

Chapter 1

General Introduction

Viruses are the most abundant biological entities in the oceans and are important mortality agents of heterotrophic and autotrophic microbial populations. These microbial hosts are numerically dominant and constitute the largest percentage (>90%) of living biomass in the ocean. Collectively microorganisms manage the pools and fluxes of energy and nutrients in the ocean. Loss factors play an essential role in controlling the activity and production of marine microbial communities and thus ocean ecosystem net productivity. Different mortality pathways influence the cycling of energy and biogeochemical elements very differently. Yet, little is known about how physicochemical factors regulate the partitioning of mortality amongst viral lysis and grazing, limiting our ability to predict how the ocean will respond to global climate change. This chapter provides a brief introduction to the different ecological and biogeochemical roles that autotrophic microorganisms, heterotrophic prokaryotes and viruses play in the marine environment, with special focus on the cycling of carbon. In addition, it provides a short overview of how global warming is expected to alter ocean stratification and what we currently know about how this will affect the structure and functioning of microbial populations. This all sets the stage for the overall aim of this thesis which is to mechanistically understand the ecological relevance of stratification in structuring microbial populations, with particular focus on losses due to viral lysis and grazing.

1.1. Phytoplankton

Marine photoautotrophic microorganisms are predominately eukaryotic and prokaryotic phytoplankton. As primary producers, phytoplankton synthesize organic compounds from aqueous carbon dioxide (CO_2) through the process of photosynthesis. Consequently, factors which regulate light and inorganic nutrient availability (e.g., nitrogen, phosphorus and iron) strongly influence the nature and activity of phytoplankton communities. Marine phytoplankton take up large amounts carbon dioxide annually ($\sim 50 \text{ Pg C y}^{-1}$; Falkowski 2002) and contribute almost half of the global net primary production occurring on the planet (Field et al. 1998). Approximately $5\text{-}10 \text{ Pg C y}^{-1}$ of this photosynthetically fixed carbon is exported from the surface into the deep ocean, via the biological pump, reducing the surface partial pressure of CO_2 which governs air-sea CO_2 exchange and therefore plays an essential role in the long term regulation of atmospheric CO_2 and climate (Ducklow et al. 2001; Jiao et al. 2010; Henson et al. 2011). As the base of most marine food chains, phytoplankton provide a significant fraction of the total organic matter (OM) available to higher trophic levels. Phytoplankton production

thus sets upper limits to both the overall activity of the pelagic food web and the quantity of organic carbon exported downwards. Accordingly, the quantification of rates, patterns and mechanisms that control uptake of CO₂ by phytoplankton and the fate of the resultant organic carbon is an important central theme in ecological and biogeochemical research.

Cell size affects many of the processes determining the growth of phytoplankton, including metabolic rates (e.g. internal metabolic transport), nutrient diffusion, uptake and requirements, excretion and light absorption (Malinsky-Rushansky and Legrand 1996; Bricaud et al. 2004; Mei et al. 2009; Finkel et al. 2010). Due to these physiological restraints of size, small cells with larger surface to volume ratios have a competitive advantage in stable and oligotrophic (nutrient poor) waters (Agawin et al. 2000). Due to the ecological relevance of size, phytoplankton are often separated based on cell size into micro- (200-20 μm), nano- (20-2 μm) and pico-phytoplankton (<2 μm). In general, the largest size classes are dominated by diatoms and dinoflagellates while the smallest size classes consist of cyanobacteria, prasinophytes and prymnesiophytes (Gibb et al. 2000; Cuvelier et al. 2010; Not et al. 2012).

Cell size also governs grazing and sinking rates, as well as the likelihood of viral infection (Murray and Jackson 1992; Kiorboe 1993; Chen and Liu 2010). Consequently, phytoplankton size largely determines trophic organization and the efficiency with which photosynthetic OM is transferred to higher trophic levels or exported to the deep ocean (Legendre and Rivkin 2002; Falkowski and Oliver 2007; Finkel et al. 2010). Biomass and production dominated by small phytoplankton is associated with high numerical abundance, slow sedimentation rates, and rapid cycling of OM through the microbial food web (e.g. dominated by bacteria and smaller zooplankton such as ciliates and flagellates), which results in low potential carbon export to the deep sea (Azam et al. 1983; Legendre and Rassoulzadegan 1996; Finkel et al. 2010). In contrast, larger phytoplankton allow for a more efficient transfer of OM through short food chains (dominated by copepods and larger zooplankton), higher sedimentation rates and therefore enhanced downward export and biological CO₂ drawdown. How environmental conditions and size affect the relative contribution of grazing and viral lysis to phytoplankton mortality remains largely unknown due to the scarcity of reports for viral induced mortality of marine phytoplankton populations, as well as direct comparisons between these different mortality pathways across broad ocean regions. As grazing and loss due to viral infection (section 1.3) affect the fate of photosynthetically fixed carbon

