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Abstract

Viruses are, along with protists, one of the major prokaryotic mortality factors in the marine environment. This study focuses on the viral impact on prokaryotes throughout the redoxcline of the central Baltic Sea. We determined the frequency of lytically-infected prokaryotic cells (FIC), frequency of lysogenically-infected cells (FLC) and virus production rates (VP). Also, determining these parameters, the difference between the virus-dilution approach and unfiltered incubations was tested. To estimate the potential contribution of *Archaea* to VP and FIC, *Bacteria* were inhibited by antibiotic treatment. The relative abundance of *Bacteria*, *Crenarchaeota*, and *Sulfurimonas* ssp. was determined by CARD-FISH. FLC determined by prophage induction through mitomycin C could not be detected, whereas FIC estimated by the virus-dilution approach ranged from 11 % in the suboxic zone to < 3 % in the transition and anoxic zone, indicating a stronger influence of viruses in the suboxic than in the anoxic zone. Incubations with antibiotics inhibiting bacterial protein synthesis demonstrated a high contribution of *Crenarchaeota* to FIC and VP in all the different zones of the water column, with a major viral influence in the suboxic zone.

Introduction

Viruses are typically seen as pathogens causing diseases by infecting humans, animals or plants (Wilhelm and Suttle, 1999). However, during the last two decades it became clear that viruses are an important component of marine microbial ecosystems as well, playing a major role in ecological processes (Fuhrman and Suttle, 1993; Jiang and Paul, 1997). Host-independent methods for enumerating viruses in marine waters revealed that viruses are about one order of magnitude more abundant than prokaryotes in surface waters (Berg et al., 1989; Bratbak et al., 1994). Over the past two decades, it became clear that viruses are the most abundant entities in marine environments with an abundance ranging from 10^5 ml^{-1} to 10^8 ml^{-1} (Bratbak et al., 1994; Fuhrman and Suttle, 1993; Paul and Jiang, 1996; Torrella and Morita, 1979). Around 10^{23} viral infections per second occur in the ocean, indicating that viruses are, along with protistan grazing, a major source of mortality for marine prokaryotic communities (Anderson et al., 2012; Heldal and Bratbak, 1991; Suttle, 2007). The impact of viruses infecting prokaryotes, termed phages, in the ocean is dependent on the viral reproductive pathway, that in most cases is either lytic or lysogenic (Weinbauer et al., 2003; Weinbauer, 2004). In the lytic cycle, the injected viral nucleic acid forces the host cell to produce new viruses, followed by the release of progeny viruses into the surrounding water (Fuhrman and Suttle, 1993; Weinbauer et al., 2003). In the lysogenic cycle, the injected viral nucleic acid integrates into the genome of the host and may remain in a dormant stage as `prophage´ (Fuhrman and Suttle, 1993; Weinbauer et al., 2003). Prokaryotes (members of the phylogenetic domains *Bacteria* and *Archaea*; no phylogenetic relationship is implied) containing a prophage are called lysogens (Paul and Jiang, 1996). Reproduction of the prophage along with the host genome can occur for many generations, until it becomes activated spontaneously or until induction of the lytic cycle by specific biochemical mechanisms (Cochran et al., 1998; Fuhrman and Suttle, 1993; Paul and Jiang, 1996; Weinbauer and Suttle, 1999; Weinbauer, 2004). It has been suggested that lysogeny constitutes a viral survival strategy when the host abundance and/or activity is low (Weinbauer and Suttle, 1999; Weinbauer et al., 2003).

However, aquatic systems are strongly influenced by microbial biomass loss due to viral lysis (Wilhelm and Suttle, 1999). A lysed host cell releases, along with viral progeny, sugars, proteins, nucleic acids, and other intracellular compounds of the host cell, and hence provides additional resources for prokaryotes in the form of dissolved organic carbon (DOC;

Bratbak et al., 1994; Winter et al., 2010). Particulate organic carbon (POC) can be utilized by grazers from higher trophic levels whereas DOC is recycled by the microbial community (Wilhelm and Suttle, 1999). Azam et al. (1983) formulated the hypothesis of the `microbial loop´, describing the flow of DOC back to the food chain via prokaryotes (Bratbak et al., 1994; Weinbauer, 2004). Hence, viral lysis constitutes a short-circuit in the flow of carbon and nutrients to higher trophic levels (Suttle, 2005). It has been shown that around one-quarter of the organic carbon flows through this `viral shunt´ to the DOC pool from where it can be utilized again by other prokaryotes (Danovaro et al., 2008; Suttle, 2005, 2007; Wilhelm and Suttle, 1999). Taken together, along with protistan grazing, viral lysis is one of the major biological loss factors in marine prokaryotic communities (Anderson et al., 2012; Heldal and Bratbak, 1991).

In our study we focused on the Baltic Sea, one of the largest (~ 377,000 km^2) brackish environments worldwide that can be considered a large estuary due to its low salinity (Brettar and Rheinheimer, 1991; Elmgren, 2001). The connection to the North Sea through shallow straits results in inflow of oxygenated and saline water (Meier et al., 2006; Reissmann et al., 2009; Stock et al., 2009). Furthermore, the Baltic Sea receives large amounts of freshwater (Reissmann et al., 2009; Stock et al., 2009) leading overall to its salinity of well below 10. Together, these mechanisms result in strong stratification with saline waters at the bottom and low salinity waters on top, generating a permanent halocline at around 60-80 m depth. This stable halocline inhibits vertical mixing, leading to oxygen deficient waters in several deep basins of the Baltic Sea (Stock et al., 2009). Such bottom waters may become re-oxygenated through major inflow events, occurring at an interval of ~ 10 years (Meier et al., 2006; Stock et al., 2009). However, due to prolonged stagnation periods between those inflow events, the bottom water becomes anoxic and hydrogen sulfide may accumulate from the sediment (Brettar and Rheinheimer, 1991; Stock et al., 2009). Furthermore, anoxic conditions can lead to anaerobic mineralization of organic matter by sulfate reducing *Bacteria*, resulting in the production of hydrogen sulfide (Grote et al., 2012). Hence, water in the deep areas of the Baltic Sea is separated into an upper oxygenated layer and an underlying anoxic sulfidic layer (Neretin et al., 2003). These two layers are separated by the oxic-anoxic transition zone, referred to as the pelagic redoxcline, which is a layer of several meter thickness with detectable but low oxygen and hydrogen sulfide concentrations (Jost et al., 2008; Labrenz et al., 2010, 2007).

One of the largest deep areas in the Baltic Sea is the Gotland Deep, a temporary anoxic basin of 248 m depth located in the central Baltic Sea (Neretin et al., 2003; Stock et al., 2009). The vertically stratified redoxcline in the Gotland Deep results in a distinct prokaryotic stratification (Labrenz et al., 2010, 2007). According to previous studies, pelagic redoxclines are hot spots for prokaryotic activity, providing an ideal habitat for nitrifying or sulfur-oxidizing prokaryotes (Anderson et al., 2012; Jost et al., 2008). The Baltic Sea redoxcline has been shown to exhibit high dark CO_2-fixation rates hence, chemolithoautotrophic production, amounting to up to ~ 30 % of surface primary production (Detmer et al., 1993; Jost et al., 2008). For example, Grote et al. (2008) determined that CO_2-fixing cells have the highest abundance in the anoxic sulfidic zone. Their study showed that ~ 29 % of total prokaryotic abundance is due to CO_2-fixing cells and that nearly all of those are *Bacteria*. Furthermore, it has been shown that ~ 70 % of these CO_2-fixing prokaryotes are *Epsilonproteobacteria* (Grote et al., 2008). Another study conducted by Grote et al. (2007) revealed that on average 94 % of these *Epsilonproteobacteria* belong to the single phylogenetic group, *Sulfurimonas* subgroup GD 17. The prokaryotic community composition throughout the redoxcline of the Baltic Sea has been well characterized (Grote et al., 2012, 2008, 2007; Jost et al., 2010, 2008; Labrenz et al., 2010, 2007). Nevertheless, data on viral assemblages and their impact on prokaryotic communities in this area are rare (Anderson et al., 2012; Weinbauer et al., 2003).

There is a variety of methods and treatments to determine the frequency of infected cells (FIC), the frequency of lysogenic cells (FLC), and viral production (VP) (Weinbauer et al., 2003; Wommack and Colwell, 2000). For example, mitomycin C is the agent of choice to induce the lytic cycle of prophages and thus, to determine FLC (Anderson et al., 2012; Danovaro et al., 2008; Jiang and Paul, 1997; Ortmann et al., 2002; Paul and Jiang, 1996; Weinbauer and Suttle, 1999; Weinbauer et al., 2003). However, according to Weinbauer et al. (2003) and Ortmann et al. (2002), not all lysogens can be induced by mitomycin C.

The aim of this study was to determine FIC, FLC, and VP throughout the redoxcline of the Gotland Deep. The potential contribution of *Archaea* to those parameters was determined by inhibiting bacteria by antibiotics. A study of Anderson et al. (2012) detected very low protistan grazing in the anoxic sulfidic zone, leading us to hypothesize that viruses may play a major role as prokaryotic loss factors. Although Anderson et al. (2012) detected low FIC in the anoxic zone, we assumed that oxygen contamination may have led to an underestimation of virus infections in their study. Thus, we hypothesized that highest FIC will

be detected in the anoxic zone. This hypothesis is supported by findings of Weinbauer et al. (2003) who detected high FIC in the anoxic zone. Nevertheless, in their study no virus-dilution approach was used. We suggested that a virus-dilution approach is crucial to obtain FIC and VP resulting from infections occurring before the onset of the experiment and hence, we compared FIC and VP in unfiltered and virus-diluted incubations (Winter et al., 2004). Furthermore, the relative abundance of *Bacteria*, *Crenarchaeota*, and *Sulfurimonas* ssp. was determined before and after the termination of the experimental incubations.

