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MASTERARBEIT

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„The influence of mixing of water masses on viral and prokaryotic communities“

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Abstract

In this study the influence of water mass mixing on prokaryotic and viral abundance, viral production, the frequency of infected cells, virus-to-prokaryote ratio, and bacterial, archaeal and viral community composition was investigated at three locations in the deep north Atlantic. Prokaryotic concentrates of two distinct water masses were incubated in virus-free seawater of both water masses for 72 h resulting in a control and a transplant treatment for each prokaryotic concentrate at each location. Prokaryotic and viral abundance of in situ samples were similar to abundances reported earlier except in Antarctic Bottom Water at station 17, where prokaryotic abundance was lower while viral abundance was higher than reported previously. In the experiments, prokaryotic abundance did not differ between control and transplant treatments in contrast to viral production, the frequency of infected cells and the virus-to-prokaryote ratio. In experiments, elevated values of viral abundance, virus-to-prokaryote ratio, viral production and frequency of infected cells were found when prokaryotes were incubated in the same virus-free seawater regardless of the prokaryotic concentrate. Therefore, differences in resource availability, which are modulating prokaryotic growth rate, seem to be the main reason for differences in viral abundance, virus-to-prokaryote ratio, viral production and frequency of infected cells. Few significant links and relations were found between community compositions of prokaryotes and viruses suggesting that stochastic processes are mainly responsible for determining prokaryotic and viral community composition in water masses undergoing mixing.

Introduction

Viruses are the greatest genetic reservoir on Earth (Breitbart et al. 2002; Pedulla et al. 2003; Rohwer 2003; Hambly & Suttle 2005; Suttle 2005). Although viruses are mainly recognized as pathogens (Wilhelm & Suttle 1999; Lopez et al. 2003; Ortmann & Suttle 2005; Haller & Weber 2007), it is well known that some viruses provide advantages for their hosts depending on the reproduction strategy (Mann et al. 2003; Villarreal 2007).

The two main reproductive strategies of viruses infecting prokaryotes are the lytic and the lysogenic cycle (Weinbauer et al. 2003; Weinbauer 2004). Lytic viruses infect their host, force the host cell to produce viral progeny until the host cell is lysed to release virions (Fuhrman & Suttle 1993; Weinbauer et al. 2003). Virions are infective viral particles outside the host cell regardless of the reproduction strategy (Weinbauer 2004). Lysogenic viruses infect their host and integrate their genome in the host genome, called prophage (Weinbauer 2004). The reproduction of a prophage is synchronized with the reproduction of the host until the prophage is induced and enters the lytic cycle (Cochran et al. 1998; Weinbauer 2004). Typical inducing agents are UV-radiation, environmental pollutants, mytomycin C or natural environmental stressors (Jiang & Paul 1996; Cochran et al. 1998; Paul 2008). The prophage is a symbiont of its host. Benefits for the virus are protection from viral decay and survival during low host abundance (Paul 2008). Benefits for the hosts are silencing of unneeded genes through phage repressors or interruption due to insertion of the prophage, anchor points for rearrangement of the host genome, immunity to infections of related phages, lysis of related strains and fitness factors (Paul 2008). Fitness factors or morons are genes encoded by a prophage which are not necessary for the reproduction of the virus e.g. genes coding for toxins in several human pathogens as *Vibrio cholera, Staphylococcus aureus* or *Clostridium botulinum* (Brussow et al. 2004).

Viral lysis is an important factor influencing prokaryotic community composition and biogeochemical processes in aquatic ecosystems as viral lysis represents a substantial prokaryotic mortality factor (Fuhrman 1999; Wilhelm & Suttle 1999; Weinbauer 2004). Viral lysis reduces the carbon flux to higher trophic levels by killing prokaryotic cells, resulting in release of dissolved organic carbon into the environment, a mechanism known as viral shunt (Wilhelm & Suttle 1999). Released lysates can be taken up by non-lysed prokaryotes (Motegi et al. 2009). To understand the influence of lytic viruses on prokaryotic communities, it is crucial to consider that host encounter is a stochastic process. Virions are non-motile entities depending on Brownian motion for host encounter. Hence, high abundance, high motility, and large cell size of the host increase the contact rate of virions with their host

(Murray & Jackson 1992). Viral host specificity supports diversity in stable environments by providing lysates from competition specialists as substrate to defense specialists, as predicted by the killing the winner hypothesis (Thingstad & Lignell 1997; Winter et al. 2010). Competition specialists are most efficient in gathering nutrients from the environment and therefore, they are expected to dominate in oligotrophic environments, but are susceptible to viral infection (Winter et al. 2010). In contrast, the defense specialist is resistant to viral infections but less efficient in gathering nutrients from the environment, which would be an advantage if viral abundance is high (Winter et al. 2010). Viral production (VP), measured as viruses produced per volume and time, is an important parameter in determining the ecology of viruses (Wommack & Colwell 2000; Wilhelm et al. 2002; Weinbauer 2004). VP increases with the growth rate of the host resulting in an increase in the number of released virions and a decrease in the latent period (Middelboe 2000).

Lysogenic viral production is determined as the differences in VP between untreated samples (control) and samples treated with an inducing agent, mostly mytomycin C (Paul 2008). High proportions of lysogenic infections are associated with low host abundance and low host growth rates (Williamson et al. 2002; Weinbauer et al. 2003; Payet & Suttle 2013) whereas a prevalence of lytic viruses is found when host growth rates are high (Weinbauer et al. 2003; Payet & Suttle 2013).

The aim of this study was to investigate the influence of mixing of water masses on VP and viral community composition in the deep North Atlantic. Intense mixing of water masses in the deep ocean is found due to the topography of the sea floor (Mccartney et al. 1991; Polzin et al. 1996; Muck et al. 2014). Muck et al. (2014) showed that only lytic VP is influenced by mixing of water masses in the Vema Fracture Zone (tropical North Atlantic). In this study, a transplant experiment was performed to investigate differences in abundance, virus-to-prokaryote-ratio (VPR), VP, frequency of infected cells (FIC), and the community composition of *Bacteria*, *Archaea* and viruses when prokaryotes were incubated in virus-free seawater of different water masses of the deep North Atlantic.

Materials & Methods

All methods used in this study are given in more detail in the appendix.

Sampling and experimental set-up

Sampling and subsequent experiments were done by Christian Winter, Nicole Köstner and Simone Muck aboard R/V Pelagia during the MEDEA-I cruise (October – November 2011). Water samples (each 160 L) of two distinct water masses were taken at three stations in the North Atlantic (Fig. 1). Three water masses were sampled, identified by their salinity and temperature characteristics. Antarctic Intermediate Water (AAIW) is characterized by low salinity (Tsuchiya et al. 1992; Tomczak 1999; Kortzinger et al. 2001) in contrast to North Atlantic Deep Water (NADW) which typically has a high salinity (Tomczak & Godfrey 1994). Antarctic Bottom Water (AABW) is found below 4000 m depth and characterized by low temperature and low salinity (Tomczak & Godfrey 1994).

The virus dilution approach was used in this study in which prokaryotes are incubated in virus-free seawater (Wilhelm et al. 2002). The rationale of this approach is to prevent new infections during incubations as host encounter is a stochastic process (Murray & Jackson 1992). Therefore, produced viruses should largely originate from infections prior to the start of the incubation (Wilhelm et al. 2002).

Several filtering steps are necessary for obtaining a prokaryotic concentrate and virus-free seawater is required for the virus dilution approach. Two steps of tangential flow filtration with pore sizes of 0.22 µm and a molecular weight cut-off of 100 kDa were used to obtain a prokaryotic concentrate and virus-free seawater, respectively, from each sample. The prokaryotic concentrate and virus-free seawater were mixed to obtain the different treatments. Each prokaryotic concentrate was incubated in virus-free seawater of the same (control) and the adjacent water mass (transplant treatment), resulting in four treatments per station. All treatments were done in duplicate. Prokaryotic concentrates of a water mass are indicated below with subscript P whereas virus-free seawater of a water mass is indicated with subscript W. Incubations were performed in duplicate in 20 L carboys over a period of 72 h. Subsamples were taken as described below.