very differently, understanding the underlying factors regulating the division of morality amongst these pathways is equally important for understanding ocean ecosystem productivity and biogeochemical cycles.

1.2 Heterotrophic prokaryotes

The term 'heterotroph' is generally applied to refer to chemoorganoheterotrophs. Heterotrophic prokaryotes are comprised of members from two domains of life - Archaea and Bacteria. Traditionally, Archaea were thought to only contribute a significant fraction of the prokaryotic community within extreme environments, however, it is now known that they can comprise greater than 30% of total microbial abundance in surface waters of the ocean (Delong 1992; Pernthaler et al. 2002; Pernthaler and Amann 2005). Thus, throughout the remainder of this thesis, 'bacteria' or 'prokaryotes' will be used synonymously, referring to Bacteria and Archaea. Heterotrophic prokaryotes have the ability to utilize organic compounds as a source of both energy and carbon, which can be incorporated directly as low molecular weight (LMW; <600 Da) or indirectly through the use of extracellular enzymes which cleave high molecular weight (HMW) molecules to LMW molecules (Gasol et al. 2008). Heterotrophic prokaryotes thus play an essential role in marine environments by remineralizing organic matter and thereby maintain the bioavailability of potential growth limiting nutrients (e.g. nitrogen, phosphorus, and carbon), as well as provide transfer mechanism of this material to higher trophic levels (i.e., microbial loop, Azam et al. 1983; Figure 1). In addition, through the mineralization of dead particulate matter (POM) and DOM, and the dissolution of sinking POM, heterotrophic prokaryotes affect the magnitude of the vertical organic fluxes and thus represent an integral part of the oceanic biological pump (Nagata et al. 2000; Ducklow et al. 2001; Jiao et al. 2010).

The relevance of heterotrophic bacteria to biogeochemical cycling of organic matter are largely determined by fluxes of bacterial production (BP) and respiration (BR) (Ducklow et al. 2010). These two fluxes are related to bacterial growth efficiency (BGE), the fraction of the total organic carbon assimilated to build up biomass. Substrate supply and complexity and inorganic nutrient availability appear to be most important factors regulating BGE in aquatic systems (del Giorgio and Cole 1998; Reche et al. 1998; Cuevas et al. 2011). However, there still remains a large amount of uncertainty in regards to what controls the magnitude and variation in BGE of ocean systems.

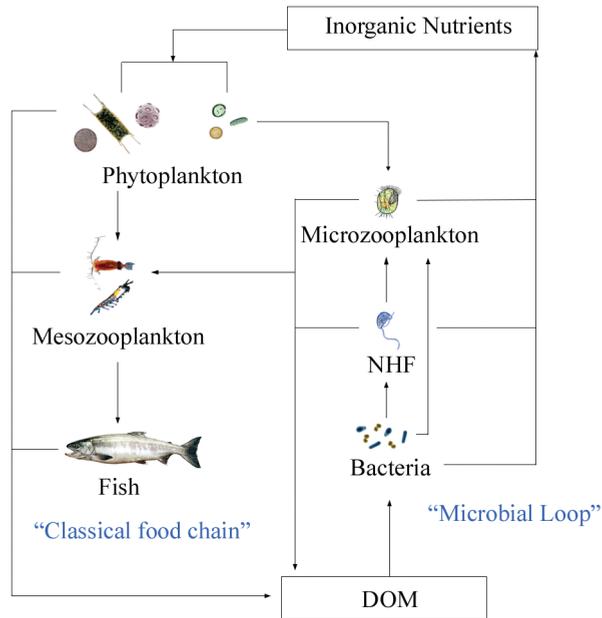


Figure 1. Simplified diagram of the flow of nutrients and organic matter through the traditional grazing food chain and microbial loop. DOM stands for dissolved organic matter, and comes from excretion, death, sloppy feeding and fecal pellets.

