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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 may be maintained in relatively homogeneous aquatic environments with only a limited number of resources. This was first noted for phytoplankton and described as the 'paradox of the plankton' by Hutchinson, later extended to aquatic prokaryotes. 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, diversity. Three major factors are thought to regulate the composition of prokaryotic communities: the availability of resources, size-selective grazing on prokaryotes by heterotrophic nanoflagellates, and viral lysis. Viral infection is a stochastic process and depends on the abundance of viruses and hosts. This is the basis for the 'killing the winner' hypothesis, suggesting that viruses selectively kill the most abundant members of the prokaryotic community and thus might be a driving force for maintaining community richness by allowing the survival of less competitive phylotypes (species).

In general, disturbance and productivity are often unimodally related to diversity. Highest diversity is found at intermediate levels of disturbance and productivity. Under the assumption that mortality caused by consumers such as viruses constitutes a form of disturbance, we hypothesized that bacterial species richness should be highest at intermediate levels of viral abundance and prokaryotic production. We tested this hypothesis by assessing the dynamics of the bacterial community composition in unfiltered and 0.8µm filtered seawater (representing free-living bacteria) in the North Sea using terminal restriction fragment length polymorphism (T-RFLP) analysis. The T-RFLP patterns were analyzed by recording the number of peaks (presence versus absence), serving as a measure of bacterial richness. The field work for this study was done during 6 cruises in the North Sea with *R/V Pelagia* between July–December 2000, June 2001, and April–May 2002.

The relationship between the parameters was further evaluated using artificial neural networks (ANNs). ANNs are computational modeling tools that can be used to develop models based on previously collected data. Conventional statistical methods to quantify the relationships between parameters such as correlation analysis or principal components analysis usually require the data to fit a pre-defined model (e.g. the normal distribution). Many complex ecological processes, however, cannot easily be constrained to fit into pre-defined conditions. The advantage of ANN-based models is that they are not *a priori* restricted to a specific class of models (e.g. linear or non-linear models). Feed-forward ANNs, as used in this study, are the most widely used types of ANNs. They consist of layers of neurons where each neuron is connected to all the other neurons in the previous and the following layer. As a consequence of this parallelism, ANNs have a high tolerance towards noise in the data. After an initial training phase, the ANN can be used for predictions on the basis of new input data.

In unfiltered seawater, bacterial richness ranged from 31–82 peaks (mean = 57 peaks) and in the 0.8µm fractions (free-living bacteria) from 27–84 peaks (mean = 51 peaks). Despite the similar range (Fig. 1), total bacterial richness in unfiltered seawater was only weakly correlated with

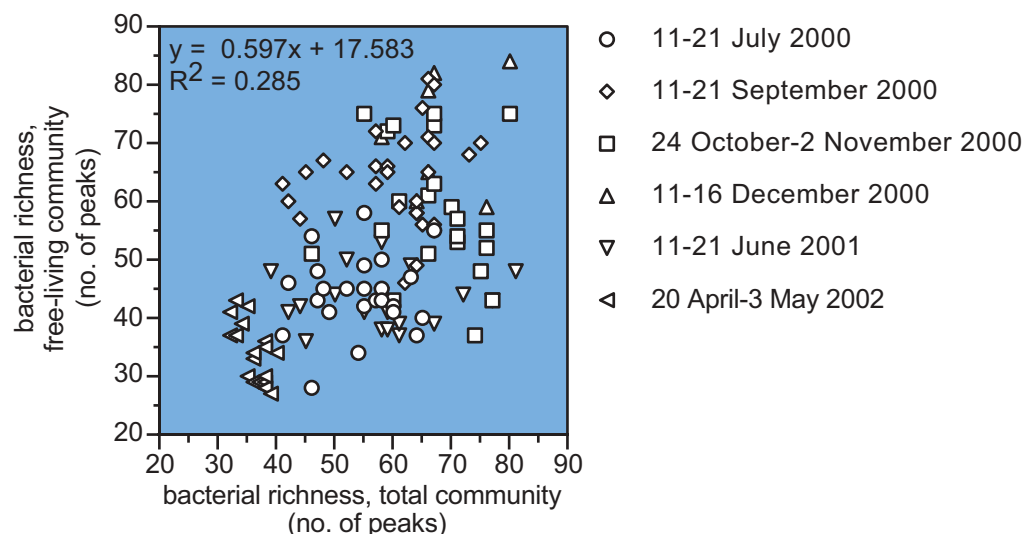


Fig. 1. Comparison of the richness of the total and free-living bacterial community as determined by T-RFLP analysis. The equation of the linear least-squares regression analysis and the coefficient of determination (R^2) are shown.

