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Linking bacterial richness with viral abundance and prokaryotic activity

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Abstract

We measured relative bacterial and archaeal richness and its covariation with physical and other biological parameters during six cruises in the North Sea over prokaryotic productivity levels ranging over two orders of magnitude. Relative bacterial and archaeal richness was estimated as the number of peaks detected by terminal–restriction fragment length polymorphism (T-RFLP) analysis of polymerase chain reaction–amplified prokaryotic 16S rRNA gene fragments in unfiltered (total community) and 0.8-μm filtered seawater (free-living community). Relative bacterial richness ranged from 20 to 57 peaks in the total community and from 16 to 56 peaks in the free-living community. Relative archaeal richness varied between 2 and 14 peaks in the total community and between 2 and 21 peaks in the free-living community. Coamplified plastid DNA might have influenced relative bacterial richness in unfiltered but not in 0.8-μm filtered seawater. Relative bacterial richness decreased with viral abundance and total and cell-specific prokaryotic production in the free-living and the total community. Stepwise multiple regression analysis revealed that temperature also influenced relative bacterial richness. Relative archaeal richness was not related to any other parameter. The data suggest that high prokaryotic productivity was sustained by a relatively small number of highly active bacterial populations that also maintained high viral abundance.

The advent of molecular techniques prompted the discovery of highly diverse prokaryotic communities in aquatic environments. As a consequence, the question arose how such high prokaryotic diversity can be maintained in relatively homogenous aquatic environments with only a limited number of resources. This problem was first noted for phytoplankton and later extended to bacterioplankton. However, it also has been argued that the high complexity of dissolved organic matter and the large number of different metabolic pathways of prokaryotes allow for a large number of niches and, thus, offer the possibility of an alternative theory where high prokaryotic diversity is maintained by substrate diversity (Thingstad 2000). Three major factors are thought to regulate the composition of bacterioplankton: the availability of resources, size-selective grazing on prokaryotes mainly by heterotrophic nanoflagellates, and viral lysis. The influence of protozoan grazing and the availability of resources on the composition of prokaryotic communities have been demonstrated by a number of studies (e.g., Šimek et al. 2003). Also, prokaryotic communities can be affected by phytoplankton blooms (Arrieta and Herndl 2002) and seasonal changes in coastal areas (Schauer et al. 2003).

Viral infection is a stochastic process and depends on the abundance of viruses and hosts. Host specificity and density dependence are the basis for the ''killing the winner'' hypothesis formulated by Thingstad and Lignell (1997), which suggests that lytic viruses might selectively kill the most abundant members of the prokaryotic community. Thus, viruses might be a major driving force for maintaining high richness in prokaryotic communities by allowing the survival of less competitive species. A number of studies have shown an influence of viruses on prokaryotic community composition, although rigorous experimental tests are sparse (Hewson et al. 2003; Schwalbach et al. 2004; Winter et al. 2004*b*). Middelboe et al. (2001) showed experimentally and in models that viral lysis also affects the clonal composition (sensitive vs. resistant strains) of prokaryotic communities. Additionally, lysogenic phages can influence prokaryotic community composition by transduction, phage conversion, and the induction of the lytic cycle (Weinbauer 2004).

In an extension of the killing the winner hypothesis, Thingstad (2000) proposed the existence of a reciprocal control mechanism between viruses and prokaryotic diversity. In his refined model, viruses act as a balancing factor that allows the coexistence of prokaryotic species with different growth rates. According to this model, viruses with fast-growing hosts should be most abundant. Overall, studies linking prokaryotic richness with production are scarce and our knowledge on the controlling mechanisms of prokaryotic richness in complex communities is poor.

In the present study we investigated the relationships between relative richness of *Bacteria* and *Archaea* and prokaryotic production, prokaryotic and viral abundance, and physical parameters in the North Sea. The relative richness of planktonic bacterial and archaeal communities was determined by T-RFLP analysis of polymerase chain reaction (PCR) amplified prokaryotic 16S rRNA gene fragments. Our

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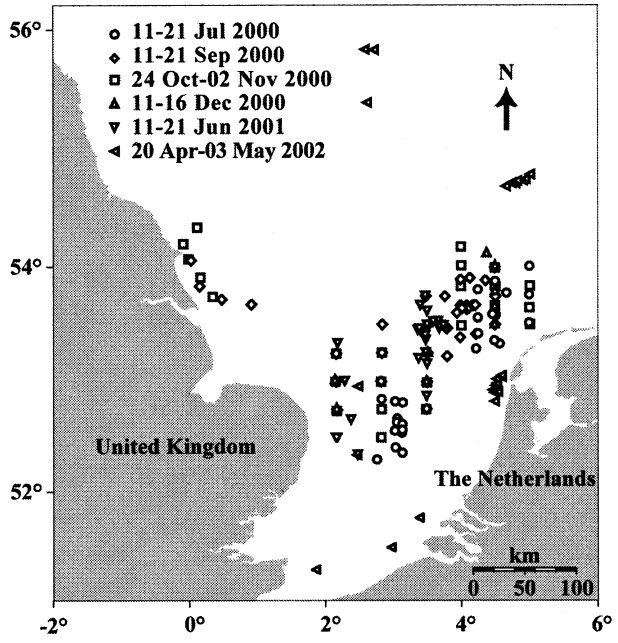

Fig. 1. Map of the sampling sites during six cruises in the North Sea.

data show a reciprocal relationship between relative bacterial richness and prokaryotic production as well as viral abundance.