Determining the abundances of prokaryotes and viruses

The abundance of prokaryotes and viruses was determined according to Brussaard (2004). Subsamples for abundance measurements of prokaryotes and viruses were taken every 4 h, fixed with glutaraldehyde (2% final concentration), flash-frozen in liquid nitrogen and stored at -80°C[1]. For abundance determinations, subsamples were thawed, diluted with TE buffer and stained with SYBR-Green I. Measurements were performed on a FACSAria II flow cytometer (Becton Dickinson). The abundance of prokaryotes and viruses was determined based on side scatter *vs.* SYBR Green I fluorescence plots.

Determining prokaryotic community composition

Subsamples to determine prokaryotic community composition were taken every 24 h[1]. Subsamples were filtered through GVWP membrane filters with a pore size of 0.22 µm[1]. Filters were put in cryovials, flash-frozen in liquid nitrogen and stored at -80°C until further analysis[1]. Prokaryotic community composition was determined using terminal restriction fragment length polymorphism (TRFLP) analysis (Moeseneder et al. 1999; Moeseneder et al. 2001). Prokaryotic DNA was extracted using an UltraClean Soil DNA Isolation Kit following the protocol of the manufacturer with minor modifications as given in detail in the appendix. PCR was performed using different fluorescently labeled primer pairs for bacterial and archaeal communities. PCR products were purified and digested using the enzyme *Hha*I (New England BioLabs). The length of the resulting fragments was determined using an automated capillary sequencer (Applied Biosystems) with laser-induced fluorescence detection.

Determining viral community composition

Viral community composition was determined using randomly amplified polymorphic DNA – PCR (RAPD-PCR) analysis (Winget & Wommack 2008). Subsamples from incubation experiments were taken every 24 h[1]. Particles larger than 0.22 µm were removed and a viral concentrate of about 50 mL was obtained using a tangential flow filter with a molecular weight cut off of 100 kDa[1]. Viral concentrates were transferred to 50 mL tubes, flash-frozen in liquid nitrogen and stored at -80°C until further analyses[1]. Viral concentrates were thawed and further concentrated to a final volume of about 200 µL using an Amicon

[1] Done by Christian Winter, Nicole Köstner and Simone Muck

Ultra Centrifugal filtering tube with a molecular weight cut-off of 100 kDa. Nucleic acids were extracted using a QIAamp MinElute Virus Spin Kit following the protocol of the manufacturer. Two RAPD-PCRs with primers OPA-13 and CRA-22 (Winget & Wommack 2008) were performed for each sample and PCR products were size-separated by agarose gel electrophoresis.

Statistics

Statistics were done using LibreOffice calc (v.4.1). Additionally, Wolfram Mathematica (v.9) was used to perform Mantel tests. Mean and standard deviation of the *in situ* abundance were calculated. Furthermore, mean and standard deviation of all abundance measurements and VPR of the incubations were calculated for comparing abundance and VPR between treatments. FIC and VP were calculated as described in Winter et al. (2004) using a burst size of 30 for calculating the FIC (Parada et al. 2006). Differences in abundance, VPR, VP, and FIC were assumed to be significant when the range of duplicates did not overlap. Spearman rank correlation coefficients of distance matrices were used as the Mantel statistic (r_M). Jaccard distance matrices were calculated from presence-absence matrices. Vectors of the upper non-diagonal values of distance matrices were used for calculating r_M. The p-value was calculated for each Mantel test based on 10,000 bootstrap replicates. The level of significance was defined as $p \leq 0.05$. Graphics were created using GraphPad Prism (v.6).

Results

Abundance of prokaryotes and viruses

Viral abundance was about one order of magnitude higher than prokaryotic abundance *in situ* and in the experiments. The prokaryotic abundance in the water column at various stations and in different water masses is given in Tab. 1. Prokaryotic abundance varied between 2.7×10^4 mL^{-1} in AAIW at Station 12 and 0.9×10^4 mL^{-1} in AABW at Station 17. Highest viral abundance was also found in AAIW at Station 12 (5.4×10^5 mL^{-1}). Lowest viral abundance (3.0×10^5 mL^{-1}) was found in AABW at Station 21. VPR varied between 19 in the NADW at Station 12 and 56 at Station 17. The prokaryotic and viral abundance in the experiments are given in Tab. 2. The standard deviation was higher for viral than for prokar-

yotic abundance. Prokaryotic abundances did not differ between control and transplant treatments. Highest prokaryotic abundance was found in $AAIW_P$ treatments of Experiment 1 (7.8×10^3 mL^{-1} to 7.9×10^3 mL^{-1}) whereas lowest values were found in the $AABW_P$ treatments of Experiment 2 (3.1×10^3 mL^{-1} to 3.2×10^3 mL^{-1}). For both prokaryotic concentrates, viral abundance was higher in $AAIW_W$ than in $NADW_W$ treatments in Experiment 1. In Experiment 2 and 3, viral abundance was higher in $NADW_W$ than in $AABW_{W'}$ treatments regardless of the prokaryotic concentrate. Differences in abundance between control and transplant treatments were higher in Experiment 1 than in Experiment 2 and 3. VPR exhibited a similar pattern as viral abundance and is presented in Fig. 2. The highest value of VPR was found in the $NADW_P$/$AAIW_W$ treatment of experiment 1 (22.8). The lowest values were found in the $AABW_P$/$AABW_W$ treatment of Experiment 3 (8). Differences in VPR were higher between $AAIW_W$ and $NADW_W$ than between $NADW_W$ and $AABW_W$ treatments (Fig. 2).

Viral production

Results on VP in the experiments are shown in Fig. 4. VP was highest in $AAIW_W$ treatments of Experiment 1 regardless of the prokaryotic concentrate. When prokaryotic concentrates were incubated in virus-free seawater of different water masses, VP differed significantly between control and transplant treatments over 32 h in 5 out of 6 cases. VP was lower in $NADW_W$ treatments in 4 out of these 5 cases with significant differences between control and transplant treatments. After 72 h, VP significantly differed in all cases between control and transplant treatments. In Experiment 1 and 2, VP was lower when prokaryotes were incubated in $NADW_W$. In Experiment 3, VP was higher in $NADW_W$ than in $AABW_W$ treatments (Fig. 4).

Frequency of infected cells

Results on the FIC are shown in Fig. 5. Highest values of FIC were found in $AAIW_W$ treatments. Within the first 32 h of incubation, the FIC significantly differed between control and transplant incubations in 4 out of 6 cases. FIC was always lower in $NADW_W$ treatments than in $AAIW_W$ or $AABW_W$ treatments. No significant differences were found in $NADW_P$ treatments of Experiment 2 and $AABW_P$ treatments of Experiment 3.

Calculating the FIC for the whole incubation period of 72 h revealed lower values of FIC in $NADW_W$ than in $AAIW_W$ treatments in Experiment 1. In $AABW_P$ treatments of Experiment 2 FIC was lower in $NADW_W$ than in $AABW_W$ when calculated for the whole incubation period. In Experiment 3 FIC was higher in $NADW_W$ than in $AABW_W$ treatments.

After 32 h of incubation no differences in FIC between control and transplant treatments were found in $NADW_P$ treatments of Experiment 2.

Community composition of *Bacteria, Archaea* and viruses

No significant relationships were found between duplicates. Significant links between the community compositions of the different treatments are presented in Table 3. In Experiment 1, the community composition obtained with the reverse bacterial primer was linked between the $NADW_P/NADW_W$ and $NADW_P/AAIW_W$ (r_M= 0.6) and between the $AAIW_P/NADW_W$ and $AAIW_P/AAIW_W$ (r_M= 0.5). In Experiment 2, no significant links were found between treatments. In the $NADW_P/AABW_W$ and $NADW_P/NADW_W$ treatments of Experiment 3, significant links were found between the community composition obtained from the bacterial forward and reverse primer (both r_M= 0.5). Furthermore, the viral community composition obtained by the primer OPA-13 was linked between $AABW_P$ treatments (r_M= 0.6). Significant links between forward and reverse bacterial and archaeal primers as well as between both viral primers are shown in Table 4. Only the community composition obtained with the bacterial primers in Experiment 3 was significantly linked with each other. The few significant links between prokaryotic and viral communities are presented in Table 5.