Materials and Methods

Study site and sampling – The samples were taken from the central Baltic Sea during a cruise with *RV Meteor* in June 2012 (station TF 271; Gotland Deep; Fig. S1). Water was collected throughout the redoxcline to compare three different zones according to oxygen and hydrogen sulfide concentrations (Table S1). Sampling was performed with Niskin bottles (24 x 10 L) mounted on a CTD rosette sampler (Seabird 911). Standard oceanographic parameters such as depth, temperature and salinity were measured with sensors on the rosette sampler.

Enumeration of prokaryotes and viruses – The abundance of prokaryotes and viruses was determined on a BD FACSAria II flow cytometer (Becton Dickinson) as previously described (Brussaard, 2004; Marie et al., 1999). Water samples and subsamples from the incubations (1.8 mL in duplicate) were fixed with glutaraldehyde (0.5 % final concentration), flash-frozen in liquid nitrogen and stored in the dark at -80°C. Prior to analysis, samples were thawed, diluted in TE buffer and stained with SYBR Green I. Each sample was analyzed in triplicate for prokaryotic and viral abundance. Prokaryotic and viral abundance were determined based on side-scatter versus SYBR Green I fluorescence plots (Fig. S2). Details of the protocol are given in Table S2.

Relative abundance of Bacteria, Crenarchaeota, and Sulfurimonas ssp. – The relative abundance of *Bacteria*, *Crenarchaeota*, and *Sulfurimonas* ssp. was determined by CARD-FISH following the protocol of Teira et al. (2004). Modifications and details about crucial steps are given in Table S3. *Euryarchaeota* were not taken into account in this study as a previous study showed that these are not found in the study area or present in very low abundance only (Labrenz et al., 2010). Original water samples (10 mL) and subsamples (~35

mL in duplicate) from the experiments taken after 40 h of incubation were fixed with formaldehyde (2 % final concentration) in the dark at 4°C for up to 24 h. Prokaryotic cells were collected on 0.2 µm pore-size membrane filters (Millipore GTTP, 25 mm diameter) and stored at -80°C until analysis. CARD-FISH was conducted using horseradish peroxidase (HRP)-labeled oligonucleotide probes (Thermo Fisher Scientific Inc.): the probe-mix consisting of EUB338 (GCTGCCTCCCGTAGGAGT; Amann et al., 1990), EUB338II (GCAGCCACCCGTAGGTGT), and EUB338III (GCTGCCACCCGTAGGTGT; Daims et al., 1999) for *Bacteria* (EUB), the probe-mix CREN537 (TGA CCA CTT GAG GTG CTG; Teira et al. 2004) and GI554 (TA GGC CCA ATA ATC MTC CT; Massana et al., 1997) for *Crenarchaeota* (CREN) and SUL90 (CGTGCGCCACTAATCATA; Grote et al., 2007) for *Sulfurimonas* ssp. (SUL). Filters were embedded in low-gelling-point agarose and stored at -20°C until further analysis. To enumerate the respective phylogenetic groups, filter slices were incubated either with lysozyme for the EUB probe-mix and the SUL90 probe or with proteinase K for the CREN probe-mix. DAPI-stained and probe-labeled cells were detected under a Zeiss Axioplan-2 epifluorescence microscope and counted as DAPI-cells compared to the fluorescein isothiocyanate signals (FITC). The relative abundance of probe-labeled cells was determined as percentage of DAPI-stained cells.

Determination of FIC, FLC, and VP – To estimate FIC and VP, a virus-dilution approach was used to reduce the background of free viruses by adding virus-free seawater to the incubations (Wilhelm et al., 2002; Winter et al., 2004). The idea behind this method is to reduce virus-prokaryote contact rates and thus, minimize the chance of new infections (Murray and Jackson, 1992; Weinbauer et al., 2010, 2002). Therefore, an increase in viral abundance during the incubation period can be attributed to infections prior to the onset of the experiment (Winter et al., 2004). FIC in % of total cells was calculated according to the equation

$$FIC = \{[((Vmax1 - Vmin1) + (Vmax2 - Vmin2))/\text{burst size}]/B_0\} \times 100$$ (Winter et al., 2004).

B_0 is the bacterial abundance at the beginning of the experiment. A burst size of 28 was chosen based on earlier studies in the Baltic Sea (Weinbauer et al., 2003, 2002). Furthermore, VP in $N \times 10^5$ mL^{-1} h^{-1} was calculated as

$$VP = [(Vmax1 - Vmin1) + (Vmax2 - Vmin2)]/(tmax2 - t\ min1)$$ (Winter et al., 2004).

Maximum and minimum values of virus abundance were determined during a 40 h incubation period (Fig. S3). If virus abundance showed more than four peaks during the incubation period, then all maximum and minimum values were added up to calculate FIC and VP (Winter et al., 2004). FLC was determined as the increase of FIC in incubations after mitomycin C addition and calculated as the difference to control incubations without mitomycin C (Weinbauer et al., 2003).

Experimental setup – For the experiments, seawater (2 L) was filtered in three filtration steps to obtain a prokaryotic concentrate, a virus concentrate and virus-free ultrafiltered seawater. First, water was pre-filtered through a 3.0 µm membrane filter (TSTP, 47mm, Millipore) to remove larger plankton and particles. Prokaryotes and viruses are passing through this filter. Prokaryotes from the filtrate were concentrated using a tangential-flow filtration device (TFF) with a pore-size of 0.2 µm (Vivaflow 200; Sartorius/Vivascience). The remaining viruses from this step were concentrated with a tangential-flow filtration device (TFF) with a molecular weight cut–off of 100 kDa (Vivaflow 200; Sartorius/Vivascience). With the last filtration step virus-free ultrafiltrate was obtained and used as growth medium for the experiments. The experiments were conducted with water samples from the suboxic zone, the transition zone and the anoxic zone (2 L of water for each treatment). Sterile glass vials (60 mL) with gas-tight lids were prepared in duplicate for 7 different treatments (Fig. 1). Two vials were filled with unfiltered water, subsequently referred to as "*unfilt*". The other vials were set-up using the virus-dilution approach (Table S4) and henceforth will be referred to as "*filt*". The idea behind this was to compare VP and FIC with and without using a virus-dilution approach. Mitomycin C was added to every other treatment to determine the abundance of lysogenic viruses (Jiang and Paul, 1997). Subsequently, filtered and unfiltered treatments with mitomycin C will be referred to as "$filt_{MitC}$" and "$unfilt_{MitC}$". Treatments without mitomycin C served to determine VP and FIC due to lytic viruses. For determining the potential contribution of *Archaea* to FIC and VP, the antibiotics streptomycin and erythromycin were added to two treatments (Könneke et al., 2005). Streptomycin (Ionescu et al., 2009) and erythromycin (Kohanski et al., 2010) inhibit the bacterial protein biosynthesis without impacting *Archaea*. Treatments with antibiotics added are referred to as "$filt_{antib}$" and "$filt_{antib,MitC}$". Treatments without antibiotics served as controls to determine VP and FIC. The last treatment consisted of a virus concentrate to estimate viral decay during the experiment.

All the glass vials were incubated in the dark under *in situ* temperature for 40 h. Subsamples were taken at regular intervals (5 h) and further processed as described above.

All filtrations, set-up of the experiments, and subsampling were performed in an anaerobic chamber filled with nitrogen gas to prevent oxygen contamination.

Significance – Differences in results were considered significant when the ranges of duplicate samples did not overlap between treatments.

Results

Characteristics of the sampling site – In situ prokaryotic abundance was 2.1×10^5 mL^{-1} (SD = 0.0) in the suboxic zone, 9.0×10^5 mL^{-1} (SD = 0.3×10^5) in the transition zone and 7.3×10^5 mL^{-1} (SD = 0.4×10^5) in the anoxic zone (Fig. S4). Thus, the abundance was highest in the transition zone and lowest in the suboxic zone. *In situ* viral abundance (measured in triplicate) was 192.2×10^5 mL^{-1} (SD = 3.4×10^5) in the suboxic zone, 165.8×10^5 mL^{-1} (SD = 15.2×10^5) in the transition zone and 147.3×10^5 mL^{-1} (SD = 4.7×10^5) in the anoxic zone (Fig. S4). Thus, virus abundance was highest in the suboxic zone and lowest in the anoxic zone.

Virus decay, FIC, FLC, and VP – No significant increase of mortality due to viruses as a consequence of mitomycin C-addition was detected thus, FLC was not measureable throughout the redoxcline. Virus decay during the experimental incubations was negligible and similar in the three depth zones (Fig. S5). FIC and VP in the *unfilt* treatments were significantly higher as compared to the *filt* treatments (Fig. 2). In the *filt* treatments, FIC was 11 % (range = ± 4.1) in the suboxic zone, 1 % (range = ± 0.6) in the transition zone, and 2 % (range = ± 1.1) in the anoxic zone (Table S5). Furthermore, FIC decreased significantly from the suboxic zone towards the anoxic zone in all treatments (Fig. 2B). VP in the *filt* treatments averaged 0.2×10^5 mL^{-1} h^{-1} (range = ± 0.0) in the suboxic zone, 0.1×10^5 mL^{-1} (range = ± 0.0) in the transition zone, and 0.2×10^5 mL^{-1} (range = ± 0.1) in the anoxic zone (Table S5). Thus, no significant difference of lytic VP was detected between the sampling zones (Fig. 2A). Comparing the treatments *filt* and $filt_{antib}$, the addition of the antibiotics streptomycin and erythromycin significantly increased VP by 0.2×10^5 mL^{-1} h^{-1} (range = ± 0.1) in the suboxic zone. Furthermore, FIC in $filt_{antib}$ increased after the addition of antibiotics as compared to *filt* by 15 % (range = ± 0.5) in the suboxic zone and by 2 % (range = ± 0.45) in the transition zone. Comparing the $filt_{MitC}$ and $filt_{antib,MitC}$ treatment, the addition of antibiotics resulted in a significant increase of VP rates in the anoxic zone and of FIC in the anoxic and transition zone (Fig. 2).