Materials and methods

Sampling and characteristics of the study sites—We conducted six cruises in the North Sea with R/V *Pelagia* between July 2000 and May 2002. The dates of the cruises were 11–21 July 2000, 11–21 September 2000, 24 October–2 November 2000, 11–16 December 2000, 11–21 June 2001, and 20 April–3 May 2002. Water samples (20 liters) from 5 m depth were collected at 146 stations (Fig. 1) with 10-liter NOEX bottles mounted on a rosette sampler, also holding the conductivity, temperature, and depth sensors and for optical backscattering to record turbidity of the water column. Optical backscatter (in arbitrary units) was only used as a relative indicator of the particle load of the water column.

Enumeration of prokaryotes and viruses—Samples (2 ml) for the enumeration of prokaryotes and viruses were fixed with formaldehyde (2% final concentration), kept at 4°C, and filtered onto 0.02-μm pore-size filters (Whatman; Anodisc, 25 mm diameter) within a few hours after collection. Prokaryotic cells and viral particles were stained on the filters using SYBR-Green I (Molecular Probes) as described by Noble and Fuhrman (1998). The filters were mounted in a solution consisting of 50% glycerol, 50% phosphate buffered saline, and 0.1% p-phenylenediamine and stored at −20°C until enumeration. A Zeiss Axiophot microscope equipped for epifluorescence microscopy was used to enumerate prokaryotic cells and viral particles as previously described (Noble and Fuhrman 1998). A minimum of 25 fields or 300 prokaryotic cells and viruses were counted per filter.

Determination of prokaryotic production by leucine incorporation—Prokaryotic production was measured by the incorporation of [^{14}C]-leucine (10×10^{-9} mol L^{-1} final concentration) (Kirchman et al. 1985) in duplicate samples and one formaldehyde-killed blank (2% final concentration) served as control. On several occasions, higher concentrations of leucine (up to 50×10^{-9} mol L^{-1} final concentration) were added to replicate samples to test substrate saturation. No indications were found that leucine concentrations higher than 10×10^{-9} mol L^{-1} result in higher leucine incorporation. After incubation in the dark at in situ temperature for 1 h, formaldehyde was added (2% final concentration) to the samples to terminate leucine incorporation. Subsequently, the samples were filtered through 0.45-μm pore-size filters (Millipore; HAWP, 25 mm diameter) and rinsed twice with 10 ml of ice-cold 5% trichloroacetic acid. Then, the filters were placed in scintillation vials and stored dry at −20°C until further processing in the lab. The radioactivity incorporated was determined by liquid scintillation counting and the disintegrations per minute were converted into the actual amount of leucine incorporated. To calculate cell production from the amount of leucine incorporated, the conversion factor 0.07×10^{18} cells produced per mol of leucine incorporated (Rieman et al. 1990) was applied and a carbon content of 20 fg cell^{-1} to convert cell production to C-biomass production (Lee and Fuhrman 1987).

During the cruises in October–November 2000, December 2000, and June 2001, prokaryotic production was also measured in 0.8-μm filtered samples (Millipore; Isopore ATTP, 25 mm diameter). The filtration procedure was performed using glass filtration funnels (Millipore) stored in 1 mol L^{-1} HCl. Prior to filtration, the assembled filtration equipment including the filter was rinsed with Milli-Q water and sample water. The vacuum did not exceed 200 mbar during filtration. Prokaryotic production in the 0.8-μm filtered samples was about 10% lower than the production in the corresponding unfiltered samples and correlated significantly (linear least-squares regression: $R^2 = 0.85$, $p < 0.0001$, $n = 47$; Reinthaler, unpubl. data) with the production of the total bacterioplankton community. Thus, prokaryotic production measured in unfiltered samples (representing the free-living plus the particle-attached community) was used to determine the relationship between prokaryotic production and relative bacterial and archaeal richness in unfiltered (total community) as well as in 0.8-μm filtered seawater (assumed to represent the free-living community).

Sample preparation for nucleic acid extraction—A total volume of 19 liters of seawater was filtered through 0.8-μm pore-size filters (Millipore; Isopore ATTP, 142 mm diameter) using a stainless steel filter holder (Sartorius) and an air-pressure pump (Verder; Contex-sot). Between sampling, the air-pressure pump and the tubing were rinsed with 1 mol L^{-1} HCl. The prokaryote size fraction was concentrated to a final volume of 400–550 ml using a tangential-flow filtration (Millipore; Pellicon filter-cassette PTGVPPC05, 0.22-μm pore-size, stored in 1 mol L^{-1} HCl between sampling stations). The ultrafiltration device was operated using a peristaltic pump (Watson Marlow; 604S) at a maximum pressure of 200 mbar. Five to ten liters of each sample were used to

rinse the filtration units before collecting the sample. A comparison between the T-RFLP patterns of the prokaryotic community in 0.8-μm filtered seawater before and after concentration showed no differences, which indicates that there were no selective losses of prokaryotic populations due to the ultrafiltration step. The filtration procedure was performed at in situ temperature within 1 h after collecting the sample. The prokaryotic cells from 1 liter of unfiltered seawater and from the concentrate of the same water sample were collected on 0.22-μm pore-size filters (Millipore; Isopore GVWP, 100 mm diameter). These filters were frozen in liquid nitrogen and stored at −80°C until further processing.

