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Ecophysiological Aspects of Algal Host - Virus Interactions in a Changing Ocean.

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Summary

Marine phytoplankton are unicellular photosynthetic microbes that are responsible for roughly fifty percent of global primary production and form the base of most of the pelagic food chains. Phytoplankton production is controlled by so called 'bottom-up' factors, i.e. physicochemical variables such as light, temperature, CO_2 and nutrient availability. However, phytoplankton abundances are also regulated by 'top-down' mortality factors, such as zooplankton grazing and viral lysis. Viruses are host-specific infectious particles, that consist of genetic material in a protein capsid, and which depend on host cells to reproduce. Upon infection they replicate inside the host cell and are then released into the extracellular environment through cell lysis, from where they can infect new host cells. As opposed to grazing of phytoplankton, viral lysis diverts matter and energy away from higher trophic levels to the dissolved organic matter pool. Changes in the impact of viruses on phytoplankton mortality might thus have subsequent impacts on food web dynamics and biogeochemical cycling. However, despite the ecological significance of the topic, knowledge on how the environment affects virus-phytoplankton interactions is still largely limited. For instance, studies thus far mostly considered the effects of nutrient deprivation, while the possible effects of nutrient supply rate, type of molecular compound, and the potentially interacting effect of two stressors (e.g. nutrient availability and light level) have been largely overlooked. The relevance of such studies is high as anthropogenic activities result in (i) changing nutrient load and N:P ratios, and (ii) increasing atmospheric and aquatic CO_2 concentrations and warming of the surface ocean. Global warming may induce and/or strengthen vertical stratification which in turn leads to a more stable light climate (high or low intensity, depending on the actual depth) and increasing nutrient limitation.

With this thesis I aimed to obtain a better understanding on how abiotic factors affect virus-phytoplankton interaction, alone and in combination with other relevant environmental variables. The main focus in this thesis is on phosphorus (P) limitation, for the primary reason that phytoplankton growth in many coastal and oceanic systems worldwide is (seasonally) limited in P, and the future (stratified) ocean is expected to become more P-limited than nitrogen (N) limited due to diazotroph N-fixation in the ocean surface. The studies in this thesis were carried out with axenic phytoplankton host-virus model systems under well-controlled experimental set-ups to obtain a mechanistic understanding and allow accurate quantification of virus growth characteristics, i.e., the viral latent period (time until first release of viruses), the viral burst size (number of viruses produced per host cell lysed) and the percentage of infective progeny viruses. Host cell physiology (e.g. photophysiology and

lipid composition) was monitored to relate differences in results between treatments to host metabolism.

In Chapter 2 of this thesis, the effects on virus-host interaction were studied in a future ocean scenario of P-limitation with elevated partial CO_2 pressure (pCO_2 of 750 µatm, representing the year 2100). Cells of the picoeukaryotic phytoplankter *Micromonas pusilla* were grown in P-limited chemostats: continuous cultures in which the growth rate (and thus the strength of limitation) is determined by the dilution rate of the medium (0.25 µM PO_4^{3-}). The P-limited cells were forced to grow at 97% and 32% of the P-replete growth rate (maximum growth rate, μ_{max} of 0.72 d^{-1}). At steady state (i.e. sustained P-controlled balanced growth with constant cell abundances), the algal cellular P-quotas, photosynthetic efficiency and net primary production rates were found severely reduced compared with the P-replete treatment. CO_2 enrichment facilitated higher *M. pusilla* abundances, due to further reduction of cellular P and N quota. Upon viral infection (with *M. pusilla* virus MpV), a higher CO_2 concentration did not affect virus proliferation. In contrast, P-limitation led to a prolonged latent period by 3 and 6h for the 0.97 and 0.32 μ_{max} cultures, respectively (4-8 h under replete conditions). Moreover, the burst size reduced 5-fold, independent of the degree of P-limitation. These results indicate that a combination of low P-availability and high pCO_2, a likely scenario for the future oceans, may support higher picophytoplankton biomass (elevated pCO_2) and reduce their mortality by viruses (P-limitation).

Chapter 3 shows that P-limitation (but not elevated pCO_2) affects the composition of intact polar lipids (IPLs) in *M. pusilla*. For the first time we show that this (i) occurs in a picoeukaryotic green alga, (ii) that the lipid remodeling depends on the strength of limitation and furthermore (iii) that lipid remodeling can be influenced by MpV infection. The ratio of phospholipids (phosphorus containing lipids) to sulfolipids and galactolipids (sulfur- and galactose containing lipids) was shown to strongly decrease along a gradient from P-replete conditions to P-controlled growth at 0.97 and 0.32 μ_{max}, and to P-starvation. When the P-starved cultures were infected with MpV, total P-lipid substitution was either lower (0.97 μ_{max}) or completely inhibited (0.32 μ_{max}). Thus the effects of viral infection on the IPL composition were dependent on the growth history of the cells. This study demonstrates that not only P-concentration, but also the P-supply rate can affect phytoplankton lipid composition, and that viral action can interfere with host lipid metabolism and as such affect the chemical composition of dissolved and particulate organic matter.