Relative abundance of Bacteria, Crenarchaeota, and Sulfurimonas ssp. – The relative abundance of *Bacteria* was 14 % (SE = 2.5) of the DAPI-stained cells in the suboxic zone, 35 % (SE = 2.4) in the transition zone, and 29 % (SE = 2.3) in the anoxic zone (Fig. S6). The relative abundance of *Crenarchaeota* was 6 % (SE = 1.8) of the DAPI-stained cells in the suboxic zone, 29 % (SE = 2.3) in the transition zone, and 22 % (SE = 2.1) in the anoxic zone (Fig. S6). The relative abundance of *Sulfurimonas* ssp. was 23 % (SE = 3.1) of the DAPI-stained cells in the suboxic zone, 39 % (SE = 3.0) in the transition zone and 33 % (SE = 2.9) in the anoxic zone (Table S6). Consequently, the relative abundance of *Bacteria*, *Crenarchaeota*, and *Sulfurimonas* ssp. was highest in the transition zone and lowest in the suboxic zone (Fig. S6).

In the suboxic zone, the addition of mitomycin C significantly decreased the relative abundance of *Bacteria* by 7 % (range ± 2.0) in $filt_{MitC}$ as compared to *filt* and by 13 % (range ± 4.7) in the treatments $filt_{antib,MitC}$ as compared to $filt_{antib}$ (Fig. 3A). In the transition zone, however, the percentage of *Bacteria* increased by 8 % (range = ± 0) in $filt_{MitC}$ as compared to *filt* (Fig. 3B). In the anoxic zone, the relative abundance of *Bacteria* remained stable in all treatments. A significant increase in the relative abundance of *Crenarchaeota* due to mitomycin C in the treatment $filt_{MitC}$ as compared to *filt* was detected in the anoxic zone (Fig. 3C).

The relative abundance of *Bacteria* increased in the $filt_{antib}$ compared to *filt* treatment by 4 % (range = ± 2.0) in the suboxic zone whereas the percentage of *Crenarcheota* was reduced by 7 % (range = ± 0.9) in the suboxic zone, by 12 % (range = ± 0.4) in the transition zone and by 8 % (range = ± 1.8) in the anoxic zone. A significant decrease was detected in $filt_{antib,MitC}$ as compared to $filt_{Mitc}$ for *Bacteria* in the transition zone and for *Crenarchaeota* in the transition and anoxic zone (Fig. 3).

Figures 4 and 5 show the relationship between VP and FIC and the relative abundance of *Crenarchaeota* and *Bacteria* in the suboxic, transition, and anoxic zone. The significant decrease of *Crenarchaeota* in the $filt_{antib}$ treatment in all zones as compared to *filt* treatment was concomitant with an increase in VP in the suboxic zone, whereas the relative abundance of *Bacteria* increased significantly with increasing VP in the same treatment. Also, the decrease in the relative abundance of *Crenarchaeota* was accompanied by an increase in FIC in the suboxic and transition zone in $filt_{antib}$ as compared to *filt*. The relative abundance of *Crenarcheota* was significantly lower in $filt_{MitC}$ as compared to $filt_{antib,MitC}$ while VP in the

anoxic zone and FIC in the transition and anoxic zone increased. The relative abundance of *Bacteria* decreased with increasing FIC in the transition zone in the $filt_{MitC}$ as compared to the $filt_{antib,MitC}$ treatment.

Discussion

The virus-dilution approach versus unfiltered incubations – In this study, we used the virus-dilution approach to estimate FIC and VP (Wilhelm et al., 2002; Winter et al., 2004). This method has been used before to estimate the impact of viruses on bacterioplankton by reducing virus numbers in the incubations (Weinbauer et al., 2002; Wilhelm et al., 2002; Winter et al., 2004). The main idea behind the virus-dilution approach is to reduce contact rates between viruses and their hosts (Murray and Jackson, 1992; Weinbauer et al., 2010). Thus, it can be assumed that an increase in the abundance of viruses during the incubation is attributable to infections that occurred prior to collecting the water sample (Wilhelm et al., 2002; Winter et al., 2004).

However, earlier studies (Weinbauer et al., 2003) reported FIC and VP based on unfiltered incubations. Hence, we compared both approaches in the Baltic Sea. For example, Weinbauer et al. (2003) detected highest FIC in the anoxic zone and high FLC throughout the redoxcline of the Gotland Deep in unfiltered incubations. The comparison between both methods performed in this study confirms that FIC and VP are significantly higher in unfiltered incubations as compared to the virus-dilution approach (Fig. 2). Thus, we suggest that the addition of virus-free water to a prokaryotic concentrate is crucial to prevent new infections during the incubations. A potential bias of the virus-dilution approach might be the alteration of the *in situ* physico-chemical conditions, such as changes in oxygen or hydrogen sulfide concentration due to the filtration steps (Anderson et al., 2012). However, this potential problem was overcome in our study by performing all filtration steps and sub-sampling in an anaerobic chamber.

Influence of lytic viruses on prokaryotes throughout the Baltic Sea redoxcline – We found highest FIC (~ 11 % of prokaryotic cells) in the suboxic zone, decreasing to < 3 % in the transition and anoxic zones (Table S5). These findings indicate that viruses may play an important role in controlling the prokaryotic community in the suboxic zone of the Baltic Sea. A previous study (Weinbauer et al., 2003) conducted throughout the redoxcline of the

Gotland Deep revealed highest FIC (up to 25 % of prokaryotic abundance) in the anoxic zone without reducing viral abundance through dilution. Highest FIC in the suboxic zone were also reported by Anderson et al. (2012) with up to 7 % of prokaryotic abundance. Furthermore, Anderson et al. (2012) examined prokaryotic mortality factors in the Gotland Deep by comparing protistan grazing and viral lysis in the suboxic, transition, and anoxic zone. They showed that prokaryotic mortality is dominated by protistan grazing in the suboxic and transition zone. However, in the anoxic, sulfidic zone, grazing was below detection limit (Anderson et al., 2012). Thus, the study by Anderson et al. (2012) indicates that neither grazing by protists nor viral lysis appear to play a substantial role as loss factor for prokaryotes in the anoxic zone. The data from our study and the study by Anderson et al. (2012) are well in agreement with each other. High FIC in the anoxic zone determined by Weinbauer et al. (2003) were not found in this study. As shown above, unfiltered incubations result in overestimations of VP and FIC and may also lead to high FLC which were not detected with the dilution method. The finding of high FIC in the suboxic zone in our study is coinciding with high virus abundance in this zone and low virus abundance in the anoxic zone. However, lowest prokaryotic abundance was detected in the suboxic zone. Together these findings may indicate that viruses have, along with protistan grazing (Anderson et al., 2012), a major influence on prokaryotes in the suboxic zone. The major prokaryotic mortality factor in the anoxic zone, however, remains unknown.

Critical evaluation of the SUL 90 probe – Significant changes in the relative abundance of *Bacteria* and *Crenarchaeota* due to viral lysis and antibiotic treatment were observed. To estimate the relative abundance of *Sulfurimonas* ssp. we used the probe SUL 90 (Grote et al., 2007). While in the study of Grote et al. (2007) the relative abundance of *Sulfurimonas* ssp. did not exceed that of *Bacteria*, in our study the percentage of SUL90-positive cells was occasionally higher than that of EUB338I-III-positive cells. Thus, the data determined by the SUL90 probe (Grote et al., 2007) need to be interpreted cautiously and will not be taken into account. It appears that the published hybridization conditions for this probe need to be further optimized. However, this was outside of the scope of this study.

Effect of Bacteria-inhibiting antibiotics on FIC and VP – The antibiotics streptomycin (Ionescu et al., 2009) and erythromycin (Kohanski et al., 2010) are inhibitors of the protein synthesis in *Bacteria*. Thus, *Archaea* should not be inhibited and hence are able to produce viruses. In a previous study (Yokokawa et al., 2012), the efficiency of streptomycin and erythromycin as bacterial inhibitors was tested by leucine incorporation. It was shown that ~

97 % of bacterial cells were inhibited due to erythromycin whereas streptomycin was less effective (Yokokawa et al., 2012). Another study revealed an inhibition of bacterial leucine uptake due to the addition of streptomycin by > 90 % (Ionescu et al., 2009). In our study, we used both antibiotics to inhibit bacterial protein synthesis and hence determine the potential contribution of *Archaea* to FIC and VP. According to previous studies (Ionescu et al., 2009; Yokokawa et al., 2012) we do not claim that *Bacteria* were totally inhibited in the antibiotic treatments and that therefore FIC and VP occurred only due to *Archaea* in these treatments. Nevertheless, our findings revealed a significant decrease (6 % to 12 %) of the relative abundance of *Crenarchaeota* after antibiotic treatment in all sampling zones. This decrease due to antibiotic addition was further observed in the mitomycin C treatments in the transition and anoxic zone. However, the percentages of *Bacteria* either increased (suboxic zone) or remained stable (transition and anoxic zone) after antibiotic addition, and only decreased in the mitomycin C treatment of the transition zone. Thus, we conclude that the mortality of *Bacteria* due to viral lysis in the antibiotic treatments was low, indicating that most of the FIC and VP in the antibiotic treatments can be attributed to *Crenarchaeota* subjected to viral lysis.

There is a conceptual difference between the interpretation of mitomycin C treatments and antibiotic treatments. Provided that prophages are present, we expected higher values after mitomycin C addition ($filt_{MitC}$) due to lytic and lysogenic viruses as compared to the control treatments with only lytic VP and FIC (*filt*). Thus, the difference between $filt_{MitC}$ and *filt* was calculated to estimate the number of lysogenic viruses. However, the antibiotic treatments ($filt_{antib}$) should result in VP and FIC only due to *Archaea* compared to the control treatments with VP and FIC resulting from both *Bacteria* and *Archaea* (*filt*). Consequently, we expected lower VP and FIC in the antibiotic treatments as compared to control treatments. Interestingly, it was observed that VP increased after addition of streptomycin and erythromycin in waters of the suboxic zone. Moreover, FIC increased after antibiotic addition by ~ 15 % in the suboxic zone and by ~ 2 % in the transition zone. This increase in VP (anoxic zone) and FIC (transition and anoxic zone) due to antibiotics was further observed in the mitomycin C treatments. Nevertheless, the detected high mortality of *Crenarchaeota* and the relatively low mortality of *Bacteria* following antibiotic addition indicate that most of the VP and FIC in those treatments can be attributed to *Crenarchaeota*. Thus, we suggest that the inhibition of the protein-synthesis in *Bacteria* results in lower competition for nutrients between *Bacteria* and *Archaea*, enabling a more rapid growth of *Archaea*. Also, we assume that antibiotics may be used as nutrients when they are not inhibiting and thus may lead to

enhanced growth compared to the control treatment. Taken together, we assume that most of the VP and FIC in the antibiotic treatments resulted from *Crenarchaeota*, indicating a relatively high contribution to those parameters in each layer with highest VP and FIC in the suboxic zone.