Nucleic acid extraction, PCR amplification, restriction digests, and T-RFLP analysis—The filters were thawed on ice and 2 ml of lysis buffer (50×10^{-3} mol L^{-1} Tris, 20×10^{-3} mol L^{-1} Na_2-EDTA [HCl, pH 8.0]) was added. Four freeze–thaw cycles were performed by placing the tubes in liquid nitrogen and subsequently thawing the filters in a water bath at 37°C. After adding another 2 ml of lysis buffer, the filters were treated with lysozyme (Sigma; catalog No. L7651) at a final concentration of 1.25 mg ml^{-1} at 37°C for 30 min. Subsequently, sodium dodecyl sulfate and proteinase K (Fluka; cat. No. 82456) at final concentrations of 1% (wt/vol) and 100 mg ml^{-1}, respectively, were added and the tubes were incubated at 55°C for 2 h. The liquid phase was extracted once with an equal volume of a mixture of phenol, chloroform, and isoamylalcohol (ratio 25 : 24 : 1) buffered with 1 × TE (10×10^{-3} mol L^{-1} Tris-HCl [pH 8.0], 1×10^{-3} mol L^{-1} Na_2-EDTA) and with chloroform-isoamylalcohol (ratio 24 : 1). After ethanol precipitation overnight at −20°C, the nucleic acid pellet was redissolved in 100 μl of ultrapure water (Sigma; cat. No. W-4502). Fifty microliters of this crude nucleic acid extract was further purified using a QIAEX II gel extraction kit (Qiagen) as recommended by the manufacturer for DNA fragments larger than 10×10^3 bp. The nucleic acids were recovered in a final volume of 20 μl elution buffer (Qiagen). One to two microliters of this cleaned nucleic acid extract was used for subsequent PCR amplification. A 1,480-bp fragment of the bacterial 16S rRNA gene was amplified by PCR using the *Bacteria*-specific primer 27F and the universal primer 1492R (Lane 1991). PCR amplification of a 920-bp fragment of the archaeal 16S rRNA gene was performed using the *Archaea*-specific primer pair 21F and 958R (DeLong 1992). The forward primers were fluorescently 5′ end-labeled with phosphoramidite fluorochrome 5-carboxy-fluorescein (5′6-FAM) and the reverse primers with 6-carboxy-4′,5′-dichloro-2′,7′-dimethoxyfluorescein (5′6-JOE; both from Interactiva; Ulm, Germany). PCR conditions and chemicals were as described in Moeseneder et al. (2001*b*). Restriction digestion of 50 ng of the PCR fragments was performed using 20 U of restriction enzyme *Hha*I (Amersham Pharmacia) at 37°C for 12 h to ensure complete digestion. Restriction digestion was stopped by heating the incubations to 65°C for 30 min immediately after digestion. Subsequent T-RFLP analysis was performed on an automated capillary sequencer (Perkin Elmer-Applied Biosystems; ABI Prism 310) as previously described (Moeseneder et al. 2001*b*).

The T-RFLP patterns were analyzed by recording the number of peaks (presence vs. absence). The data were carefully checked and corrected for ''bleed through'' artifacts of fluorescence signals into other channels including the channel for the TAMRA 2500 size standard (Perkin Elmer-Applied Biosystems) by visual inspection of the T-RFLP patterns. It has been suggested that it is necessary to combine the results of the forward and reverse primer to fully characterize a clone library by T-RFLP analysis (Moeseneder et al. 2001*a*). However, we found no differences in the trends of the relationships of relative richness with other parameters when using the combined results of the forward and reverse primer and the forward primer alone. Thus, we chose to use the results from the forward primer alone as a measure of relative richness.

Regression analysis and statistical tests—Linear least-squares regression analysis was performed to assess covariation of parameters. Data were log-transformed except when noted otherwise. The coefficient of determination (R^2) was used as performance measure of the regression analysis. Analysis of variance (ANOVA) in combination with Scheffe's F multiple comparison test was used to evaluate differences in relative bacterial and archaeal richness between the cruises. To determine the most suitable set of parameters that explains best the variability of relative bacterial richness, stepwise multiple regression analysis was performed on log-transformed data except for salinity, which was not transformed. Tests showed that the stepwise multiple regression analysis was not affected by colinearity of the parameters. The effects of the parameters on the multiple regression models with a p value ≤ 0.05 were assumed to be significant. The significance of differences in relative richness between the total and free-living community was tested using paired t-tests.

Results

Variation of physical and biological parameters—Salinity ranged between 31.2 and 35.1, while temperature varied from 8.0 to 17.4°C and turbidity ranged over two orders of magnitude (Table 1). Prokaryotic production and viral abundance in unfiltered seawater varied over two orders of magnitude, whereas prokaryotic abundance varied only over one order of magnitude (Table 1).