The effect of viral infection on host IPL composition was further investigated for the haptophyte *Phaeocystis globosa* (Chapter 4). Viral infection of *P. globosa* with its virus PgV

did not lead to changes in IPL composition. This is in itself remarkable because literature shows that lipid profiles of the closely related haptophyte *Emiliania huxleyi* are strongly affected by viral infection. A closer look to the IPL bound fatty acids (FAs; Chapter 5), however, reveals that viral infection did lead to a decrease of polyunsaturated FAs. Organisms in higher trophic levels are dependent on phytoplankton for these highly nutritional compounds and hence these results suggest that viral infection of *P. globosa* has the potential to affect ecosystem dynamics by reducing the availability of PUFAs in the system (via grazing on infected cells or via lysed dissolved organic matter). Chapters 3, 4 and 5 furthermore describe that MpV and PgV possess lipid membranes, which resemble host lipid composition, but that they are impoverished in thylakoid membrane specific galacto- and sulfolipids. The profile of PgV showed shorter and more saturated FAs than the average FAs of the host. Both viruses might selectively recruit their membranes from their phytoplankton host, i.e. from specific cell compartments.

In Chapters 6 and 7 the effects of P-limitation on *M. pusilla* and *P. globosa* were investigated in relation to light availability and N-limitation, respectively. Chapter 6 shows that P-limitation in combination with relatively low or high light level (respectively 25 and 250 µmol quanta m^{-2} s^{-1}) resulted in severely reduced photosynthetic efficiencies for both phytoplankton species (compared to medium light level of 100 µmol quanta m^{-2} s^{-1} and the P-replete treatment). The low and high light treatments did not affect virus proliferation in infected *M. pusilla*. In contrast, the viral burst size of PgV decreased by 55 and 23%, respectively. Remarkably, only 2 and 4% of the virus progeny were infective, as compared to 62% for P-limited under standard light level. This study clearly shows that light level can drastically strengthen the negative effects of P-limitation on virus infectivity and that this effect is host species-specific.

Simultaneously low availability of P and N (Chapter 7) did not lead to such cumulative constraints on viral proliferation in *M. pusilla* and *P. globosa*. Although for both species the steady state maximum growth rate under N- and NP-limitation was equal to P-limitation, the NP-limited treatment showed most resemblance to the N-limited cultures in mean cell size, cellular mean chlorophyll fluorescence, and photosynthetic efficiency. All infected N- and NP-limited cultures showed similarly prolonged viral latent periods as under P-limitation. While MpV burst sizes were equally reduced under N- and P-limitation (i.e. ±70%), the burst sizes of PgV were further reduced by 94% under N- and NP-limitation (as compared to 70% for P-limitation). The results demonstrate that N-limitation can be equally inhibiting or be an even stronger inhibitor of viral proliferation than P-limitation. This is in

shear contrast to the thus far prevailing perception that algal virus production is hardly affected by host N-limitation.

The previous chapters indicate that the availability of P is an important regulating factor of algal virus proliferation and that the outcome of infection depends on the strength of the metabolic limitation of the host prior to infection (i.e. the supply rate of P). The latter is an important finding as primary producers in seas and oceans experience different degrees of P-limitation. Furthermore, primary production in oligotrophic pelagic systems depends strongly on a continuously low supply of nutrients by nutrient recycling. I therefore studied the effect of near-continuous supply of the limiting nutrient (i.e. P) to infected *M. pusilla* (Chapter 8). A low supply of the soluble reactive P (SRP) to P-limited *M. pusilla* during the infection cycle resulted in a doubling of the burst size as compared to no addition, independent of the level of P-limitation (0.97 or 0.32 μ_{max}). Delaying this supply up to 18h post infection still increased the burst size to a similar level. Supply with an organic P-source (soluble non-reactive P, SNP) led to a similar burst size stimulation as with SNP, illustrating efficient utilization of SNP compounds by *M. pusilla* during infection. Viral burst sizes were even stimulated by natural SNP in virus-free lysate (also SRP-free) of previously lysed P-limited *M. pusilla*. This study shows the importance of the supply rate of ecologically relevant low concentrations of P for virus production in P-limited phytoplankton hosts. The data suggest that remineralization (illustrated by the SRP-supply) and viral lysis of neighboring cells (mimicked by the SNP treatment) can promote viral proliferation and thus phytoplankton mortality by viral lysis even though SRP concentrations in the water are depleted.

Overall, the research presented in this thesis provides new insights into how the type and level of nutrient limitation determines the outcome of the lytic virus infection of phytoplankton-hosts. Moreover, depending on the species, light level can force an additional constraint on already relatively low viral burst sizes of nutrient-limited phytoplankton. The success of lytic viral infection (speed of production and number of produced viruses) is thus dependent on the metabolic state of the host cells and variability in environmental factors has a strong potential to influence virus production and subsequently viral-induced phytoplankton mortality. No direct role for IPLs and FAs in the interaction between the physicochemical environment and phytoplankton proliferation was observed, but viral infection was found to have the potential to interfere with how the environment affects host IPL composition, possibly altering the nutritional (FA) value of phytoplankton. Knowledge on how the environment affects phytoplankton viral lysis becomes more important as the human population drastically influences the earth's atmosphere and oceans. This thesis strengthens

the hypothesis that virus activity and viral-induced phytoplankton mortality might be strongly affected by global change processes, with consequent potential effects on food web dynamics and biogeochemical cycling. Further study, in line with this thesis, is needed to investigate the effects of the environment on virus-host interaction, with particular emphasis on differences between host species, virus strains infecting the same species, and examination under natural conditions.