Conclusion

In the present study, we showed a clear pattern of FIC throughout the redoxcline of the Gotland Deep with declining FIC from suboxic (~ 11 %), to transition and anoxic zone (< 3 %). Thus, a decrease in viral mortality with increasing depth was detected, whereas the FLC remained below the detection limit. Also, we showed significant differences between the virus-dilution approach and unfiltered incubations leading us to conclude that reducing virus abundance by dilution is essential for estimating FIC and VP. To our knowledge, this study represents the first report on the archaeal contribution to FIC and VP. The decrease of relative abundance of *Crenarchaeota* by, on average, ~ 9 % after antibiotic treatment indicates a potentially high contribution of *Archaea* to FIC and VP in all the zones, with strong viral influence in the suboxic zone. In summary, viruses appear to play a role as prokaryotic mortality factor in the suboxic zone but do not have a strong influence on prokaryotes in the anoxic zone.

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First of all, I would particularly like to thank Nicole Köstner, Ph.D. student at the Department of Limnology and Oceanography (University Vienna) working on the project “Virus-host dynamics in the Baltic Sea redoxcline” of Christian Winter, supported by the Austrian Science Fund (FWF). I want to give my sincere thanks to Nicole for spending countless hours with me in the lab, explaining me whatever I wanted to know, helping me with all questions and insecurities and let me be part of her interesting work. I also want to thank Christian Winter for his helpful advice and support during the last month and for leading me through writing the thesis. Further thanks to Gerhard Herndl for the great opportunity to write my Master thesis at the Department of Limnology and Oceanography (University Vienna) and for his suggestions. Finally, I want to thank Franziska Eibenberger for her support in the lab and

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APPENDIX

Figures

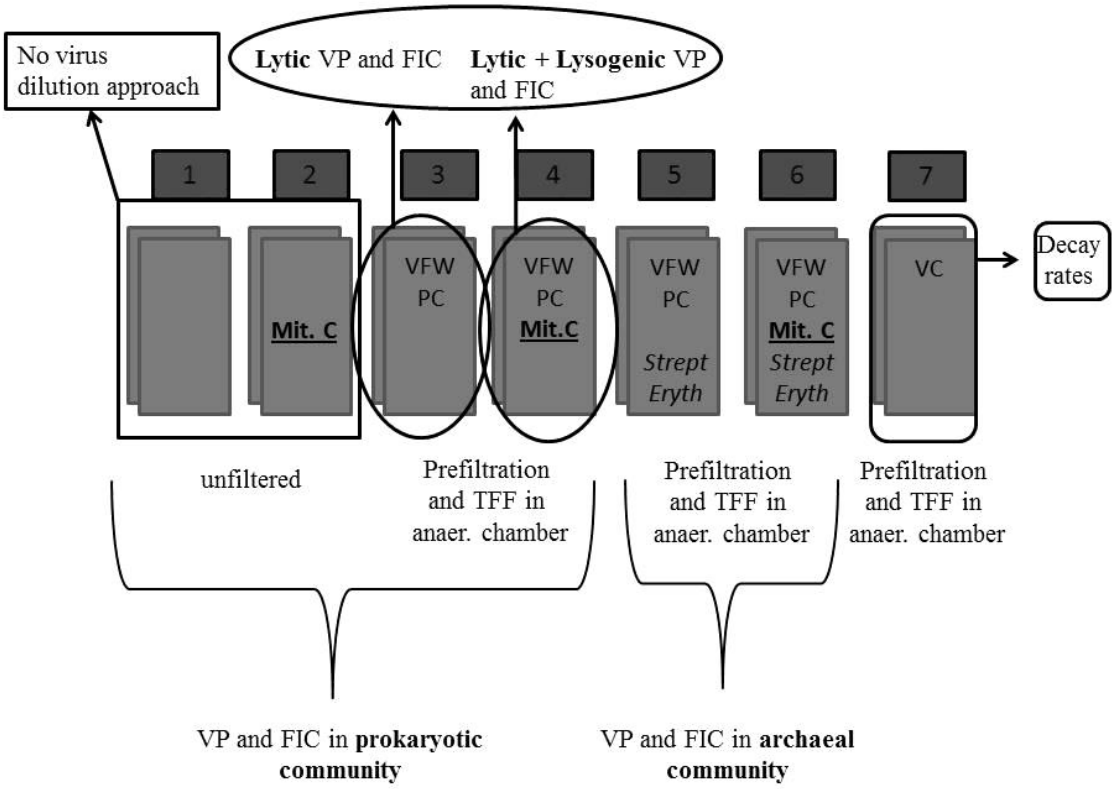


Fig. 1. Setup of the experiment with 7 treatments in duplicate (VFW = virus-free water, PC = prokaryotic concentrate, VC = virus concentrate, Mit. C = mitomycin C, Strept. = streptomycin, Eryth. = erythromycin, VP = virus production, FIC = frequency of infected cells).

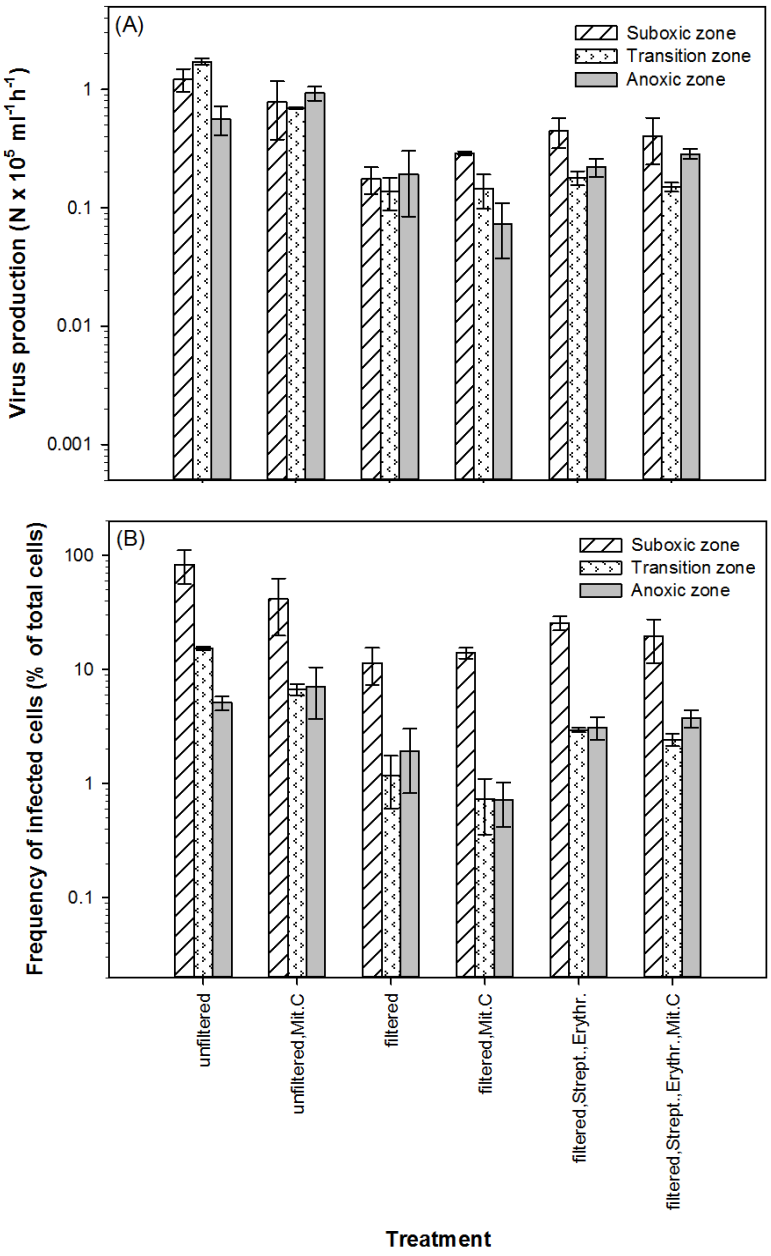


Fig. 2. Virus production (A) and frequency of infected cells (B) (log scale) in the suboxic, transition, and anoxic zone. Data are given as the average of duplicate incubations for the treatments *unfilt*, $unfilt_{MitC}$, *filt*, $filt_{MitC}$, $filt_{antib}$ and $filt_{antib,MitC}$. Error bars represent the ranges of duplicate incubations.