Relative bacterial and archaeal richness of the total and free-living community—Relative bacterial richness averaged 38 in the total community and 33 in the free-living community (Table 1), and this 15% difference was significant (paired t-test: $p < 0.0001$). Relative bacterial richness of the total and the free-living community were weakly but significantly related to each other ($R^2 = 0.35$; Fig. 2). Relative archaeal richness averaged eight in the total and the free-living community (Table 1). However, *Archaea* could be detected in only 25% of the unfiltered samples. A summary of the data on relative bacterial and archaeal richness for each cruise is shown in Table 2. The relative bacterial and archaeal richness differed significantly between cruises (ANOVA: total bacterial community, $F = 32.843$, $p < 0.0001$; free-living bacterial community, $F = 51.302$, $p < 0.0001$;

Table 1. Summary of physical and biological parameters recorded during the cruises in the North Sea.

Parameters	Units	Mean	Minimum	Maximum	SD	*n*
Salinity	dimensionless	34.2	31.2	35.1	0.8	146
Turbidity	not calibrated, arbitrary units	2.9	0.1	24.6	4.2	144
Temperature	°C	13.2	8.0	17.4	2.8	146
Bacterial richness: total community	number of peaks	38	20	57	9	133
Bacterial richness: free-living community	number of peaks	33	16	56	10	133
Archaeal richness: total community	number of peaks	8	2	14	3	30
Archaeal richness: free-living community	number of peaks	8	2	21	3	122
Viral abundance	10^6 ml^{-1}	22.3	2.1	184.0	24.1	140
Prokaryotic abundance	10^6 ml^{-1}	1.0	0.3	4.3	0.6	142
Prokaryotic production	μg C L^{-1}d^{-1}	9.0	0.6	63.1	11.3	124

free-living archaeal community, $F = 25.575$, $p < 0.0001$) except for the total archaeal community for which no test could be performed due to the low number of *Archaea*-positive samples. Scheffe's F multiple comparison test revealed that relative bacterial richness in the total community did not differ significantly ($p > 0.05$) between June and July; from September through October-November to December; or between June and September. Relative bacterial richness in the free-living community did not differ significantly ($p > 0.05$) between June and July; between September and October-November; or between September and December. Relative archaeal richness in the free-living community was not significantly different ($p > 0.05$) between July and December; between April-May and July; between September and October-November; or from December through April-May to June.

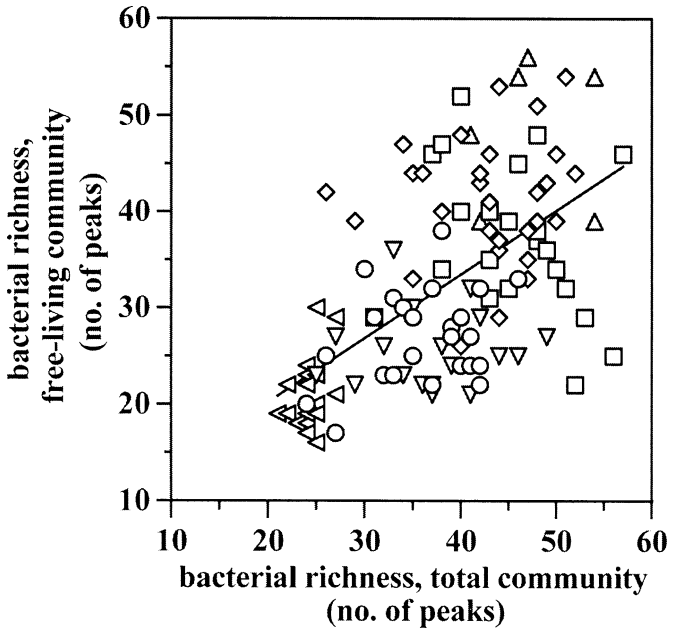

Fig. 2. Comparison of relative bacterial richness in the total and the free-living community as determined by T-RFLP analysis. The line represents a linear least-squares regression analysis fitted to the data. $y = 0.66x + 6.90$, $R^2 = 0.35$, $n = 122$. Symbols as in Fig. 1.

Covariation of physical and biological parameters—Salinity was not related to any other parameter (data not shown). The relationships of turbidity and temperature to prokaryotic activity, prokaryotic and viral abundance, and relative bacterial richness were weak (data not shown; in all cases $R^2 \leq 0.30$). Viral abundance increased significantly with prokaryotic production ($R^2 = 0.65$; Fig. 3) and with cell-specific prokaryotic production ($R^2 = 0.46$, $p < 0.0001$, $n = 119$).

Relative bacterial richness of the total community decreased with increasing prokaryotic production and viral abundance (prokaryotic production, $R^2 = 0.34$, $p < 0.0001$, $n = 114$; viral abundance, $R^2 = 0.38$, $p < 0.0001$, $n = 128$). For the free-living community, the negative relationships of prokaryotic production and viral abundance with relative bacterial richness were more pronounced (prokaryotic production, $R^2 = 0.43$; viral abundance, $R^2 = 0.45$; Fig. 4) than for the total community. When using the smaller data set of prokaryotic production in the 0.8-μm fraction, prokaryotic production was related to relative bacterial richness (e.g., free-living community: $R^2 = 0.52$, $p < 0.0001$, $n = 45$) and viral abundance ($R^2 = 0.57$, $p < 0.0001$, $n = 45$) in a similar way as was total prokaryotic production. A significant negative relationship was also found for relative bacterial richness and cell-specific prokaryotic production in the total and free-living communities (total community, $R^2 = 0.40$, $p < 0.0001$, $n = 111$; free-living community, $R^2 = 0.49$, $p < 0.0001$, $n = 113$).