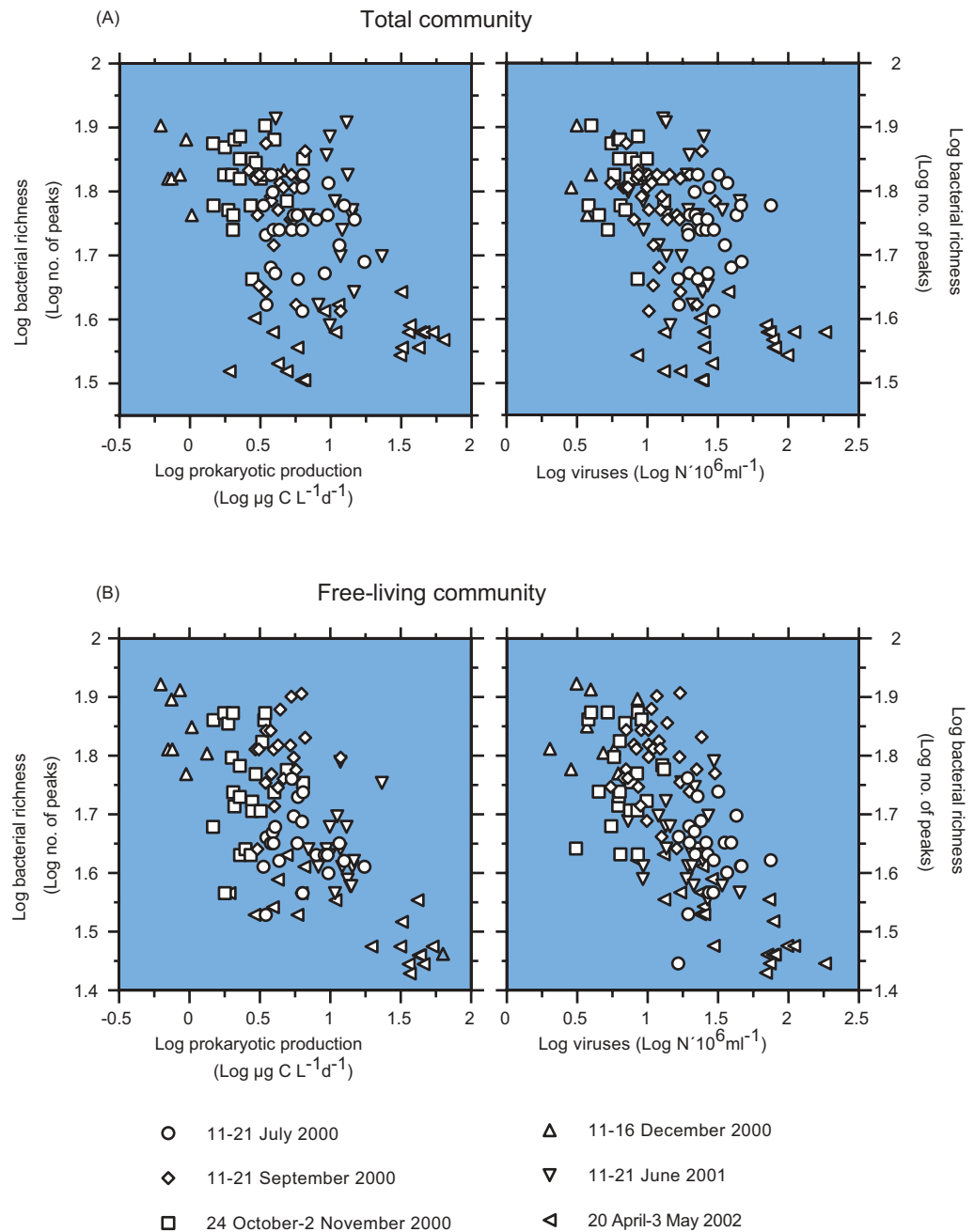


Fig. 2. Relationship of the prokaryotic production (left panels) and viral abundance (right panels) with bacterial community richness as determined by T-RFLP analysis for the total (A) and the free-living bacterial community (B).