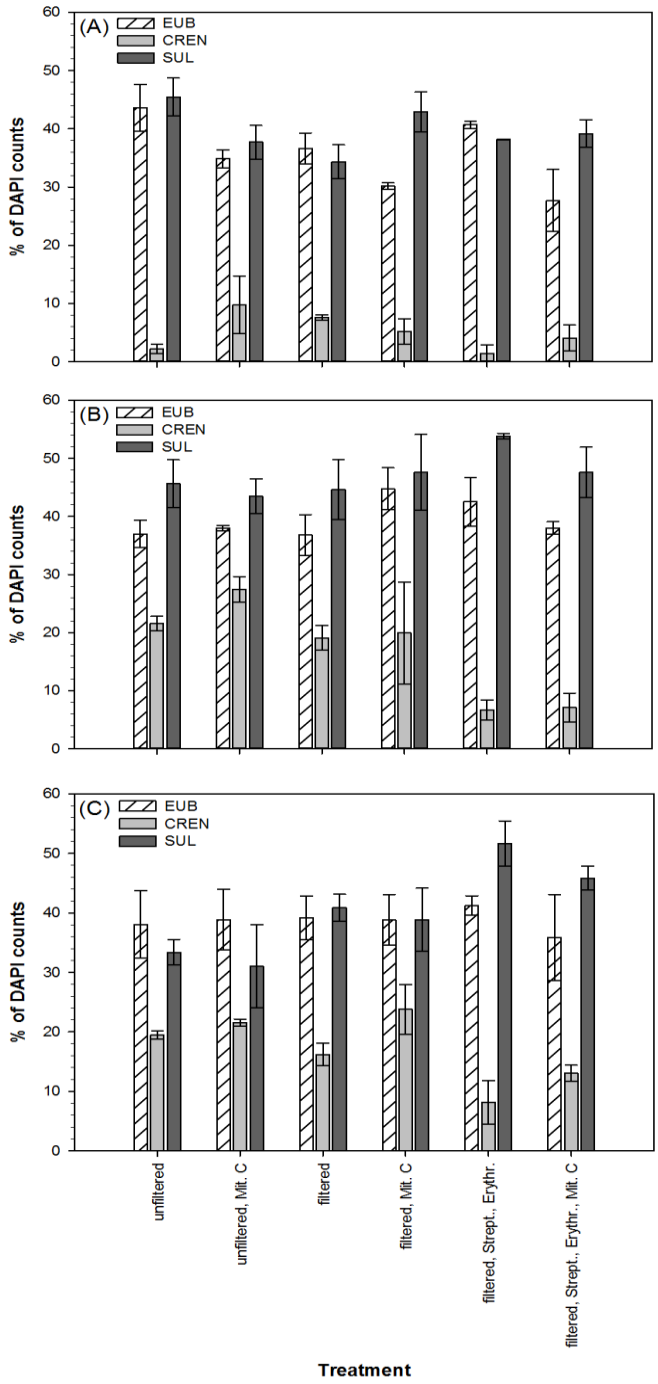


Fig. 3. Relative abundance of *Bacteria*, *Crenarchaeota*, and *Sulfurimonas* ssp. at the end of the incubation time with water from the suboxic (A), transition (B), and anoxic (C) zone in the experimental treatments *unfilt*, $unfilt_{MitC}$, *filt*, $filt_{MitC}$, $filt_{antib}$ and $filt_{antib,MitC}$. The data are average values and the error bars represent the ranges.

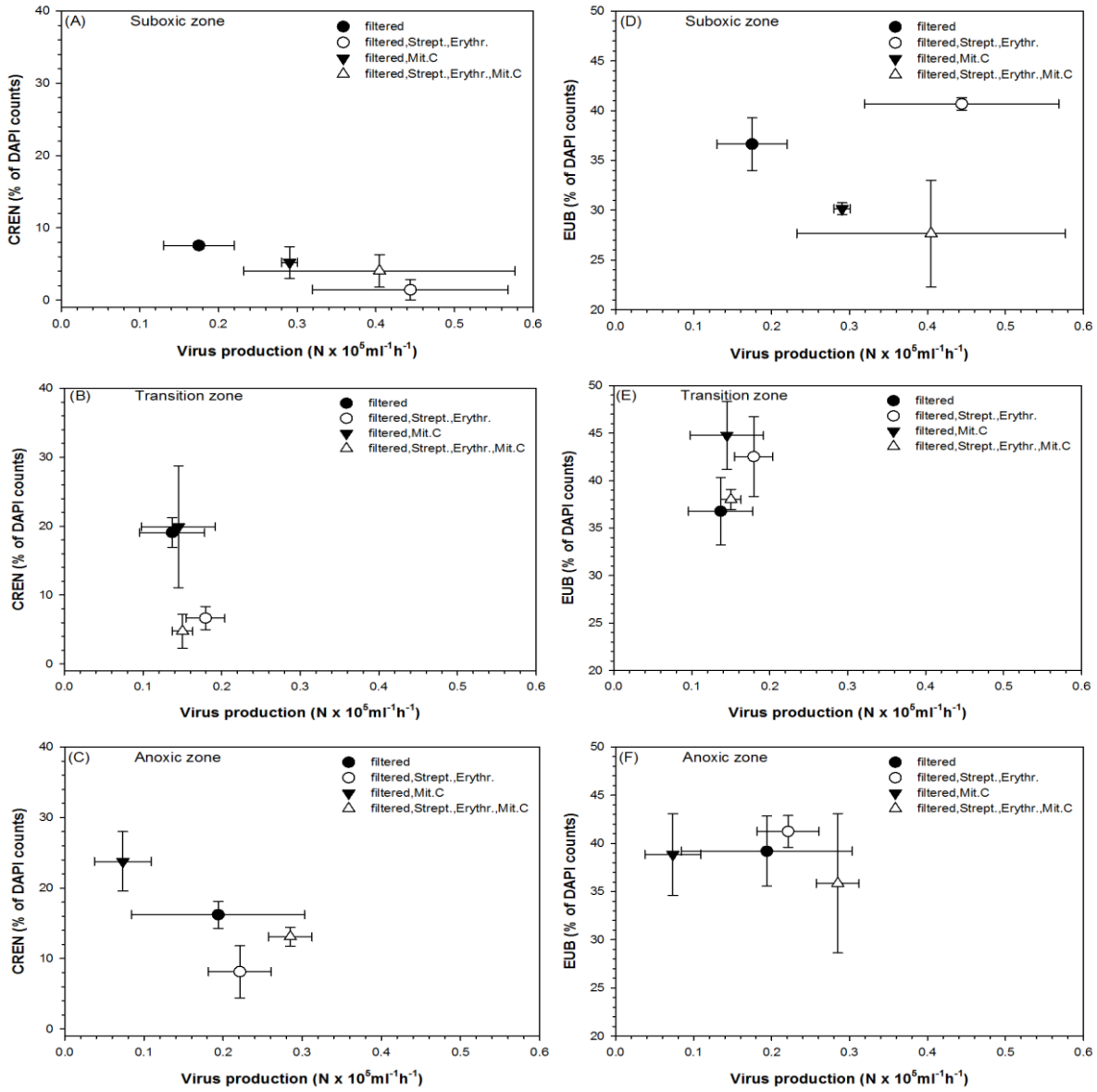


Fig. 4. Relationship between virus production and relative abundance of *Crenarchaeota* (A-C) and *Bacteria* (D-F) in the suboxic, transition, and anoxic zone of the experimental treatments *filt*, $filt_{MitC}$, $filt_{antib}$ and $filt_{antib,MitC}$. The data are average values and the error bars represent the ranges.

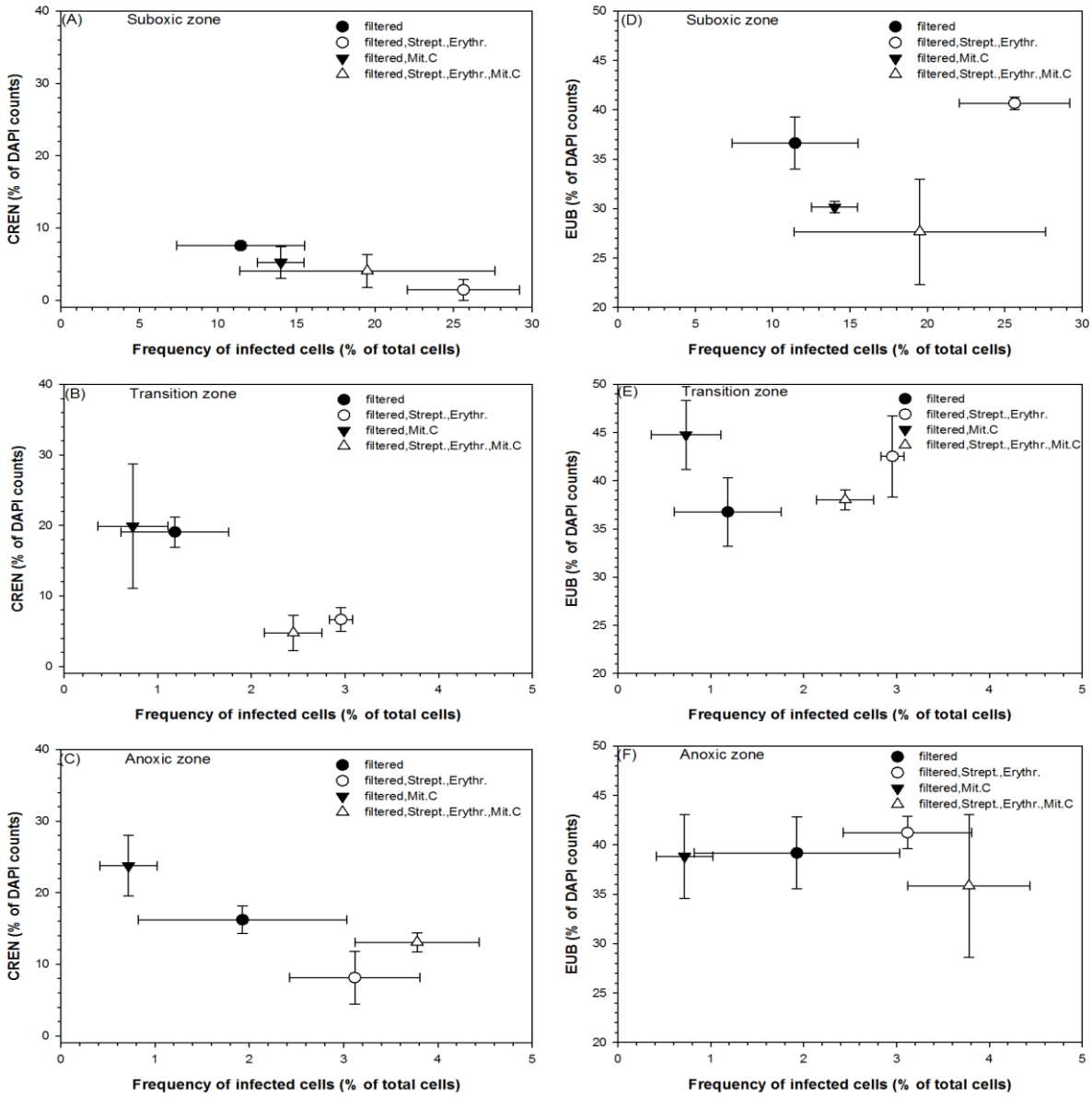


Fig. 5. Relationship between frequency of infected cells and relative abundance of *Crenarchaeota* (A-C) and *Bacteria* (D-F) in the suboxic, transition, and anoxic zone of the experimental treatments *filt*, $filt_{MitC}$, $filt_{antib}$ and $filt_{antib,MitC}$. The data are average values and the error bars represent the ranges.