No relationship was found between relative bacterial richness and prokaryotic abundance (total community, $R^2 = 0.001$, $p = 0.75$, $n = 130$; free-living community, $R^2 = 0.02$, $p = 0.15$, $n = 132$). Relative archaeal richness in the total and free-living community was not related to any other parameter (data not shown; in all cases $R^2 < 0.20$). Also, when data from single cruises were analyzed, no relationships between any of the parameters were found (data not shown).

Table 2. Summary of relative bacterial and archaeal richness of the total and free-living community for each cruise.

Parameters	Cruise	Mean	Minimum	Maximum	SD	*n*
Total community						
Bacterial richness	Jul 2000	36	24	47	6	26
	Sep 2000	43	26	52	6	32
	Oct-Nov 2000	44	20	57	8	23
	Dec 2000	47	41	54	5	9
	Jun 2001	37	25	55	8	21
	Apr–May 2002	25	21	30	2	22
Archaeal richness	Jul 2000	—	—	—	—	—
	Sep 2000	9	8	12	2	5
	Oct–Nov 2000	8	5	9	2	5
	Dec 2000	—	—	—	—	—
	Jun 2001	9	2	14	4	10
	Apr–May 2002	6	3	14	3	10
Free-living community						
Bacterial richness	Jul 2000	27	17	38	5	24
	Sep 2000	41	26	54	7	32
	Oct–Nov 2000	37	22	52	8	24
	Dec 2000	47	39	56	7	9
	Jun 2001	27	21	37	5	23
	Apr–May 2002	21	16	30	4	21
Archaeal richness	Jul 2000	8	3	13	3	24
	Sep 2000	10	7	15	2	32
	Oct–Nov 2000	12	7	21	4	24
	Dec 2000	6	5	8	1	9
	Jun 2001	5	2	8	2	22
	Apr–May 2002	6	3	7	1	11

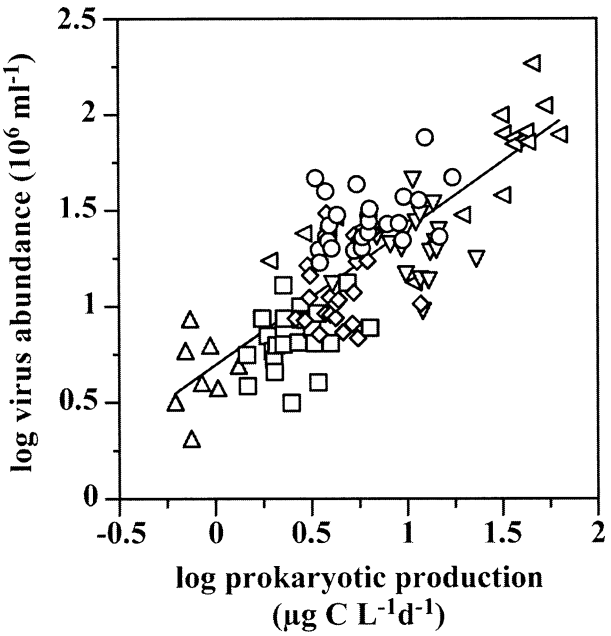

Fig. 3. Relationship of prokaryotic production with viral abundance. The line represents a linear least-squares regression analysis fitted to the data. $y = 0.71x + 0.69$, $R^2 = 0.65$, $n = 119$. Symbols as in Fig. 1.

Stepwise multiple regression analysis—The parameters shown in Table 1 were used in stepwise multiple regression analysis to obtain multiple linear regression models for relative bacterial richness of the total and the free-living community. The combined effects of viral abundance, temperature, and prokaryotic production explained 62% of the variability of relative bacterial richness in the total community (Table 3). Viral abundance, temperature, prokaryotic production, and salinity explained 65% of the variability of relative bacterial richness in the free-living community (Table 3). In the total and the free-living community, viruses alone accounted for 60% of the variability of relative bacterial richness.