the richness in the free-living bacterial fraction. Based on the slope (Fig. 1), bacterial richness was, on average, ca. 40% higher in unfiltered seawater than in the free-living fraction of the bacterial community. Filtration through 0.8µm pore-size filters removed particles and prokaryotic cells attached to them. Generally, free-living bacterial communities are distinctly different from particle-attached communities. Additionally, the particle load of the water column in the North Sea varied considerably during the study period. Thus, the differences in bacterial richness between unfiltered seawater (total community) and the 0.8µm fraction (free-living community, Fig. 1) reflect the exclusion of particle-attached *Bacteria* in the 0.8µm fraction.

Prokaryotic production and viral abundance in unfiltered seawater varied over two orders of magnitude between 0.6–63.1µg C L⁻¹d⁻¹ (mean = 9µg C L⁻¹d⁻¹) and 2.1–184×10⁶ml⁻¹ (mean = 22.3×10⁶ml⁻¹), respectively, while prokaryotic abundance varied only over one order of magnitude (0.3–4.3×10⁶ml⁻¹, mean = 1×10⁶ml⁻¹). Bacterial richness of the total and the free-living communities decreased with increasing prokaryotic production and viral abundance (Fig. 2).

Bacterial richness of the total community decreased with viral abundance and with prokaryotic production. An even closer negative correlation was obtained for the richness of the free-living bacterial community and prokaryotic production and viral abundance. However, prokaryotic

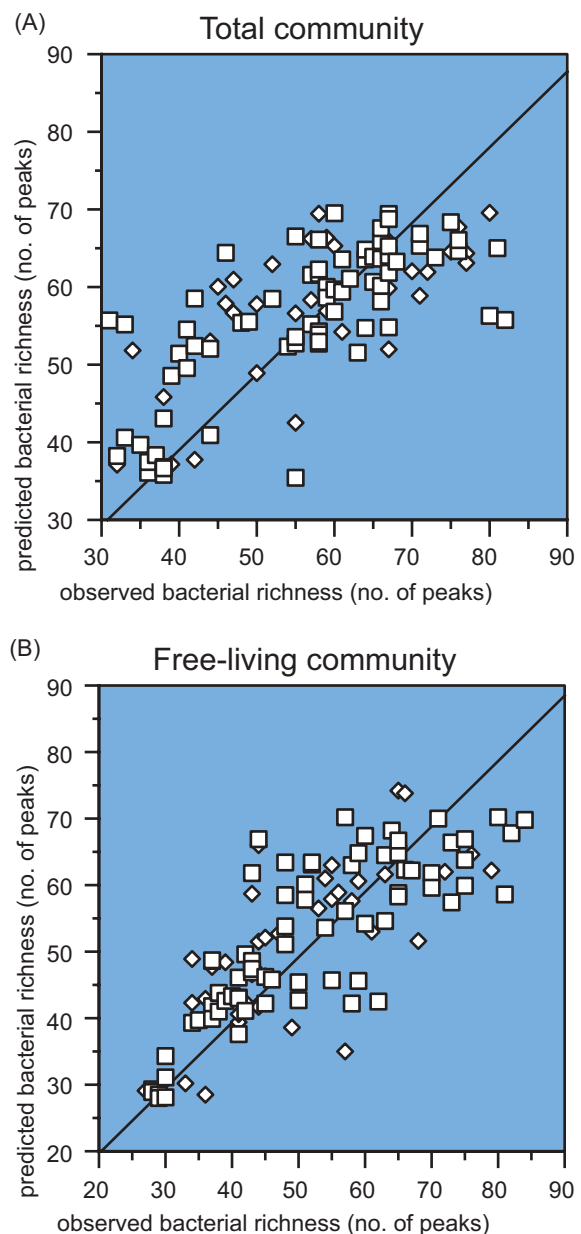


Fig. 3. Comparison between observed and predicted values of the bacterial community richness in the total (A) and free-living bacterial community (B). Bacterial community richness was determined by T-RFLP analysis and the predictions were computed using the artificial neural networks (ANNs). The formulas and the coefficients of determination (R^2) of the lines representing the linear least squares fit to the data are shown.

abundance was not correlated with bacterial richness. Additionally, we found a strong positive correlation of viral abundance and prokaryotic production.