Discussion

Abundance of prokaryotes and viruses

Prokaryotic and viral abundance and VPR in the different deep-water masses were similar to abundances found in previous studies except in the AABW at Station 17 (De Corte et al. 2010; Muck et al. 2014). In the AABW at Station 17, prokaryotic abundance was lower and viral abundance higher than previously reported (De Corte et al. 2010; Muck et al. 2014). Prokaryotic abundance was not influenced by transplantation in water from a different water mass in contrast to viral abundance and VPR. Viral abundance was related to prokaryotic abundance and heterotrophic production (Middelboe 2000; De Corte et al. 2010). As prokaryotic abundance did not differ between treatments, differences in viral abundance and VPR between treatments with the same prokaryotic concentrate could be explained by differences in host growth rates. Bacterial and consequently, viral production depend on resource availability. Therefore, the results indicate that a more growth limiting resource

exists in $NADW_W$ compared to $AAIW_W$ in Experiment 1. In Experiments 2 and 3, $AABW_W$ is more limited in a resource than $NADW_W$. Taking together all experiments, limitation of resources seem to increase with depth, leading to decreasing viral abundance with depth. However, this should be taken with reservations as no depth profile with several sampling depth were created at a single location.

VP and FIC

VP and FIC were less in the water mass of greater depth in the majority of the significant cases. Host growth rate modulation through resource limitation might be the most probable reason for this pattern. Contradictory to Experiment 1 and 3, VP and FIC increase with depth in all significant cases of Experiment 2. The easiest explanation is that prophages were induced in $AABW_W$ of Experiment 2. This would be consistent with prokaryotic and viral abundance in AABW at station 17 in which prokaryotic abundance was low but viral abundance was untypically high. This was probably caused by lysis of prokaryotes containing a prophage.

Muck et al. (2014) suggest that lytic VP is influenced by mixing of NADW and AABW in the Vema Fracture Zone. However, in contrast to Muck et al. (2014), we found differences in FIC. In most cases elevated FIC and VP were found in the same water regardless of the origin of the prokaryotic concentrate. Therefore, modulation of host growth rate through resource availability seems to be most important factor influencing VP and FIC.

Community composition of *Bacteria*, *Archaea* and viruses

We found no significant relationships between bacterial, archaeal and viral community composition in duplicates or among treatments. A lack of significant relationships between duplicates indicates that treatment effects do not exist or are not strong enough to influence community composition.

Overall, as the majority of the Mantel tests were not significant, stochastic effects seem to be most important for determining prokaryotic and viral community composition in our experiments.

Critical evaluation of FIC and the virus dilution approach

FIC exceeded 100 % in some treatments when calculated for the whole incubation period, which clearly is not possible. Apparently, FIC exceeding 100 % results from inappropriate estimations of the burst size.

In the virus dilution approach the abundance of free viruses should increase steadily during the incubation period. Frequently, however this is not observed. One of the reasons for a non-steady increase in viral abundance and multiple viral abundance peaks in the incubations might be re-infection during the incubation as discussed in Winter et al. (2004). It has been suggested that multiple peaks during incubation appear due to different viral populations (Winter et al. 2004). Viral latent period is related to the generation time of their prokaryotic hosts (Fuhrman & Suttle 1993; Proctor et al. 1993; Binder 1999). Re-infection during the incubation is less likely as it was shown that the generation time for bacteria in oligotrophic environments ranges between 5-15 days (Fuhrman & Suttle 1993).

Reported burst sizes exhibit a large range varying from about 10-80 for bacteria with average of ≈ 30-40 (Weinbauer & Suttle 1999; Hwang & Cho 2002; Parada et al. 2006). Additionally, burst size depends on host growth rates (Middelboe 2000). Taking together all the factors influencing the VP and FIC estimates, it appears that most of the factors determining VP and FIC are poorly constraint.

Conclusion

Taken together, the abundance of prokaryotes and viruses in the deep water masses of the North Atlantic was similar to previous studies from these regions except in AABW at station 17 where prokaryotic abundance was lower and viral abundance higher than previously reported. In experiments prokaryotic abundance was not influenced by the origin of water mass in contrast to viral abundance, VPR, VP and FIC. Differences in VPR, VP, and FIC between control and transplant treatments are likely caused by variations in host growth rates. Few significant links were found between community composition of prokaryotes and viruses indicating that stochastic processes are determining their composition.

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Figures

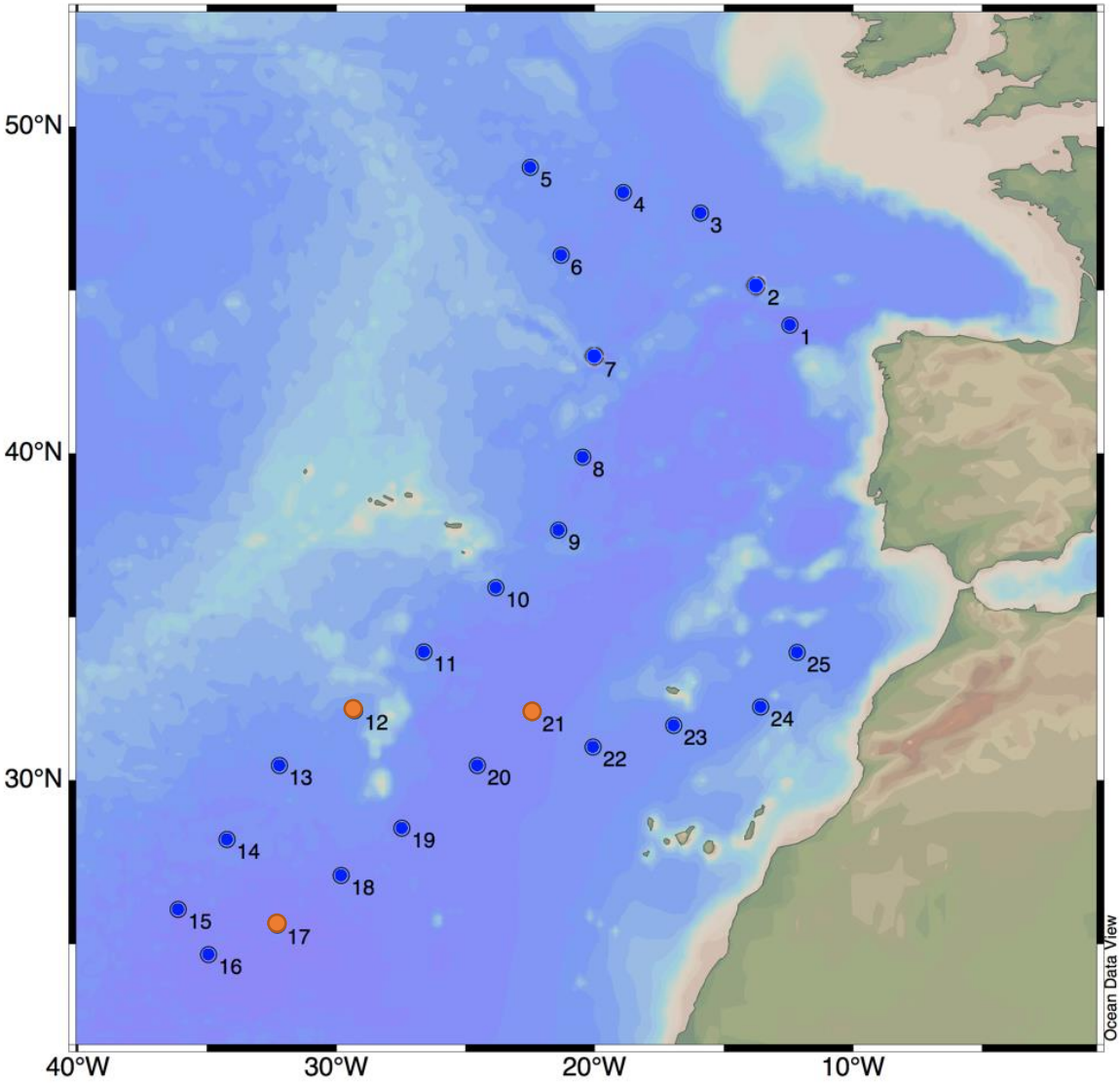


Figure 1: Sampling sites of the MEDEA-I cruise. Red dots indicate the sampling sites for this study.