Discussion

Relative bacterial and archaeal richness in the total versus free-living community—Filtration through 0.8-μm pore-size filters removed particles and prokaryotic cells attached to them. A number of studies have shown that free-living bacterial communities are distinctly different from particle-attached communities (e.g., Moeseneder et al. 2001*b*). Since the particle load of the water column in the North Sea as estimated via turbidity changed dramatically during the study period (Table 1), the contribution of attached prokaryotes to relative bacterial and archaeal richness in the total community should vary strongly. Moreover, it is possible that the bacterial PCR primers have amplified 16S rRNA genes of chloroplasts from phytoplankton in our unfiltered samples (Rappé et al. 1997). However, phytoplankton were

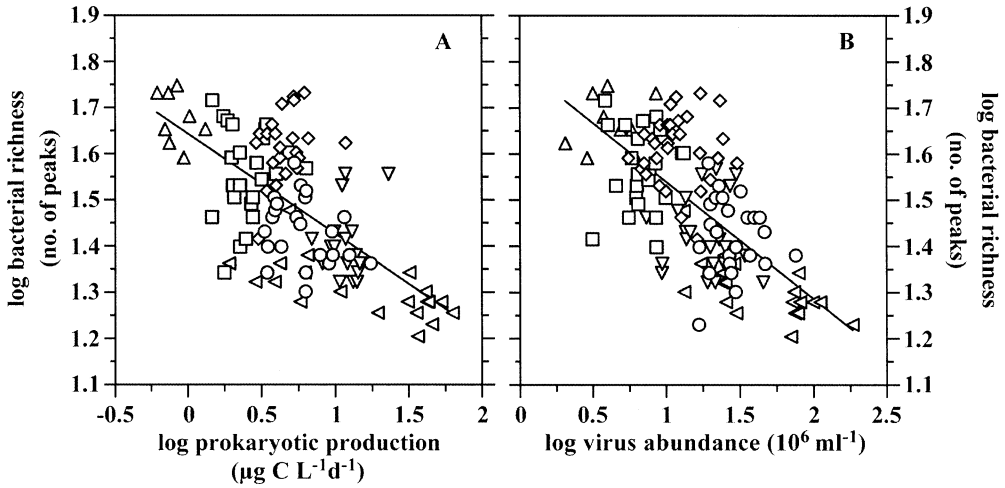

Fig. 4. (A) Relationships of prokaryotic production and (B) viral abundance with relative bacterial richness of the free-living community as determined by T-RFLP analysis. (A) $y = -0.22x + 1.64$, $R^2 = 0.43$, $n = 113$. (B) $y = -0.25x + 1.80$, $R^2 = 0.45$, $n = 131$. Symbols as in Fig. 1.

removed by prefiltration and relative bacterial richness was on average only 15% higher in the total than in the free-living community (Table 1).

It has been shown that, in terms of abundance, *Archaea* are of minor importance in the North Sea (Pernthaler et al. 2002). The finding that *Archaea* could be detected in almost all 0.8-μm filtered samples but only in 25% of the unfiltered samples indicates that prefiltration improves the resolution for studies on prokaryotic richness. Since the amplification product was ca. 35% shorter for *Archaea* than for *Bacteria*, relative archaeal richness might have been underestimated. However, we only used the results of the forward primers, which target nearly the same region of the 16S rRNA gene (bacterial, 27F; archaeal, 21F), to determine relative archaeal and bacterial richness. Moreover, fragments in the T-RFLP patterns of the forward primers for *Bacteria* and *Archaea* did not exceed 920 bp (the amplicon size for the archaeal primers) when using *Hha*I as restriction enzyme (this study, data not shown; Winter et al. 2004*b*). Thus, it is unlikely that a larger archaeal amplification product would have increased the number of peaks detected in the archaeal T-RFLP patterns. Since relative bacterial richness of the total community might be influenced by the potential coamplification of plastid DNA and *Archaea* are not numerically important in the study area, the following discussion focuses mainly on the relative bacterial richness of the free-living community.

The link of relative bacterial richness with viral abundance and prokaryotic production—It is possible that the inverse relationships between relative bacterial richness and viral abundance or prokaryotic production were driven by spatial heterogeneity rather than representing a link between these parameters. Since the contribution of salinity to the stepwise multiple regression analysis of relative bacterial richness of the free-living community was small and turbidity did not contribute significantly to the models of relative bacterial richness (Table 3), spatial differences are apparently not important. Instead, in both regression models, the first parameters added by the forward stepwise procedure were viral abundance, temperature, and prokaryotic production. Thus, the stepwise multiple regression analysis confirms the importance of viral abundance and prokaryotic production for the relative bacterial richness and indicates that seasonal (temperature related) rather than spatial differences are driving this relationship. Seasonal changes and changes in relative bacterial richness such as those in conjunction with phytoplankton blooms are well known for coastal areas (Arrieta and Herndl 2002; Schauer et al. 2003).

The observed trend toward lower relative bacterial richness with increasing prokaryotic production suggests a shift in evenness toward a relatively small number of dominant (in the sense of detectable) phylotypes rather than lower overall richness. Less abundant populations could drop below the detection limit of T-RFLP analyses when other populations become more abundant. Indeed, it seems unlikely that the total number of different bacterial populations fluctuates extensively on a spatial or seasonal scale in an environment such as the southern North Sea, characterized by a permanently mixed water column. However, we cannot completely exclude the possibility of periodic introduction of bacterial populations by river discharge or their removal by settling particles.

Since highly active prokaryotic communities were characterized by low relative bacterial richness (Fig. 4), the high prokaryotic activity found during spring–summer in the North Sea is potentially a consequence of a relatively small number of prokaryotic populations capable of using available substrate and becoming numerically dominant over less adapted populations. This is also indicated by the inverse relationship between relative bacterial richness and cell-specific prokaryotic production. The positive relationship between viral abundance and prokaryotic production (Fig. 3) as well as cell-specific prokaryotic production suggests that high viral abundance in the study area did not only concur with high productivity as has been reported previously for other environments (e.g., Corinaldesi et al. 2003; Weinbauer et al. 2003) but also with highly active prokaryotic cells.