Supplementary Information

Table S1. Vertical zonation of the water column at the Gotland Deep in the central Baltic Sea.

Zone	Oxygen	Hydrogen sulfide	Depth of sampling
Oxic zone	> 30 µ M	-	-
Suboxic zone	< 30 µ M	-	80 m
Transition zone	< 4 µ M	< 2 µ M	90 m
Anoxic zone	n.d.	> 2 µ M (< 20 µ M)	95 m

Table S2. Extended protocol for determining prokaryotic and viral abundance.

Process	Method and Notes
Fixation samples	Glutaraldehyde (25%); 1.8 mL sample + 36 µL Glutaraldehyde; 0.5 % final concentration
Storage samples	Flash frozen in liquid nitrogen; storage at -80°C
Preparation TE buffer	5 mL 1M Tris-HCL + 1 mL 0.5M EDTA + 494 mL Milli-Q Filtration of 60-120 mL daily (before use) with filter syringe (Terumo Syringe, 50 mL; Acrodisc 0.2 µm)
Preparation SYBR Green I	Storage at -20°C; dilution in Milli-Q (1:200); sterile filtration with filter syringe (Acrodisc 0.2 µm); kept in dark
Measurement blanks	1000 µL TE buffer + 10 µL SYBR Green I; measured like samples with every newly prepared TE buffer
Measurement flow rate	Run of Milli-Q for 10 min at flow rate 2, 5 and 9; weigh Milli-Q before and after measurement; calculation of flow rate in µL min^{-1}
Preparation samples	10 min of thawing in warm water bath; triplicates; dilution in TE buffer (1:2, 1:5, 1:10 or 1:50) to 500 µL + 5 µL SYBR Green I (final volume of 505 µL)
Incubation samples	Prokaryotes: 10 min in dark Viruses: 10 min at 80°C; cooling for 5 min Heating at 80°C enhances the staining efficiency of viruses (Brussaard et al., 2010; Marie et al., 1999)
Measurement samples	1 min; 200-1000 events s^{-1}; flow mode 1-7; > 10 000 events/ sample; measured on BD FACSAria II

Table S3. Extended protocol for determining relative abundance of *Bacteria*, *Crenarchaeota*, and *Sulfurimonas* ssp. (Teira et al., 2004).

Step	Details about filter treatment
Embedding	0.1 % [wt/vol] Agarose; 37 °C hybridization oven (15 min); dehydration in 99 % (vol/vol) ethanol; drying; cut filters
Permeabilization	Incubation with either lysozyme (10 mg/mL) for *Bacteria* and *Sulfurimonas ssp.* or with proteinase K (10.9 mg/mL) for *Crenarchaeota* in 10 mL solution (0.1 Tris HCL, 0.05 EDTA, Milli-Q); 37 °C (1 hour); wash in Milli-Q (1x for lysozyme, 3x for proteinase K); incubation in HCL (0.01 M) (25 min); wash 2x in Milli-Q; dehydration in 99 % ethanol; drying
Hybridization	Hybridization buffer (10 % [wt/vol] Dextran sulfate, 0.9 M NaCl, 20 mM Tris-HCl, 0.05 % Triton X100 – 40-60 °C water bath [30 min.] – cooling – Formamide [CREN: 20 %, EUB, SUL: 55 % vol/vol], 1 % blocking reagent [Boehringer-Mannheim], Sigma Water to final concentration 10 mL); 15 µl of each HRP probe (final volume 300 µl; 1:20); 35 °C (15 – 16 h)
Washing	Washing buffer (20 mM Tris-HCL, 5 mM EDTA, 0.01 % [wt/vol] SDS, NaCl [CREN: 145mM, EUB,SUL: 13mM], Milli-Q [final volume 50 mL]); pre-warming at 37 °C (30 min); filters in washing buffer in 37 °C (15 min)
Amplification	Phosphate-buffered saline (PBS)-Triton-Mix (0.05 % TritonX100, PBS [final volume 50 mL]); incubation in dark (15 min); Amplification buffer (10 % [wt/vol] Dextran Sulfate, 2M NaCl, 0.1 % [wt/vol] blocking reagent, PBS [2950µl]); filter AMP through 0.2 µm Acrodisc; Substrate mix: 493 µl amplification buffer + 5 µl of AMP/H2O2 mix [200 µl AMP/ 1 µl 0.0015 % H2O2] + 5 µl Tyr (Alexa488m); 37 °C hybridization oven (45 min); Drying in dark (20 min); washing in PBS-T-Mix (10 min) - Milli-Q - 99 % ethanol; drying
DAPI- staining	4’,6-diamidino-2-phenylindole (DAPI) [1 µg/ml] in Milli-Q [1:1000]; hybridization in dark (3 min); filters in PBS:VectaShield:Cititfluor Mix (PBS [0.5µg/mL], Vectashield [1µg/mL], Cititfluor [5.5µg/mL] on slides
Counting	Zeiss Axioplan-2 epifluorescence microscope For each filter piece: 20 microscopic fields; counting in 3 x 3 fields of view (12 µm x 12 µm per field) on images

Table S4. Detailed experimental setup.

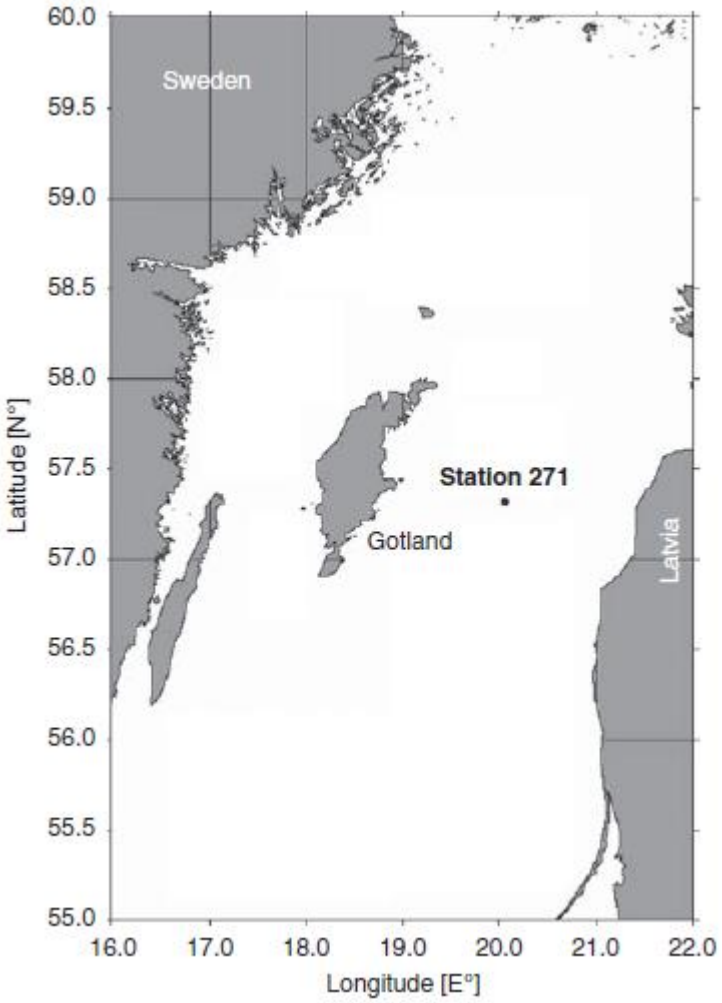
Treatment	Dilution
Unfiltered	50 mL original water
Unfiltered, mitomycin C	50 mL original water + 1 µg / mL mitomycin C
Filtered	45 mL virus-free water + 5 mL prokaryotic concentrate
Filtered, mitomycin C	45 mL virus-free water + 5 mL prokaryotic concentrate + 1 µg / mL mitomycin C
Filtered, streptomycin, erythromycin	45 mL virus-free water + 5 mL prokaryotic concentrate + 100 µg / mL streptomycin + 10 µg / mL erythromycin
Filtered, mitomycin C, streptomycin, erythromycin	45 mL virus-free water + 5 mL prokaryotic concentrate + 1 µg / mL mitomycin C + 100 µg / mL streptomycin + 10 µg / mL erythromycin
Virus concentrate	50 mL virus concentrate

Table S5. Virus production (VP) and frequency of infected cells (FIC) in the experimental treatments taken from the suboxic, transition, and anoxic zone. Values for VP and FIC are expressed as mean values with the range calculated from duplicate incubations.

Treatment	VP (N x 10^5 ml^{-1} h^{-1})		FIC (%)	
	mean	range (±)	mean	range (±)
Suboxic zone				
Unfiltered	**1.2**	0.3	**84**	27.6
Unfiltered,Mit.C	**0.8**	0.4	**41**	21.6
Filtered	**0.2**	0.0	**11**	4.1
Filtered,Mit.C	**0.3**	0.0	**14**	1.5
Filtered,Strept.,Eryth.	**0.4**	0.1	**26**	3.6
Filtered,Strept.,Eryth.,Mit.C	**0.4**	0.2	**20**	8.1
Transition zone				
Unfiltered	**1.7**	0.1	**15**	0.5
Unfiltered,Mit.C	**0.7**	0.0	**7**	0.8
Filtered	**0.1**	0.0	**1**	0.6
Filtered,Mit.C	**0.1**	0.1	**1**	0.4
Filtered,Strept.,Eryth.	**0.2**	0.0	**3**	0.1
Filtered,Strept.,Eryth.,Mit.C	**0.2**	0.0	**2**	0.3
Anoxic zone				
Unfiltered	**0.6**	0.2	**5**	0.7
Unfiltered,Mit.C	**0.9**	0.1	**7**	3.4
Filtered	**0.2**	0.1	**2**	1.1
Filtered,Mit.C	**0.1**	0.0	**1**	0.3
Filtered,Strept.,Eryth.	**0.2**	0.0	**3**	0.7
Filtered,Strept.,Eryth.,Mit.C	**0.3**	0.0	**4**	0.7

Table S6. Relative abundance (% of DAPI-stained cells) of *Bacteria* (EUB), *Crenarchaeota* (CREN), and *Sulfurimonas* ssp. (SUL) in the original water sample and in the treatments from the suboxic, transition, and anoxic zone taken after 40 h incubation time. Values for the relative abundance are expressed as mean values (except original filters) with the ranges calculated from duplicate incubations.