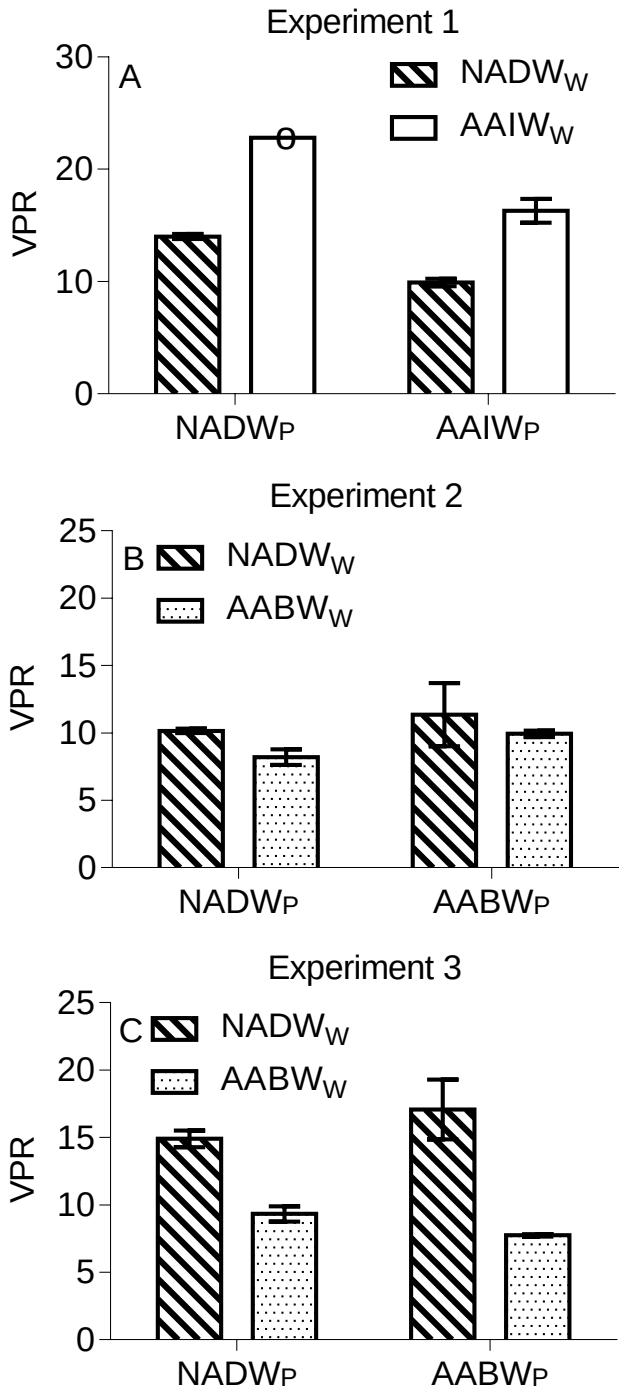


Figure 2: Virus-to-prokaryote ratio in Experiment 1 (A), Experiment 2 (B) and Experiment 3(C). VPR: Virus-to-prokaryote-ratio. NADW$_P$: Prokaryotic concentrate of North Atlantic deep water; NADW$_W$: Virus-free seawater of North Atlantic Deep Water; AAIW$_P$: Prokaryotic concentrate of Antarctic Bottom Water; AAIW$_W$: Virus-free seawater of Antarctic Bottom Water.

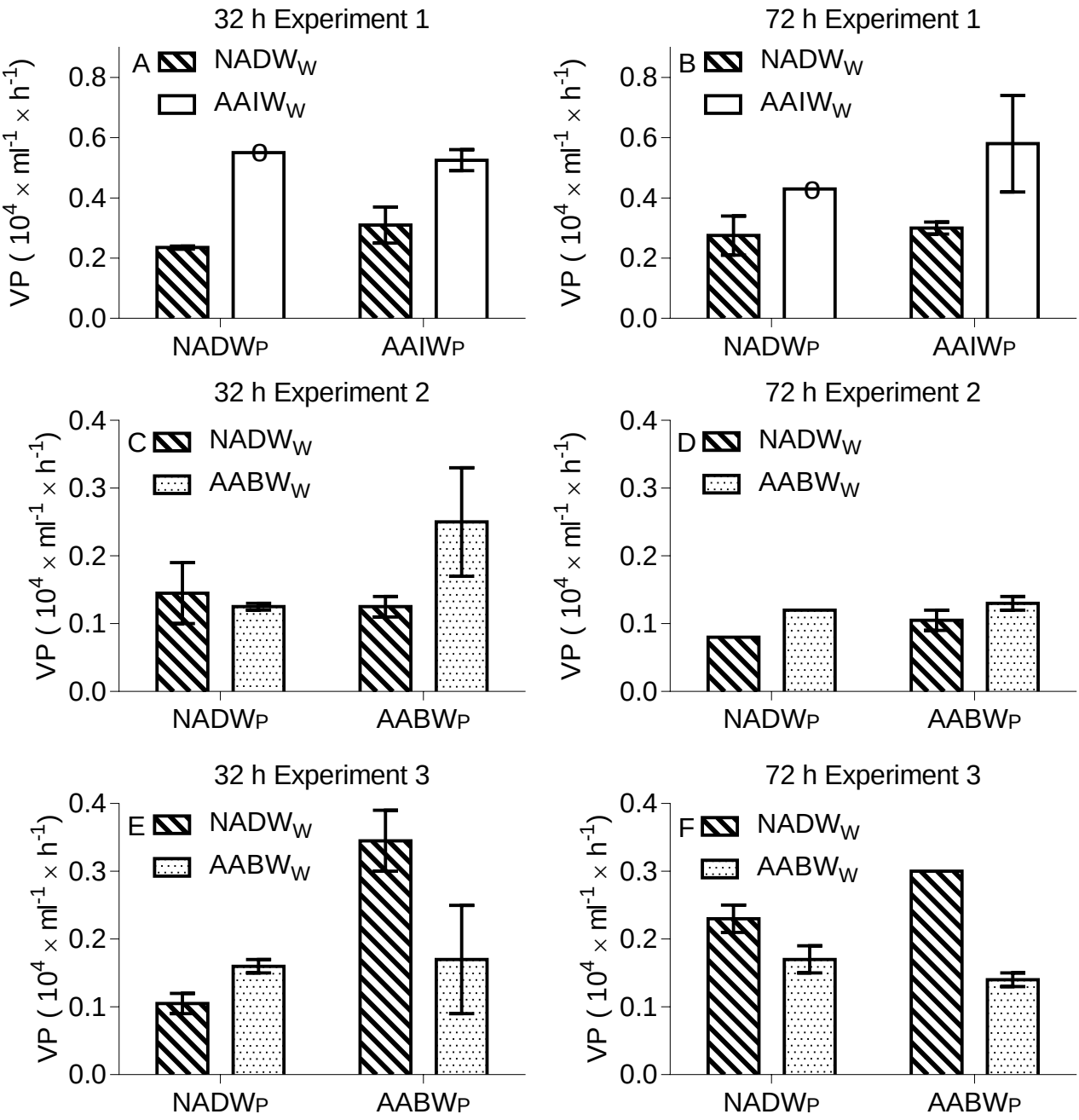


Figure 3: Viral production in Experiment 1 (A, B), Experiment 2 (C, D) and Experiment 3 (E, F) for 32 h (A, C and E) and 72 h (B, D and F). NADW$_P$: Prokaryotic concentrate of North Atlantic Deep Water; NADW$_W$: Virus-free seawater of North Atlantic Deep Water; AAIW$_P$: Prokaryotic concentrate of Antarctic Bottom Water; AAIW$_W$: Virus-free seawater of Antarctic Bottom Water.