Table 3. Stepwise multiple regression analysis of the bacterial richness of the total and the free-living community. The parameters comprising the best performing models are indicated in bold.

Parameters	*n*	R^2	*F* ratio	*p*	Coefficient	SE	*p*	Intercept	SE
Total community									
Viruses	107	0.41	72	<0.0001	−0.20	0.02	<0.0001	1.81	0.03
Viruses	107	0.60	76	<0.0001	−0.17	0.02	<0.0001	1.17	0.10
Temperature					0.51	0.08	<0.0001		
Viruses	107	0.62	56	<0.0001	−0.10	0.03	0.0013	1.15	0.10
Temperature					0.54	0.08	<0.0001		
Prokaryotic production					−0.07	0.03	0.0151		
Viruses	107	0.63	43	<0.0001	−0.10	0.03	0.0014	1.70	0.46
Temperature					0.57	0.08	<0.0001		
Prokaryotic production					−0.10	0.03	0.0090		
Salinity					−0.02	0.01	0.2689		
Free-living community									
Viruses	111	0.50	109	<0.0001	−0.26	0.03	<0.0001	1.80	0.03
Viruses	111	0.60	82	<0.0001	−0.24	0.02	<0.0001	1.24	0.11
Temperature					0.48	0.09	<0.0001		
Viruses	111	0.63	61	<0.0001	−0.15	0.11	<0.0001	1.21	0.11
Temperature					0.48	0.09	<0.0001		
Prokaryotic production					−0.10	0.03	0.0025		
Viruses	111	0.65	50	<0.0001	−0.14	0.04	0.0002	2.45	0.51
Temperature					0.57	0.09	<0.0001		
Prokaryotic production					−0.15	0.04	<0.0001		
Salinity					−0.04	0.02	0.0115		
Viruses	111	0.66	40	<0.0001	−0.16	0.04	<0.0001	2.46	0.51
Temperature					0.48	0.11	<0.0001		
Prokaryotic production					−0.16	0.04	<0.0001		
Salinity					−0.04	0.02	0.0193		
Prokaryotes					0.08	0.06	0.2563		

Combined with the finding that low relative bacterial richness coincides with high viral abundance (Fig. 4), this indicates that a small number of highly active, primarily free-living bacterial populations maintain high viral abundance.

This scenario is based on the following assumptions: (1) High prokaryotic production is mainly due to a relatively small number of dominant, i.e., detectable, populations. Such a situation is well known for phytoplankton blooms, where the majority of primary production is due to a small number of species. During a phytoplankton bloom in the North Sea, Zubkov et al. (2001) sorted bacterioplankton into three fractions by flow cytometry. The genus *Roseobacter* as determined by fluorescence in situ hybridization accounted for 70% of prokaryotic abundance in one of the fractions, and 75% of the clones obtained from this fraction were identical in sequence and belonged to *Roseobacter*. *Roseobacter* spp. accounted for 24% of total prokaryotic abundance and was the only detected group that significantly correlated with prokaryotic production. Similarly, Šimek et al. (2001) found that in a freshwater reservoir a subgroup of *β-Proteobacteria* contributed 33% of prokaryotic abundance and showed growth rates ca. three times higher than the average prokaryotic community. A small number of different sequences of these *β-Proteobacteria* were detected in clone libraries and by denaturing gradient gel electrophoresis, which indicates a small phylogenetic diversity of this subgroup. Both studies suggest that prokaryotic abundance and production can be dominated by a single group, thus supporting our interpretation that high prokaryotic production is mainly due to a few dominant populations (Fig. 4A). (2) In the North Sea, the majority of the viruses infect prokaryotes, whereas phytoplankton viruses do not contribute significantly to the virus pool. This statement is underlined by the strong positive relationship between prokaryotic production and viral abundance (Fig. 3) and the lack of any relationship between viral abundance and chlorophyll *a* concentration in the North Sea (Winter et al. unpubl. data). The same trend has been reported for other environments in a number of studies (*see* review of Wommack and Colwell 2000). This suggests that the majority of the viruses in the North Sea infect prokaryotes, although episodic influences by algal viruses such as during phytoplankton blooms cannot be excluded. (3) The populations constituting the majority of prokaryotic production also produce most of the viruses. Several studies have shown that the frequency of infected cells can increase with prokaryotic production (Wommack and Colwell 2000). At times of high prokaryotic production such as in June, more than 30% of bacterioplankton were on average in a lytic stage of infection (Winter et al. 2004*b*). This is not compatible with the idea that rare but active populations drive viral production.

Viral production can be due to lytic viruses or induction of lysogenic cells. Although the relative contribution of these mechanisms to viral abundance and virus-induced mortality of prokaryotes remains unknown, the few available data suggest that lysogenic virus production is typically not important in surface waters (Weinbauer 2004). Thus, it is likely that lytic infection maintained high viral abundance. However, for the interpretation of the data as outlined above it is not relevant whether high viral abundance was due to lytic infection of abundant and active populations or due to the induction of lysogenized populations, e.g., due to high growth rates. Both viral replication strategies could result in the relationships found in our study.