Artificial neural network-based models of the richness of the total and the free-living communities were developed independently from each other. The data-set was divided into a training data-set used to adapt the ANNs, and a validation data-set used to test the performance of the ANNs (Fig. 3). The models mimicked the actual bacterial richness best if prokaryotic production and prokaryotic and viral abundance were used as input parameters. The high correlation coefficients calculated from the combined training and validation data sets between predicted and observed values indicate that the training strategy was successful. Additionally, the slope of linear least-squares regression analysis between observed and predicted bacterial richness was close to one for both models (Fig. 3).

The simulations based on the ANNs revealed that predicted richness of the total and the free-living bacterial community was affected differently by the input parameters (Fig. 4). This finding is not evident from Fig. 2 or the correlations between bacterial richness and prokaryotic production and viral abundance. Different types of particles (i.e., inorganic particles with organic coating versus detrital particles) might be colonized by different bacterial phylotypes. Silt plumes originating from the British coast, sediment resuspension, and decaying phytoplankton blooms constitute major sources of particles in the North Sea. Thus, changes in the particle load and the source of the particles might cause changes in the particle-attached bacter-

ial community and would explain the differences between the models for the total and the free-living bacterial consortia.

The first conclusion that can be drawn from our results is that highly active bacterial communities are characterized by low bacterial richness (Fig. 2). In the study area, prokaryotic activity was high in June-July and low in December. Thus, the high prokaryotic activity found during early summer in the North Sea might be a consequence of a small number of bacterial species capable of utilizing available substrate and out-competing less adapted species. This is further supported by the negative correlation between the richness of the free-living bacterial community and the cell-specific prokaryotic production ($r = -0.666$, $p < 0.0001$). However, bacterial richness of the total community was only weakly correlated with prokaryotic production (or cell-specific prokaryotic production: $r = -0.478$, $p < 0.0001$). This suggests that the richness of the total (particle-attached and free-living) bacterial community is related to fluctuating concentrations of inorganic particles colonized by bacteria.

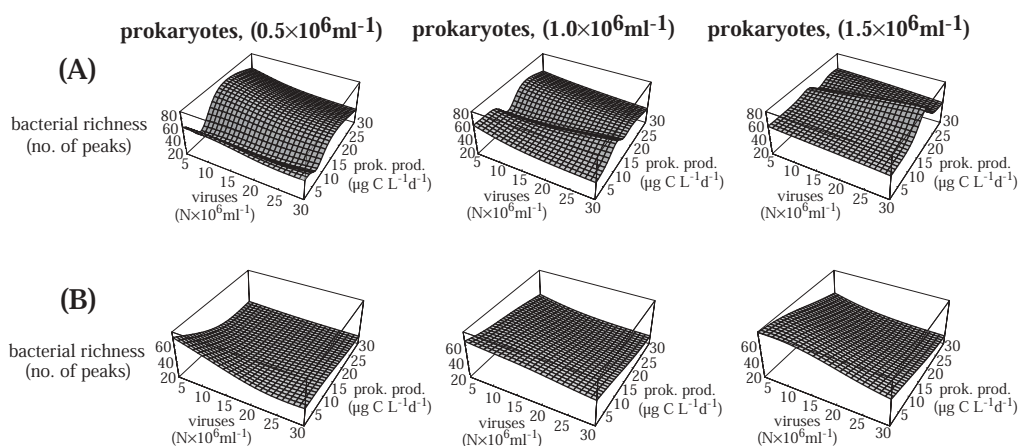
The second conclusion, which can be drawn from our results is that low bacterial richness corresponds to high viral abundance (Fig. 2). The strong correlation between viral abundance and prokaryotic production suggests that high viral abundance is maintained by an active prokaryotic community. Taken together, these findings indicate that a small number of highly active, primarily free-living bacterial phylotypes maintain high viral abundance (Fig. 2B). Thus, there is a link between bacterial species richness, prokaryotic production, and viral abundance.