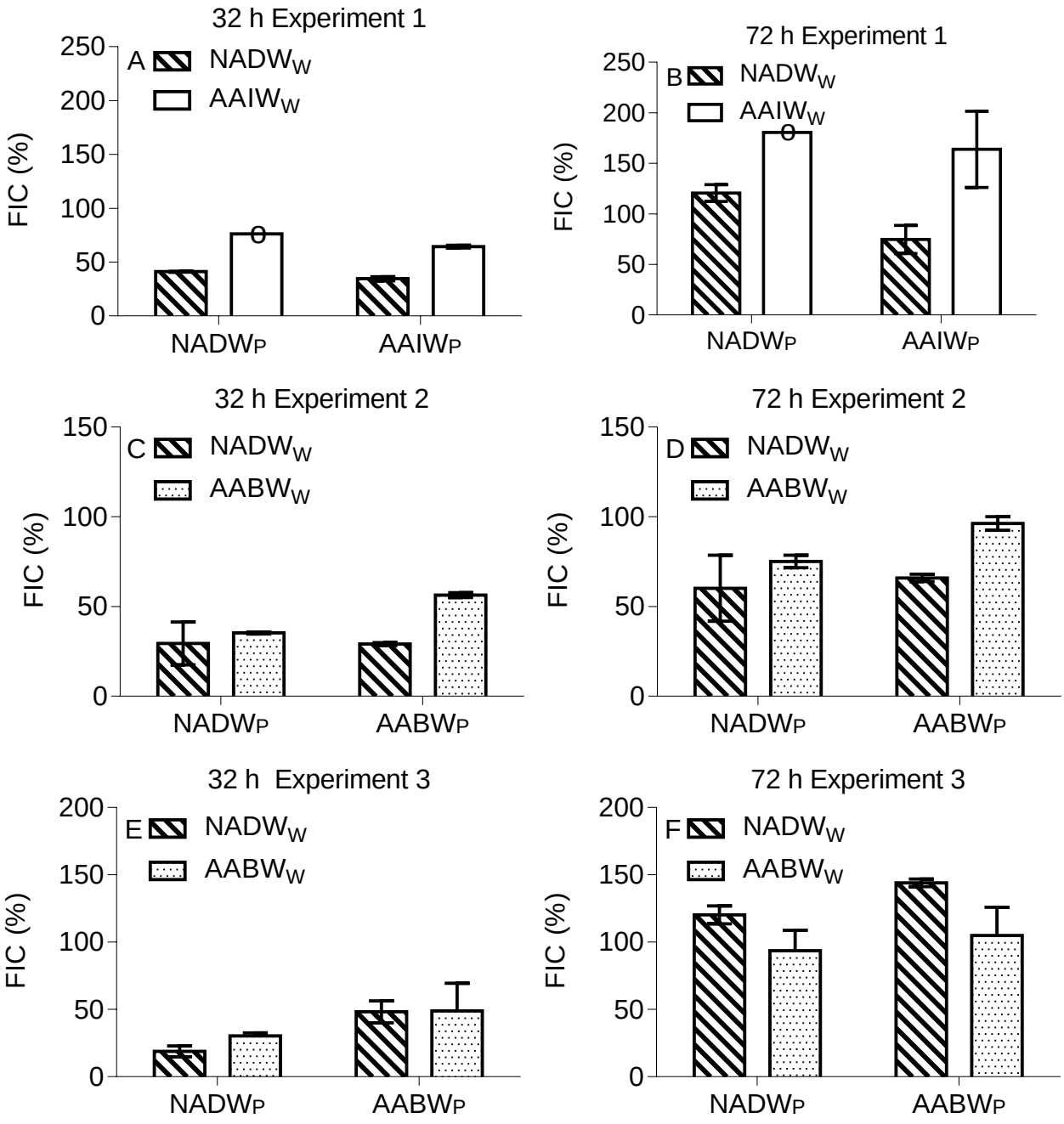


Figure 4: Frequency of infected cells in Experiment 1 (A, B), Experiment 2 (C, D) and Experiment 3 (E, F) for 32 h (A, C and E) and 72 h (B, D and F). FIC: Frequency of infected cells; NADW$_P$: Prokaryotic concentrate of North Atlantic Deep Water; NADW$_W$: Virus-free seawater of North Atlantic Deep Water; AAIW$_P$: Prokaryotic concentrate of Antarctic Bottom Water; AAIW$_W$: Virus-free seawater of Antarctic Bottom Water.

Table 1: Prokaryotic (PA) and viral abundance (VA) in specific depth layers of the North Atlantic. The standard deviation of abundance is given in brackets. VPR: Virus-to-prokaryote ratio; STD: standard deviation, for Station numbers see Fig. 1; AAIW: Antarctic Intermediate Water; NADW: North-Atlantic Deep Water; AABW: Antarctic Bottom Water

Experiment	Station	Depth	Water mass	PA (10^4 mL^{-1})	STD	VA (10^5 mL^{-1})	STD	VPR	STD
1	12	1700	AAIW	2.7	<0.1	5.4	0.4	20.0	1.7
1	12	2750	NADW	1.8	<0.1	3.5	1.0	19.1	5.9
2	17	2750	NADW	1.1	0.1	3.1	0.7	27.0	2.4
2	17	5000	AABW	0.9	<0.1	5.1	2.2	55.7	23.4
3	21	2750	NADW	1.6	0.1	3.7	0.2	23.5	0.9
3	21	5000	AABW	1.1	0.1	3.0	0.4	27.2	3.6

Table 2: Prokaryotic and viral abundances in the different treatments. Mean standard deviation is given for duplicate A and B. Significant differences were assumed when the range of the means of the duplicates do not overlap. X indicates missing values. STD: standard deviation. Subscript P denotes prokaryotes of the specific water mass, subscript W the origin of the virus-free seawater; abbreviations for the water masses are given in Table 1.

	Prokaryotes (10^3 mL^{-1})				Viruses (10^4 mL^{-1})			
Experiment 1	A	STD	B	STD	A	STD	B	STD
$NADW_P/NADW_W$	4.7	0.3	4.8	0.2	6.5	2.2	6.8	1.8
$NADW_P/AAIW_W$	4.8	0.3	X	X	10.9	3.0	X	X
$AAIW_P/NADW_W$	7.8	0.4	7.8	0.4	8.0	2.7	7.5	2.1
$AAIW_P/AAIW_W$	7.9	0.5	7.8	0.3	13.7	3.4	11.8	5.2
Experiment 2	A	STD	B	STD	A	STD	B	STD
$NADW_P/NADW_W$	3.7	0.4	3.8	0.2	3.8	0.6	3.8	1.0
$NADW_P/AABW_W$	3.8	0.5	3.8	0.3	2.9	0.7	3.3	0.8
$AABW_P/NADW_W$	3.2	0.2	3.2	0.2	2.9	0.7	4.4	0.9
$AABW_P/AAIW_W$	3.1	0.3	3.2	0.3	3.2	1.0	3.1	0.9
Experiment 3	A	STD	B	STD	A	STD	B	STD
$NADW_P/NADW_W$	3.8	0.4	3.9	0.3	5.4	1.5	6.0	2.1
$NADW_P/AABW_W$	3.8	0.3	4.0	0.4	3.7	1.3	3.5	1.3
$AABW_P/NADW_W$	3.5	0.4	3.5	0.7	5.2	1.8	6.7	1.5
$AABW_P/AAIW_W$	3.6	0.3	3.3	0.3	2.8	1.0	2.6	1.0

Table 3: Mantel tests (r_M) between treatments. Only significant results are shown. - indicates no significant result, x indicates a not defined Mantel test; abbreviations for the water masses are given in Table 1.

Experiment 1	$NADW_P/NADW_W$	$AABW_P/NADW_W$
	$NADW_P/AAIW_W$	$AABW_P/AAIW_W$
Forward bacteria	-	-
Reverse bacteria	0.6	0.5
Forward archaea	-	-
Reverse archaea	-	X
CRA-22	-	-
OPA-13	-	-
Experiment 2	$NADW_P/NADW_W$	$AABW_P/NADW_W$
	$NADW_P/AABW_W$	$AABW_P/AABW_W$
Forward bacteria	-	-
Reverse bacteria	-	-
Forward archaea	-	-
Reverse archaea	-	-
CRA-22	-	-
OPA-13	-	-
Experiment 3	$NADW_P/NADW_W$	$AABW_P/NADW_W$
	$NADW_P/AABW_W$	$AABW_P/AABW_W$
Forward bacteria	0.5	-
Reverse bacteria	0.5	-
Forward archaea	-	-
Reverse archaea	-	-
CRA-22	-	-
OPA-13	-	0.6

Table 4: Mantel tests (rM) between forward and reverse primers of bacteria and archaea, and between CRA-22 and OPA-13. Only significant results are shown. - indicates no significant results, x not defined Mantel tests. FB: forward bacterial primer; RB: reverse bacterial primer; FA: forward archaeal primer; RA: reverse archaeal primer, CRA: viral primer CRA-22; OPA: viral primer OPA-13; abbreviations for the water masses are given in Table 1.