Grazing by phagotrophic protists, particularly bacterivorous nanoflagellates, and viral lysis are the main loss factors regulating bacterial populations in aquatic environments (Hahn and Hofle 2001; Pernthaler 2005). However, grazing and viral lysis have differential effects on prokaryotic communities. Protists prey on a wide range of prokaryotic species in a size-selective manner (Pernthaler 2005; Glucksman et al. 2010), while viruses typically have a narrow host range (see section 1.3) and thus regulate prokaryotic species (or even strain) diversity (Thingstad 2000; Weinbauer 2004). Studies comparing microzooplankton grazing and viral lysis reveal both contribute similarly to bacterial mortality, but their relative importance can vary with season or environmental conditions (Fuhrman and Noble 1995; Weinbauer and Peduzzi 1995; Pernthaler 2005; Tsai et al. 2012). The underlying factors regulating the interplay between viruses and protist in the control of marine prokaryotes are still poorly understood, especially with respect to the role of physical processes such as water column stratification.

1.3 Marine viruses

Viruses are the most abundant biological entities in the oceans (Bergh et al. 1989; Fuhrman and Suttle 1993; Suttle 2007). Currently, it is estimated that viruses range from $\sim 3 \times 10^6$ viruses ml^{-1} in the deep sea to $\sim 10^8$ ml^{-1} in productive coastal waters (Suttle 2005). Viruses are biological particles comprised of ribonucleic acid (RNA) or deoxyribonucleic acid (DNA) genome protected by a protein coat (i.e., capsid) (Hurst 2000). They are considered as obligate parasites due to their reliance upon a host to provide the energy and metabolic machinery necessary for replication. Viruses typically have a narrow host specificity, with the majority of viruses infecting only one host species. However, within a host species strain-specificity can vary widely (Brussaard 2004; Holmfeldt et al. 2007).

Due to their small size (~ 100 nm; 10-200 fg; Breitbart 2012), transport is governed by the random wandering of Brownian motion and therefore they obey the laws of diffusion in their approach to larger particles such as hosts (Murray and Jackson 1992). Contact rates are directly dependent on viruses and host abundance; but also can be effected by host size, motion and morphology (Murray and Jackson 1992). Once contact between a viable host and infective virus is accomplished, viral replication can proceed through different life strategies; lytic, lysogenic, chronic and pseudolysogeny. In the lytic cycle, viral replication proceeds immediately after infection and terminates with the lysis of the host and release of viral progeny and host cell content into the surrounding water (Figure 2A). During lysogenic infection, the genetic material of temperate phages (prophage) is stably incorporated into the host genome, and the host continues to live and reproduce normally, transmitting the prophage vertically to daughter cells during each subsequent cell division, until an event triggers the virus to enter the lytic pathway (Figure 2B). Lysogeny appears to be mostly restricted to prokaryotic hosts (Van Etten et al. 2002; Paul 2008), where it is theorized to represent a survival strategy under conditions of low host productivity and abundance (Williamson et al. 2002; Weinbauer et al. 2003; Payet and Suttle 2013). However, the importance of the different life strategies and mechanisms regulating selection over large ocean scales remain largely unknown. While lytic and lysogenic life styles have received the most attention, viral replication has also been shown to occur through chronic infection where viruses are released through budding or extrusion without killing their host (Mackinder et al. 2009; Thomas et al. 2011) or through pseudolysogeny which differs from true lysogeny in that the viral genome does not integrate into the host genome (Williamson et al. 2001).