One of the consequences of the 'killing the winner' hypothesis is that viral lysis should sustain high bacterial richness. The finding that bacterial richness is low at high viral abundance contradicts the original 'killing the winner' hypothesis. However, a refined model proposes the existence of a reciprocal mechanism by which lytic viruses and bacterial richness are controlling each other. In this model, viruses act as balancing factor that facilitates the coexistence of bacterial species with different growth rates and viruses with fast-growing hosts should be most abundant. Thus, the inverse relationship of viral abundance and prokaryotic activity with bacterial richness found in our study is probably driven by a few phylotypes dominating at a given time and producing high numbers of viral progeny. The negative correlations of bacterial richness with prokaryotic production and viral abundance contradict our initial hypothesis that highest bacterial species richness should be found at intermediate levels of productivity and viral abundance. It might be argued that the ranges of prokaryotic production and viral abundance might not be wide enough to test this hypothesis. However, it seems unlikely that this impacts our conclusion since prokaryotic production and viral abundance ranged over two orders of magnitude (Fig. 2) covering the entire seasonal range found in the North Sea.

The strong correlation between prokaryotic production and viral abundance suggests that viral lysis of prokaryotes is the major source of viruses in the North Sea. Thus, viral abundance might serve as a measure of consumer pressure on bacterioplankton via viral lysis. Prokaryotic production measured by leucine incorporation served as a proxy for resource availability to prokaryotes. A previously published multivariate model on the influence of productivity and disturbance on diversity predicts that the effect of physical disturbance on diversity depends on productivity and *vice versa*. The predictions of this model have recently been supported by the results of an experimental study manipulating rocky shore communities of algae suggesting that the influence of disturbance and consumer pressure on diversity is similar. The study showed that consumers decrease diversity at low productivity levels but increase diversity at high productivity.

Applying the theoretical framework of this multivariate model to our study, bacterial richness should decrease with viral abundance at low prokaryotic production and increase with viral abundance at high prokaryotic production. However, our results (Fig. 2) and the ANN-

Fig. 4. Simulations of the bacterial community richness in the total (A) and the free-living bacterial community (B). The artificial neural networks (ANNs) developed in our study were used to simulate bacterial community richness at prokaryotic production ranging between $1\text{--}30\ \mu\text{g C L}^{-1}\text{d}^{-1}$, prokaryotic abundance between $0.5\text{--}1.5 \times 10^6\text{ml}^{-1}$, and viral abundance between $5\text{--}30 \times 10^6\text{ml}^{-1}$. The results of the simulations for 3 different values of prokaryotic abundance are shown.



based model of bacterial richness of free-living *Bacteria* (Fig. 4B) show that bacterial richness decreases with increasing prokaryotic production and viral abundance. Prokaryotes have the ability to quickly acquire resistance to co-occurring viruses, especially under favorable growth conditions. The phenomenon of resistance to viral infection at high prokaryotic production could explain the differences between the multivariate model and our results. However, since viral abundance was significantly correlated to prokaryotic production in the North Sea, this seems rather unlikely. Viruses can influence the ratio of sensitive to resistant clones of prokaryotic populations. Thus, if viruses influence the clonal composition rather than the community composition of prokaryotes, the effect of viruses might not be detectable by T-RFLP analysis, especially at high prokaryotic production when the community appears to be dominated by a small number of phlotypes (Fig. 4). Furthermore, as viruses depend entirely on the metabolism of their hosts for viral replication it is unlikely to find high consumer control of bacterial richness in the form of viral lysis at low prokaryotic production.

Our results show that bacterial richness is negatively correlated with prokaryotic production and viral abundance. Thus, we conclude that a small number of highly active bacterial phlotypes maintain high viral abundance. Additionally, ANN-based models indicate differences in the relationship of bacterial richness with prokaryotic production, and prokaryotic and viral abundance between the particle-attached and free-living bacterial communities. This is probably due to differences in the composition of the bacterial communities colonizing different types of particles. In the North Sea, low prokaryotic production and viral abundance coincide with high bacterial richness. Thus, the results are in contrast to our initial hypothesis that bacterial richness should be highest at intermediate levels of viral abundance and prokaryotic production.