Experiment 1		$NADW_P$/ $NADW_W$	$NADW_P$/ $AAIW_W$	$AAIW_P$/ $NADW_W$	$AAIW_P$/ $AAIW_W$
FB	RB	-	-	-	-
FA	RA	-	-	X	-
CRA	OPA	-	-	-	-
Experiment 2		$NADW_P$/ $NADW_W$	$NADW_P$/ $AABW_W$	$AABW_P$/ $NADW_W$	$AABW_P$/ $AABW_W$
FB	RB	0.9	-	-	0.8
FA	RA	-	-	-	-
CRA	OPA	-	-	-	-
Experiment 3		$NADW_P$/ $NADW_W$	$NADW_P$/ $AABW_W$	$AABW_P$/ $NADW_W$	$AABW_P$/ $AABW_W$
FB	RB	0.8	0.8	0.5	0.8
FA	RA	-	-	-	-
CRA	OPA	-	0.5	-	-

Table 5: Mantel tests (r_M) between prokaryotic and viral communities. Only significant results are shown. - indicates no significant results, x indicates not defined Mantel tests. FB: forward bacterial primer; RB: reverse bacterial primer; FA: forward archaeal primer; RA: reverse archaeal primer, CRA: viral primer CRA-22; OPA: viral primer OPA-13.

Experiment 1		$NADW_P$/ $NADW_W$	$NADW_P$/ $AAIW_W$	$AAIW_P$/ $NADW_W$	$AAIW_P$/ $AAIW_W$
FB	CRA	-	-	-	-
RB	CRA	-	-	0.6	-
FA	CRA	-	-	-	-
RA	CRA	-	-	X	0.9
FB	OPA	0.5	-	0.5	-
RB	OPA	-	-	-	-
FA	OPA	-	-	-	-
RA	OPA	-	-	x	-
Experiment 2		$NADW_P$/ $NADW_W$	$NADW_P$/ $AABW_W$	$AABW_P$/ $NADW_W$	$AABW_P$/ $AABW_W$
FB	CRA	-	-	-	-
RB	CRA	-	0.5	-	-
FA	CRA	-	0.5	-	0.5
RA	CRA	-	-	-	-
FB	OPA	-	-	-	-
RB	OPA	-	-	-	-
FA	OPA	-	0.6	-	-
RA	OPA	-	-	-	-
Experiment 3		$NADW_P$/ $NADW_W$	$NADW_P$/ $AABW_W$	$AABW_P$/ $NADW_W$	$AABW_P$/ $AABW_W$
FB	CRA	0.5	-	-	-
RB	CRA	0.6	-	-	-
FA	CRA	-	-	-	-
RA	CRA	-	-	-	-
FB	OPA	-	-	-	-
RB	OPA	-	-	-	-
FA	OPA	-	-	-	-
RA	OPA	-	-	-	-

Appendix

Determining prokaryotic and viral abundance

1. TE buffer and SYBR Green I (Invitrogen, Cat.No.: S-7563) were prepared as follows:
    a. For TE buffer 5 mL 1M Tris-HCl and 1 mL 0.5M EDTA were added to 494 mL MilliQ water. Around 50 mL of TE buffer were filtered every day before measuring, using an Acrodisc syringe filter with a pore size of 0.2µm (Pall, Part Number: 4612). TE buffer was stored at room temperature.
    b. 5 µL SYBR Green I were added to 195 µL MilliQ (1:200 of commercially available stock solution). Diluted SYBR Green I was filtered through an Acrodisc syringe filter with a pore size of 0.2µm (Pall, Part Number: 4602). SYBR Green I was kept in dark and stored at -20 °C
2. Before measuring flow rates, the optical set-up of the FACS-Aria II flow cytometer (Becton Dickinson) was checked. Flow rate checks were performed using the weight of MilliQ water before and after 10 min of measuring at flow rate 2, 5, and 9. The resulting data was used for calculating all flow rates. Lasers were checked using one drop of BD™ Cytometer Setup and Tracking Beads (Becton Dickinson, Cat.No.: 641319) in 350 µL MilliQ water. Data acquisition and analyses was performed using FACSDiva software (BD, v.6.1.3).
3. Samples were thawed in warm water. Two tubes with 500 µL TE buffer, 500 µL sample and 10 µl SYBR Green I were prepared, one for viral counts and another one for prokaryotic counts. The tube for viral counts was incubated at 80 °C in the dark for 10 min. Afterwards the tube was cooled down while the prokaryotes were counted. The tube for prokaryotic counts was incubated in the dark at room temperature for 10 min before measurement. The following settings were used for prokaryotic counts: Forward scatter (FSC): 470; Side scatter (SSC): 500; SYBR Green I: 200. For viral abundance measurements the same settings were used for FSC and SSC, but SYBR Green I was set to 570. Depending on the event rate, measurements were done for 1 to 3 min. Each day blanks were measured for 1 min using TE buffer instead of sample.

4. Side scatter versus SYBR Green I Plots were used for determining prokaryotic and viral counts (S1). A threshold of 200 was used for SYBR Green I.
5. Abundance, FIC and VP were calculated using LibreOffice Calc (v. 4.1).
   Abundance were calculated based on the following measurements: $N_{measured}$: Measured events; N_{blank}: Measured events during blank measurement of the used flow rate; FR: flow rate; t: acquisition time; DF: dilution factor; V_{total}: total volume; $V_{measured}$: measured volume. Abundance plots were created for each trial (S2)
   Equoation for abundance based on FACS ARIA II measurements:

$$\text{Abundance} = \frac{N_{measured} - N_{blank}}{FR \times t} \times DF \times \frac{V_{total}}{V_{measured}}$$

6. VP and FIC were calculated according to Winter et al. (2004) using LibreOffice Calc (v.4.1).

$$VP = \frac{((Vmax_1\text{-}Vmin_1)+(Vmax_2+Vmin_2)\ldots+(Vmax_i\text{-}Vmin_i))}{t_{maxi}\text{-}t_{min1}}$$

$$FIC = \left(\frac{((Vmax_1\text{-}Vmin_1)+(Vmax_2\text{-}Vmin_2)\ldots+(Vmax_i\text{-}Vmin_i))/\text{burst size}}{B_0}\right) \times 100$$

TRFLP

1. Extraction of prokaryotic DNA was performed using an UltraClean Soil DNA Isolation Kit (MoBio Laboratories, Cat.No.: 12800-100) following the manufacturer's protocol with the following minor modifications. In step 2 and 4 tubes were shaken 20 times instead of vortexing them. Step 6 was replaced by an alternative lysis method starting with shaking the tubes 20 times and afterwards incubating them at 70°C for 5 min. This was repeated before tubes were shaken another 20 times. This alternative lysis method proofed to reduce shearing. In steps 9 and 12 vortexing was replaced by 5 inversions of the tubes. DNA extracts were stored at -80°C.
2. All DNA extracts were checked. For that, gel electrophoresis of DNA extracts on 1% agarose Gel (Biozym LE Agarose, Art. No.: 840004) was performed for 50 min using a voltage of 75 V. Gels were stained with SYBR Gold (Invitrogen, Cat.No.: S-11494) for 30 min. For Gels and staining of Gels 1x TBE buffer was used (1.34 M Tris, 0.44 M boric acid and 0.025 M EDTA, pH: 8.4). Gels were visualized using a Gel Doc EQ digital gel

imaging system (BioRad) combined with the software Quantity One (v. 4.6.8).