Treatment	**EUB (%)**		**CREN (%)**		**SUL (%)**	
	mean	range (±)	mean	range (±)	mean	range (±)
Suboxic zone						
Original filter	**14**	-	**6**	-	**23**	-
Unfiltered	**44**	4.0	**2**	0.8	**45**	3.3
Unfiltered,Mit.C	**35**	1.5	**10**	5	**38**	2.9
Filtered	**37**	2.6	**8**	0.5	**34**	2.9
Filtered, Mit.C	**30**	0.6	**5**	2.2	**43**	3.4
Filtered,Strept.,Eryth.	**41**	0.6	**1**	1.4	**38**	0.1
Filtered,Strept.,Eryth.,Mit.C	**28**	5.3	**4**	2.2	**39**	2.4
Transition zone						
Original filter	**35**	-	**29**	-	**39**	-
Unfiltered	**37**	2.4	**22**	1.3	**46**	4.1
Unfiltered,Mit.C	**38**	0.4	**27**	2.2	**43**	3
Filtered	**37**	3.6	**19**	2.1	**45**	5.2
Filtered,Mit.C	**45**	3.6	**20**	8.8	**48**	6.5
Filtered,Strept.,Eryth.	**43**	4.2	**7**	1.7	**54**	0.5
Filtered,Strept.,Eryth.,Mit.C	**38**	1.1	**7**	2.5	**48**	4.4
Anoxic zone						
Original filter	**29**	-	**22**	-	**33**	-
Unfiltered	**38**	5.6	**19**	0.7	**33**	2.1
Unfiltered,Mit.C	**39**	5.1	**22**	0.6	**31**	7.0
Filtered	**39**	3.6	**16**	1.9	**41**	2.3
Filtered,Mit.C	**39**	4.3	**24**	4.2	**40**	5.3
Filtered,Strept.,Eryth.	**41**	1.6	**8**	3.7	**52**	3.8
Filtered,Strept.,Eryth.,Mit.C	**36**	7.2	**13**	1.3	**46**	2.0



(Labrenz et al., 2010)

Fig. S1. Location of the sampling site (TF 271) in the Gotland Deep of the central Baltic Sea (57°19.20´N; 20°03´E).

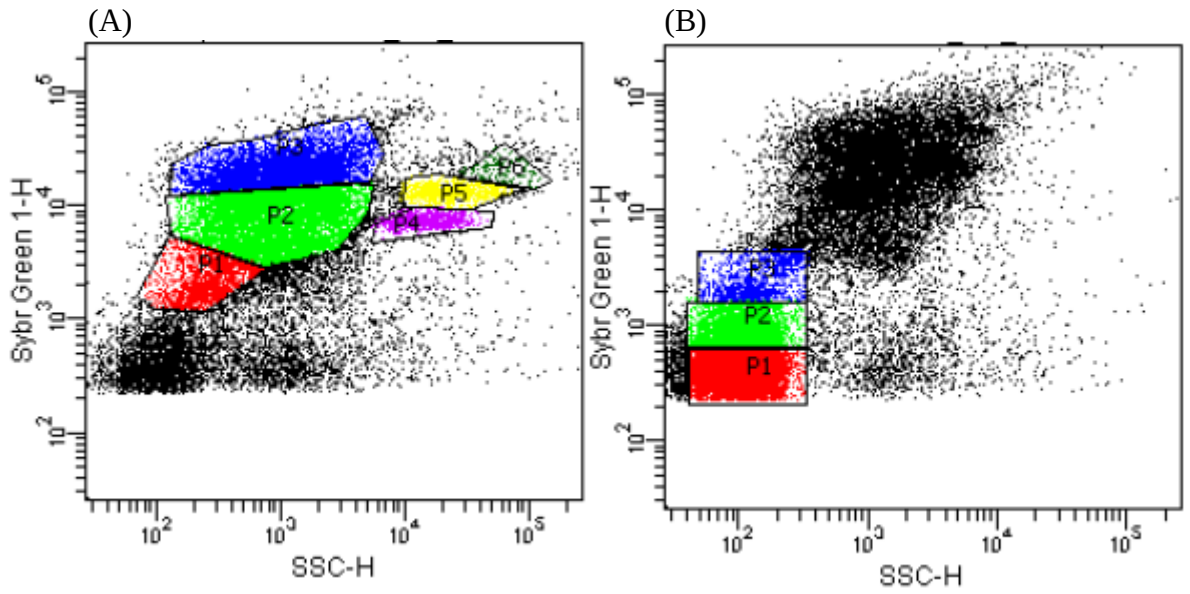


Fig. S2. Typical examples of cytograms with side-scatter versus SYBR Green I fluorescence for (A) prokaryotes and (B) viruses.

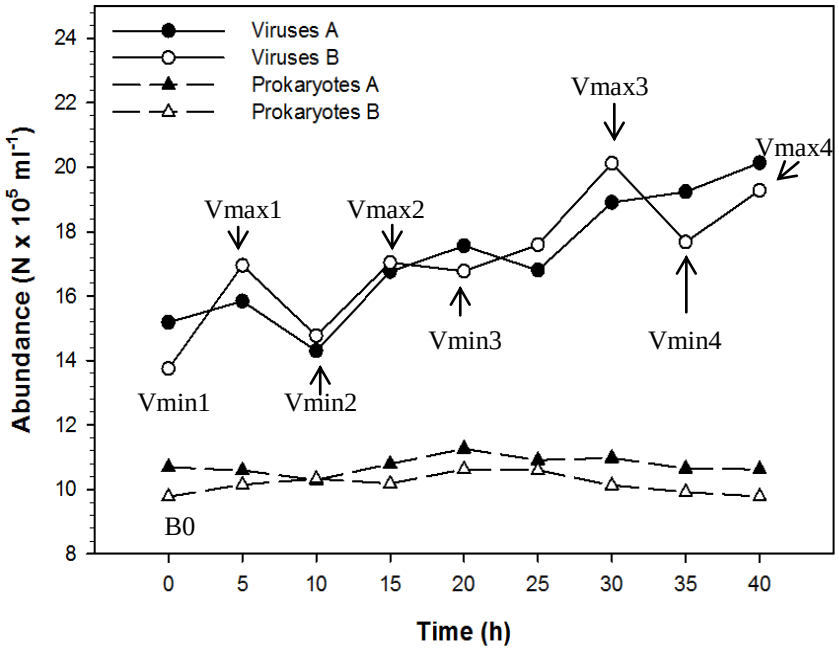


Fig. S3. Example of viral and prokaryotic abundance in an experimental incubation treated with the antibiotics streptomycin and erythromycin taken from the anoxic zone. A and B denote duplicate incubations. Minima and maxima used to calculate FIC and VP are indicated by arrows.

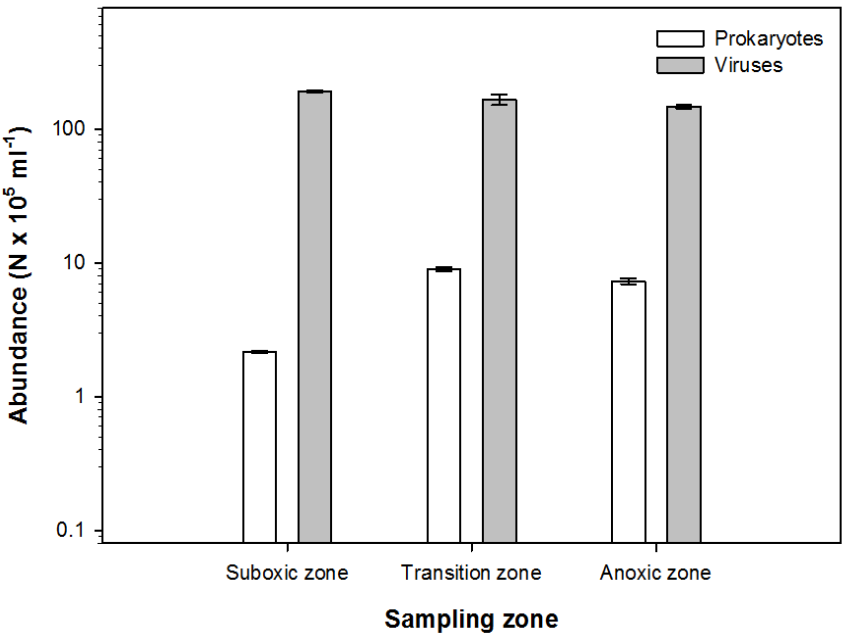


Fig. S4. Abundance of prokaryotes and viruses (log scale) in the suboxic, transition, and anoxic zone of the Gotland Deep. Data are average values of triplicate measurements and error bars represent standard deviations.