The killing the winner hypothesis—The refined model of Thingstad (2000) builds upon an earlier model (Thingstad and Lignell 1997) and predicts two key mechanisms for viruses to interact with richness and productivity: (1) viruses allow for the coexistence of competing prokaryotes by killing the winner (the numerically dominant populations) and (2) prokaryotic diversity (in the sense of different growth rates of populations) controls viral abundance. These two mechanisms act in parallel and led to the proposition of a reciprocal feedback mechanism between lytic viruses and prokaryotic richness (Thingstad 2000). Our data support this model in a general sense by suggesting a link between relative bacterial richness, prokaryotic production, and viral abundance.

Experimental studies designed to detect the response of prokaryotic communities to the addition of viruses in comparison to virus-free treatments have shown that viruses can influence richness of *Bacteria* and *Archaea* (Hewson et al. 2003; Schwalbach et al. 2004; Winter et al. 2004*b*) and prokaryotic production (Wommack and Colwell 2000; Weinbauer 2004). In contrast to these studies, viruses were always present (10^6–10^8 ml^{-1}) during our in situ study. Our data suggest that relative bacterial richness decreased with increasing viral influence. Since it is one of the consequences of the killing the winner hypothesis (Thingstad and Lignell 1997) that viral lysis should sustain high bacterial richness, our data seem to contradict a specific prediction of this hypothesis. However, if there are threshold abundances necessary for viral control of populations and if the lower relative bacterial richness was on average indeed due to higher relative abundance of some populations, a shift to more abundant populations would be conceivable before viral control can become significant.

Additional virus-mediated mechanisms affecting relative bacterial richness—Resistance against infection allows for circumventing viral control but has to be paid for, e.g., with metabolic constraints (Weinbauer 2004; Wommack and Colwell 2000). Thus, the ratio of sensitive to resistant cells in a population might explain why populations can be abundant and significant producers of viruses (Middelboe et al. 2001). The high values of frequency of infected cells and viral abundance at high prokaryotic production would suggest a relatively high number of sensitive cells. During situations with high prokaryotic production, the reduction of uptake capacities for nutrients caused by resistance to viral infection might become unfavorable and allow for a high number of sensitive cells. It has been suggested that grazing can increase the proportion of sensitive cells (Middelboe et al. 2001), and studies with natural communities have indeed confirmed that viral infection was stimulated by the presence of grazers (Šimek et al. 2001). In addition, the high cell-specific prokaryotic production at high prokaryotic production might also stimulate viral production and result in increased viral abundance.

Ultraviolet radiation, suspended particles, and high molecular weight dissolved organic matter (>30 kDa) are the main known mechanisms for viral decay (Suttle and Chen 1992; Noble and Fuhrman 1997). Particle load (Table 1) and ultraviolet radiation (summer–winter transition) varied significantly during the study period. In addition, it is likely that the composition and concentration of high molecular weight dissolved organic matter changed during our seasonal study. Strong viral decay has been found in ultrafiltered water amended with prokaryotes and kept in the dark during summer in the North Sea (Winter et al. 2004*a*). Consequently, high prokaryotic production in summer might have increased viral decay by ectoenzymatic activity or activity of free enzymes, which destroy viral protein capsids.

Recently, Thyrhaug et al. (2003) found that the recovery of algal cultures in infection experiments occurred earlier at high initial virus-to-host ratios than at lower ratios. These authors suggested that excess host recognition molecules such as incomplete viruses produced during viral reproduction could, once released by lysis, compete with intact viruses for receptor sites on the host cell surface. This would result in a reduction of successful adsorption of viruses on host cells. Such a mechanism could also lead to reduced viral adsorption coefficients in prokaryotic virus–host systems. In addition, it has been argued that inhibitors such as endolysins released during lysis may sweep receptors on prokaryotes (Marsh and Wellington 1994), also resulting in reduced viral adsorption coefficients. As inhibitors are probably released concomitantly with viruses, such mechanisms should be strongest in summer when viral abundance and viral infection rates were high.

It is likely that the mechanisms mentioned above act differently on different virus populations. For example, decay rates differ between viral types (Wommack and Colwell 2000). This might affect prokaryotic production and richness as well. How this is related to the low relative bacterial richness at high viral abundance and prokaryotic production remains unknown. It is possible that mechanisms such as resistance, decay, and receptor blocking/sweeping relaxes viral control of bacterial populations and allows some populations to become more dominant.

Conclusions—At the current state of knowledge, we can only speculate about the mechanisms that govern viral control of prokaryotic richness and how high viral abundance is maintained at low prokaryotic richness in natural communities. Methodological progress to determine the abundance and dynamics of viruses and hosts in situ and to assess viral host range and the role of resistance to viral infection is necessary for answering that. Nevertheless, the reported negative relationship between relative bacterial richness with prokaryotic production and viral abundance might provide the basis for developing new hypotheses on the role of viruses for prokaryotic richness and production. Based on the presented arguments, our interpretation of the data is that a small number of active bacterial populations is maintaining high viral abundance when prokaryotic production is high.

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