3. PCR was performed using a Master Cycler Pro (Eppendorf, Hamburg, Germany). Different Primer pairs were used for Bacteria and Archaea:
    a. Bacteria:
        i. Forward: 27f-FAM (5’ – AGA GTT TGA TCC TGG CTC AG - 3’)
        ii. Reverse: 1492r-VIC (5’- GGT TAC CTT GTT ACG ACT T – 3’)
    b. Archaea:
        i. Forward: 21f-FAM (5’ – TTC CGG TTG ATC CYG CCG GA – 3’)
        ii. Reverse: 958r-VIC (5’ – YCC GGC GTT GAM TCC AAT T – 3’)

    Both forward primers were labeled on the 5’-end with 6-carboxyfluorescin (FAM) whereas both reverse primers were labeled with the fluorescent dye VIC. A master mix was prepared fresh for each run containing 5 µl 10×Taq buffer (provided with Taq DNA polymerase, containing 200 mM Tris-HCl [pH 8.4] and 500 mM KCl), 4 µl $MgCl_2$ (25 mM, provided with Taq DNA polymerase), 1.25 µl dNTP (10µM each, Invitrogen Cat.No.: 10297-018), 2.5 µl 27F-FAM, 2.5 µl 1492 R-VIC, 0.25 µl Taq DNA polymerase (5 U $µl^{-1}$, Thermo Scientific, Cat.No.:180380018) for each reaction. 1-5 µl prokaryotic nucleic acid extract were added. Tubes were filled up to a total volume of 50 µl using autoclaved and UV-sterilized Milli-Q water. For each run a negative control was performed using autoclaved and UV-sterilized Milli-Q water instead of nucleic acid extract.
4. The PCR program started at 95°C for 5 min to denature DNA. Afterwards 30 cycles followed, each consisting of 1 min at 95°C for denaturation, 1 min at 55°C for annealing and 1 min at 72°C for elongation. A final step at 72°C was performed for completing elongation before cooling down to 4°C until PCR products were removed. PCR products were stored at -20°C.
5. PCR products were checked with a Gel electrophoresis (See 2.)
6. PCR products were purified using a 5Prime PCR Extract Mini Kit (Ref. No. 2300610) following the protocol of the manufacturer.
7. The concentration of Nucleic acids in the purified PCR products were determined using a NanoDrop spectrophotometer.
8. The restriction Enzyme H*ha*I (New England BioLabs, Cat.No.: R0139S) was used for digestion of purified PCR products. For each reaction 1.5 µl CutSmart buffer (provided, containing 50 mM Potassium Acetate, 20mM Tris-acetate, 10mM Magnesium Acetate, 100µg/ml BSA; pH: 7.9 at 25°C), and 0.5µl *Hha*I (20.000 U/ml) were used. Depending on NanoDrop measurements, 1–12 µl PCR product were digested. All tubes were filled

up to a total volume of 15 µl with autoclaved and UV sterilized MiliQ water. Tubes with fresh master mix were prepared before adding the purified PCR product. Digestions were done using a Master Cycler Pro (Eppendorf, Hamburg, Germany) at 37°C for 12 h. Afterwards it was heated up to 65°C for 20 min to inactivate the restriction enzyme before cooling down to 4°C until the digested PCR products were removed from the cycler. Digested PCR products were stored at -20°C.

9. For sequencing the master mix contained 165µl Hi-Di™ Formamide (Applied Biosystems, Cat.No.: 4311320) and 5 µl 1200 LIZ size marker (Applied Biosystems, Part No. 4379950) for 16 samples. The master mix was prepared freshly for each run. 1 µl digested PCR product was added to 10 µl master mix on a plate for the capillary sequencer before denaturation was performed at 95°C for 3 min. Afterwards the plate was put on ice immediately. After cooling down the plate was stored at 4°C until sequencing.
10. An automated capillary sequencer (Applied Biosystems 3130XL) was used for analyses.
11. Data were analyzed using the software Peak Scanner 1.0 (Applied Biosystems). For statistical analysis binary matrices were created.

RAPD-PCR

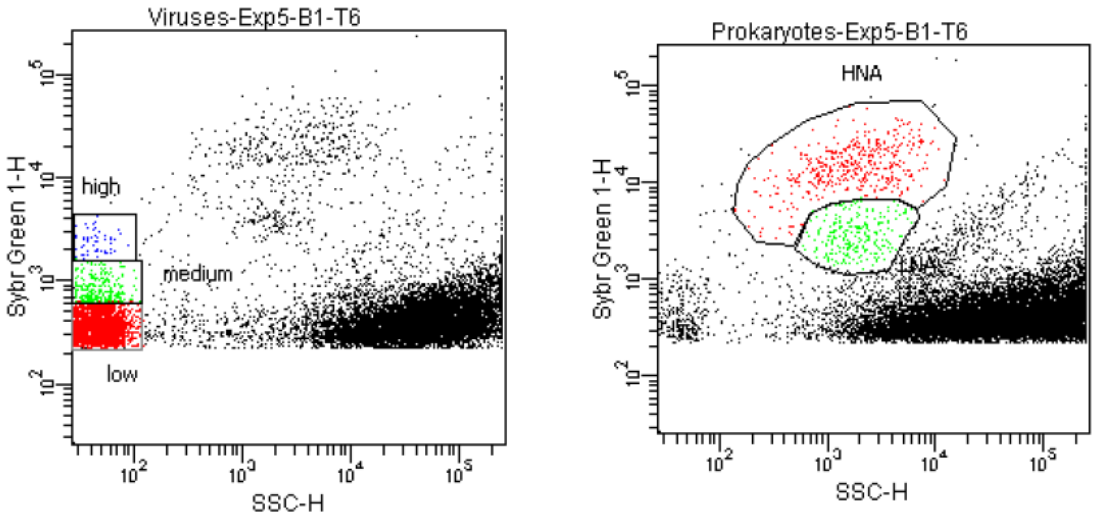
1. Viral concentrates were thawed in water and filtered using an Acrodisc syringe filter with a pore size of 0.2µm (Pall, Part Number: 4612). Further steps were done on ice if possible.
2. Amicon Ultra Centrifugal filtering tubes (100 kDa; Cat. No. UFC 910024) were used to further concentrate viruses to ~ 200 µl. For concentration a cooled centrifuge was used (Eppendorf). Viral concentrates were stored at -80°C.
3. Extraction of viral DNA was done with a QIAamp MinElute Virus Spin Kit (Qiagen, Cat. No. 57704) following the manufacturers protocol. Viral DNA extracts were stored at – 80°C.
4. Two RAPD-PCR's were performed for each sample with the following primers:
    a. OPA-13 (5'- CAG CAC CCA C – 3')
    b. CRA-22 (5'- CCG CAG CCA A – 3').

   A master mix was prepared freshly and put in PCR tubes before template DNA was added. For one reaction, the master mix contain 5 µl 10x PCR buffer (provided with Platinum Taq DNA polymerase), 1.5 µl $MgCl_2$ (50 mM, provided with Platinum Taq

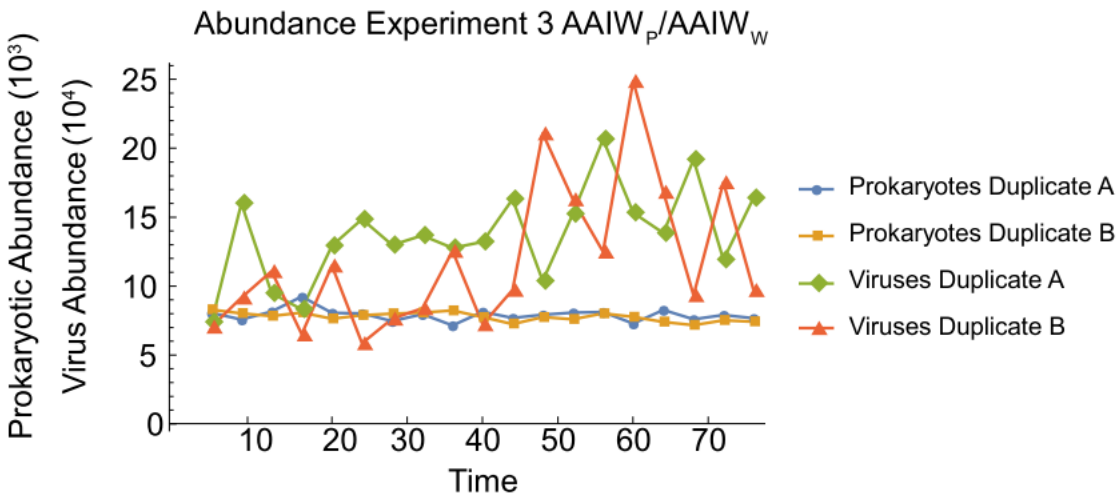
DNA polymerase), 1 µl dNTP mix (See TRFLP step 3), 5 µl CRA-22 or 10 µl OPA-13, 0.4 µl Platinum Taq (Invitrogen, Cat.No.: 10966-026 and 10966-018) and autoclaved and UV sterilized water for reaching a total volume of 50 µl (including viral nucleic extract). 1 µl viral DNA extract were added. If RAPD-PCR was not functioning, RAPD-PCR were repeated with different amounts of viral nucleic extract between 0.5 and 5µl. For each run a negative control with autoclaved and UV-sterilized MiliQ water was performed. RAPD-PCR was performed with a Master cycler Pro (Eppendorf). The program started with the denaturation of DNA and activation of the Platinum Taq DNA polymerase at 94°C for 10 min. Afterwards 30 cycles followed, each with 30 sec denaturation at 94°C, 3 min annealing at 35°C and 1 min elongation at 72°C. Temperature was held at 72 °C for a final elongation step before cooling down to 4°C until RAPD-PCR products were removed from the cycler. RAPD-PCR products were stored at – 20 °C.