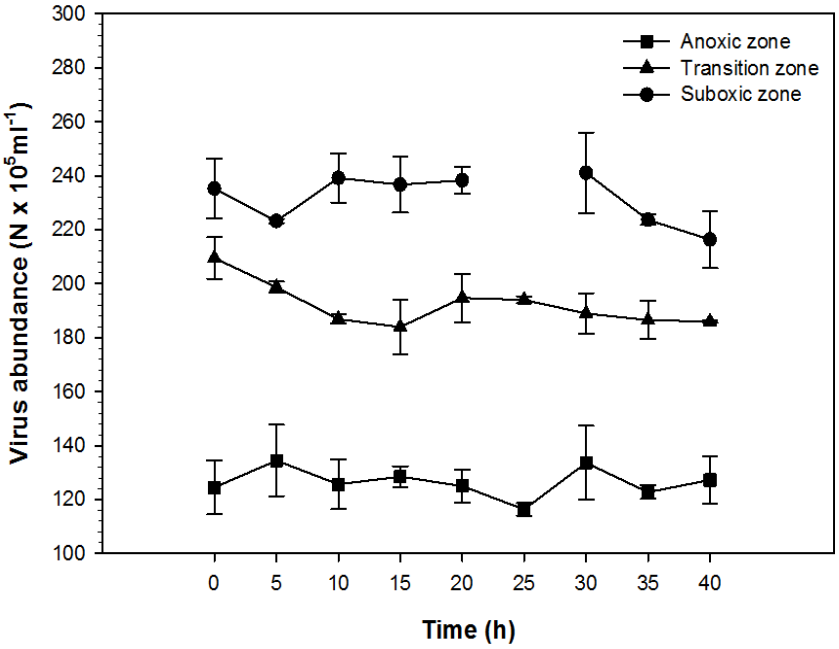


Fig. S5. Virus abundance in the suboxic, transition, and anoxic zone to estimate virus decay over a 40 h incubation period. Data are average values of duplicate incubations and bars represent the ranges.

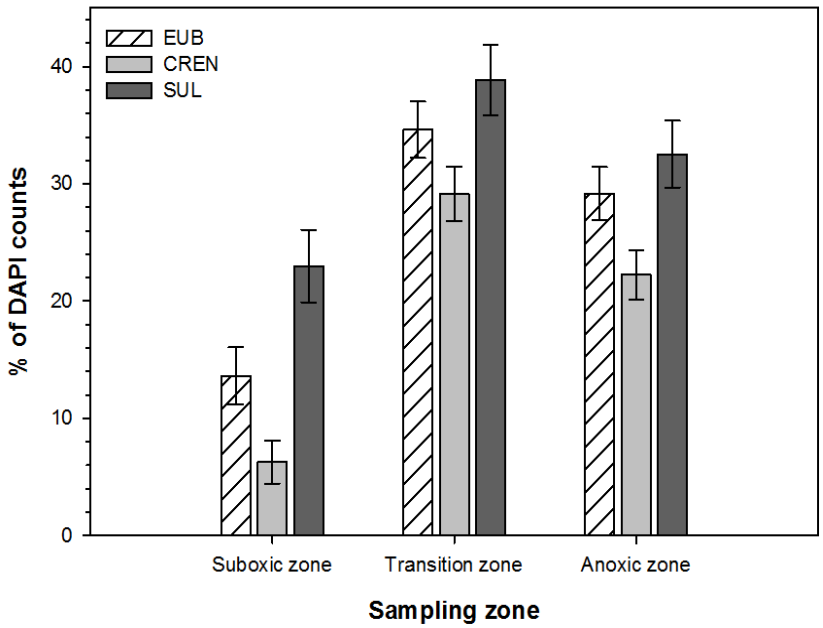


Fig. S6. Relative abundance of *Bacteria* (EUB), *Crenarchaeota* (CREN), and *Sulfurimonas* ssp. (SUL) in the suboxic, transition and anoxic zone. Bars represent the standard errors.

Summary

Viruses are the most abundant entity in marine environments with an abundance ranging from 10^5 ml^{-1} to 10^8 ml^{-1}. Over the last two decades, substantial progress was made elucidating the ecological role of viruses in marine environments. After injection of the viral nucleic acid, a lytic virus forces the host cell to produce new viruses, whereas lysogenic viruses integrate their nucleic acid into the genome of the host and are reproduced together with the host cell until induction of the lytic cycle. In the lytic cycle, the host cell bursts and viral progenies are released into the environment. This cell lysis is an important mechanism, releasing organic material from the host cytoplasm into the environment. Released particulate organic matter (POM) is grazed by higher trophic levels, whereas dissolved organic matter (DOM) is taken up by prokaryotes. Thus, viral lysis contributes, besides the production of viral progeny, to the dissolved organic carbon (DOC) pool and provides a positive feedback loop for heterotrophic prokaryotic growth.

This study focused on the impact of viruses on prokaryotes throughout the redoxcline of the Gotland Deep in the central Baltic Sea. The Baltic Sea is a large brackish environment with a stable halocline, leading to strong stratification with anoxic bottom waters. The frequency of infected cells (FIC) and virus production (VP) was determined in the suboxic, transition, and anoxic zone. The lytic cycle in lysogens (bacteria containing a viral genome) was induced with mitomycin C. However, no lysogenic viruses were detected. FIC was highest in the suboxic zone, with up to 11 % of prokaryotic abundance. Protistan grazing pressure in the anoxic zone seems to be low thus, we expected high FIC in the anoxic zone to explain prokaryotic mortality due to viral lysis. Contrary to our expectations, low FIC was detected in the anoxic zone and consequently, the major loss factors for prokaryotes in the anoxic zone remain unknown. To determine the contribution of *Archaea* to the FIC and VP, we inhibited *Bacteria* with the antibiotics streptomycin and erythromycin. We could show that most of the *Bacteria* were inhibited and that high FIC and VP result from *Crenarchaeota*. We also compared the virus-dilution approach with the previously used approach using undiluted waters to estimate FIC. The virus-dilution method is used to prevent new infections during an experiment through free viruses in the surrounding water. Thus, detected FIC and VP can be attributed to infections occurring before the onset of the experiment. Higher FIC and VP in our unfiltered treatments revealed that the virus-dilution approach is crucial to prevent overestimating FIC and VP in this environment.

Zusammenfassung

Viren stellen mit einer Abundanz von 10^5 ml^{-1} bis zu 10^8 ml^{-1} die zahlenmäßig größte Einheit in marinen Lebensräumen dar. Während der letzten zwei Jahrzehnte wuchsen das Wissen über die große Bedeutung von Viren, über ihren Einfluss auf ökologische Prozesse und mikrobielle Gemeinschaften im Meer. Ein lytischer Virus zwingt die Zelle des Wirts, nach erfolgreicher Injektion der viralen Nukleinsäure, neue Viren zu produzieren. Lysogene Viren hingegen integrieren ihre injizierte Nukleinsäure in das Genom des Wirts und können dort über Generationen hinweg gemeinsam mit dem Genom des Wirts reproduziert werden, bis der lytische Zyklus induziert wird. Im lytischen Zyklus lysiert die Wirtszelle und die neuen Viren werden in die Umgebung entlassen. Diese Lyse der Zelle, in diesem Fall eine Prokaryotenzelle, entlässt zusätzlich zu den Virennachkommen partikuläres organisches Material (engl. particulate organic material = POM), welches von Organismen aus höheren trophischen Ebenen aufgenommen werden kann, und gelöstes organisches Material (engl. dissolved organic material = DOM), welches von anderen Prokaryoten genutzt und somit wiederverwertet wird. Daher ist die Lyse durch Viren wichtig um gelösten organischen Kohlenstoff (engl. dissolved organic carbon = DOC) zum ursprünglichen DOC Pool zurück zu transportieren, wo das Material erneut von Prokaryoten aufgenommen werden kann.

Diese Arbeit konzentrierte sich auf den Einfluss von Viren auf Prokaryoten in der Redoxkline des Gotlandtiefs in der Ostsee. Die Ostsee ist ein Brackwassermeer mit einer permanenten Halokline, welche zu starker Schichtung und sauerstoffarmen bzw. anoxischen Tiefenwasser führt. Die Frequenz von infizierten Zellen (engl. frequency of infected cells = FIC) und die Virenproduktion (engl. virus production = VP) wurden in der suboxischen, anoxischen und in der oxisch-anoxischen Übergangszone ermittelt. Der lytische Zyklus in Lysogenen (Bakterien die ein virales Genom enthalten) wurde durch Mitomycin C induziert. Es konnten jedoch keine lysogenen Viren detektiert werden. Mit 11 % war die FIC am höchsten in der suboxischen Zone. Laut einer früheren Studie gibt es in der anoxischen Zone nur einen sehr geringen Fraßdruck durch Protisten. Daher wurde bei Beginn unserer Studie davon ausgegangen, dass Viren vor allem in dieser Zone einen sehr wichtigen Mortalitätsfaktor darstellen. Jedoch war die FIC in der anoxischen Zone relativ gering, weshalb Lyse durch Viren dort keine große Rolle zu spielen scheint. Somit bleibt die Frage nach dem Hauptmortalitätsfaktor in der anoxischen Zone ungeklärt. Um FIC und VP von *Archaeen* zu bestimmen, wurden *Bakterien* mit den Antibiotika Streptomycin und

Erythromycin inhibiert. Dabei konnten wir zeigen, dass *Crenarchaeen* einen signifikanten Anteil an FIC und VP im Bereich der Redoxkline der Ostsee haben.

Ein weiterer wichtiger Punkt in dieser Arbeit war es, die Notwendigkeit des Viren-Reduktions-Ansatzes zu testen. Diese Verdünnungs-Methode wird in einem Experiment verwendet um Infektionen durch freie Viren im umgebenden Wasser zu verhindern. Daher kann man davon ausgehen, dass die am Ende des Experiments ermittelte FIC und VP von Zellen stammen die bereits vor Beginn des Experiments infiziert waren. Im Vergleich von Viren-Reduktions- Ansatz und dem Ansatz mit ungefiltertem Wasser konnten wir zeigen, dass diese Methode unbedingt notwendig ist um korrekte Werte für FIC und VP zu ermitteln.

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CURRICULUM VITAE

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Education

1993 – 1997	Primary school in Losenstein/ Upper Austria
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2005 – 2010	Bachelor study of Biology with specification on "Biodiversity and Ecology" at the Karl-Franzens-University of Graz
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Work experience

2009	Intern at Marine School in Pula/ Croatia
2010	Intern and assistant at Marine School in Pula/ Croatia
2011 – 2013	Course instructor at Marine School in Pula/ Croatia
2013	Tutor at University of Vienna for the course "Introduction in fauna and flora of marine habitats" in Rovinj/ Croatia

Skills

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