in Delaware Bay phytoplankton community structure. Mar Biol 52:207–215

Wheeler PA, Kirchman DL (1986) Utilization of inorganic and organic nitrogen by bacteria in marine systems. Limnol Oceanogr 31:998–1009

Yilmaz P, Iversen MH, Hankeln W, Kottmann R, Quast C, Glöckner FO (2012) Ecological structuring of bacterial and archaeal taxa in surface ocean waters. FEMS Microbiol Ecol 81:373–385

Editorial responsibility: Jennifer Martiny, Irvine, California, USA

Submitted: August 5, 2016; Accepted: November 1, 2016
Proofs received from author(s): December 15, 2016