5. 2.5 % agarose gels were used for size separation of RAPD-PCR products (44 V, 135 min, staining and visualizing as in TRFLP step 2.
6. Analysis of bands were done using the software GIMP (v. 2.8.0). For sizing, bands were put in relation to two different size markers, Two standard ladders were used for sizing: SmartLadder (Eurogentec, Cat.No.: MW-1700-10) and GeneRuler (Thermo Scientific, Cat. No. SM0311) (S2).
7. Binary matrices were created for each experiment using LibreOffice calc (v.4.1).

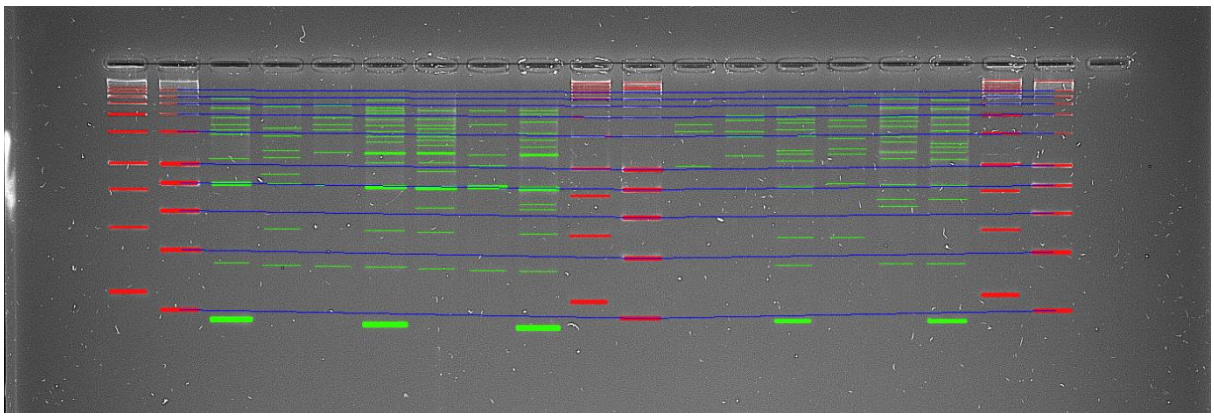
Supplementary Figures



Supplementary Figure 1: Typical scatter plot derived from abundance measurements using FACS Aria II.



Supplementary Figure 2: Typical Abundance Plot of a treatment.



Supplementary Figure 3: Analyzed Gel of RAPD-PCR fragments. Red stripes indicate bands of size marker. Green stripes indicate bands from RAPD-PCR fragments. Blue lines are for appropriate sizing according to the size marker.

Zusammenfassung

In dieser Studie wurde untersucht wie sich das Mischen von benachbarten Wasserschichten des Nord Atlantik auf die Abundanz von Prokaryoten und Viren, sowie der Virenproduktion, dem Anteil der infizierten Zellen (engl.: Frequency of infected cells), dem Verhältnis von Viren zu Prokaryoten (engl.: virus-to-prokaryote ratio), sowie auf die Prokaryoten- und Virengemeinschaften auswirkt. Es wurde das Mischen von je zwei Wassermassen an drei verschiedenen Stellen im Nordatlantik untersucht. Von jeder Wassermasse wurde die Prokaryoten- sowie die Virenabundanz bestimmt. Durch Filtration wurde von jeder Wassermasse ein Prokaryotenkonzentrat und viren-freies Seewasser gewonnen. Jedes Prokaryotenkonzentrat wurde je einmal in virus-freiem Wasser der eigenen Wassermasse, sowie in virus-freiem Wasser der anderen Wassermasse für 72 h inkubiert. Die Prokaryoten und Virenabundanz der *in situ* Wasserproben waren den bereits vorliegenden Werten in dieser Region ähnlich, mit Ausnahme des antarktischen Tiefenwassers an der Station 17, an welcher die Prokaryotenabundanz niedriger war, während die Virenabundanz über den Literaturwerten lag. In den Experimenten konnte zwischen den Inkubationen mit dem jeweils selben Prokaryotenkonzentrat kein Unterschied in der Prokaryotenabundanz gefunden. Jedoch gab es deutliche Unterschiede in der Virenabundanz, dem Verhältnis von Viren zu Prokaryoten, der Virenproduktion und dem Anteil der infizierten Zellen. Da erhöhte Werte der genannten Faktoren immer in derselben Wassermasse auftraten scheint die unterschiedliche Verfügbarkeit von limitierten Ressourcen für die gefundenen Unterschiede verantwortlich zu sein, welche die Wachstumsrate der Wirtsorganismen verändern und somit auch Faktoren wie z.B. die Virenproduktion oder dem Verhältnis von Viren zu Prokaryoten.

Es wurden nur wenige Unterschiede in der Zusammensetzungen der Prokaryoten- und Virengemeinschaften zwischen den Inkubationen gefunden. Das deutet darauf hin, dass die Inkubationen nur wenig Einfluss auf die Zusammensetzung der Prokaryoten- und Virengemeinschaften hatten, sondern stochastische Effekte diese bestimmten.

Curriculum Vitae

Education

2010-2015
- Master of Science study “Ecology” with specification on “Marine Biology” at the University of Vienna/ Austria
  M.Sc. thesis title: “The influence of mixing of water masses and its influence on prokaryotic and viral communities”

2010-2014
- Master study “Behavior, Neurobiology and Cognition” at the University of Vienna/ Austria
  M.Sc. thesis title: “The role of public information on nest defense for nest site choice in *N.caudopunctatus*”

2008-2010 Bachelor study of Biology with specification of “Ecology” at the University of Vienna/ Austria

2000-2008 Secondary School in Dresden/ Germany

1996-2000 Primary School in Burkhardswalde/ Germany

Additional References

2010-2012 Project assistant at the FWF-Project „Habitat selection and male mating success in a colonial fish“ at the Konrad Lorenz Institute of Ethology

- sampling and processing of data
- animal care
- preliminary studys
- tutor at student courses

2009-2011 Volunteer of the ÖWR (Austrian water rescue services)

2010 Intern at „IDUS“ biological analytical environmental lab (Ottendorf-Okrilla/Germany)

2005 Intern at „Aquarium Kiel“ of the „GEOMAR“ Kiel/ Germany

Publications

Martins, CIM; Schaedelin, FC; Mann, M; Blum, C; Mandl, I; Urban, D; Grill, J; Schosswender, J; Wagner, RH (2012): Exploring novelty: a component trait of behavioural syndromes in a colonial fish. Behaviour (149), 2 215-231.

Skills

Languages: German (first language), English (fluent), Latvian (basic user), Spanish (basic user)

Computer Skills: MS-Office, Libre Office, Mathematica, SPSS, Observer, Virtual dub, Sigma Plot

Additional Qualifications: Driving license (B), Diving license (*/ VDTL)), Lifeguard

Interests

Behavioral, Neural and Marine Science, Playing music, diving, snorkeling, swimming