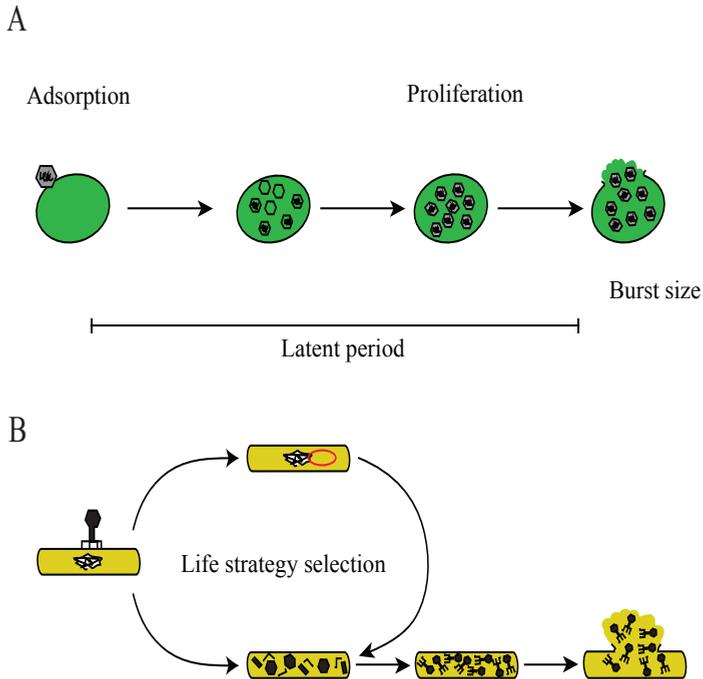


Figure 2. Simplified schematic of how viral replications occurs through the (A) lytic viral replication in autotrophic eukaryotes and (B) through both lytic and lysogenic infection in prokaryotic hosts.

The lysis of microbes diverts energy and biomass away from the classical food web towards microbial-mediated recycling and the dissolved organic matter pool. In this manner, the ‘viral shunt’ reduces the transfer of carbon and nutrients to higher trophic levels, while enhancing the recycling of potential growth-limiting nutrients (Fuhrman 1999; Wilhelm and Suttle 1999) (Figure 3). Theoretical models have been used to estimate that between 6 and 26% of the photosynthetically fixed carbon (PFC) is shunted to the DOM pool by the activity of viruses (Wilhelm and Suttle 1999). However, our ability to confirm estimates and thus understand the true magnitude of viruses in the marine biogeochemical cycles has been restricted by a lack of quantitative estimates of viral lysis in marine phytoplankton populations (Weitz and Wilhelm 2012), as well as by information regarding how viral lysis rates compare to grazing. In addition, little is known about the existence of large-scale patterns in virus-phytoplankton biogeography (Breitbart 2012) and the factors regulating viral activity and distribution of phytoplankton viruses on global ocean scales.

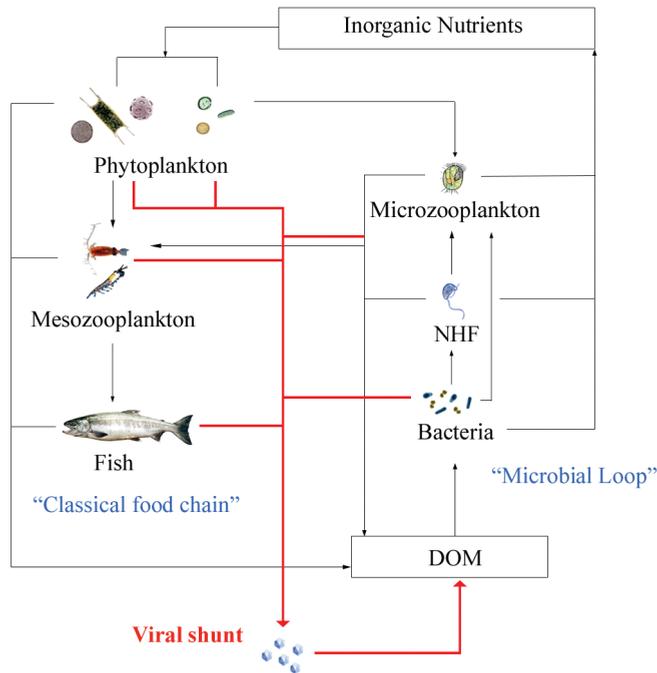


Figure 3. Simplified diagram illustrating the role of viruses in the marine food web. The ‘viral shunt’ (highlighted by red arrows) moves organic matter and energy away from the higher trophic levels towards the DOM (and after bacterial remineralization to the inorganic nutrient pool). Thus viral lysis is separated from the traditional flow of DOM (largely excretion).

1.4. Global warming and vertical stratification

Global climate has been changing over the last few decades due to anthropogenic-induced increases in atmospheric concentrations of key greenhouse gases such as CO_2 and CH_4 (Meehl et al. 2007). The oceans play an essential role in regulating global climate through the storage and transportation of heat (Levitus et al. 2000; Barnett et al. 2005; Hallegraeff 2010; Hoegh-Guldberg and Bruno 2010) and uptake and sequestration of CO_2 (Hallegraeff 2010), providing an important buffer against climate change. More than 90% of the increase in the global heat content which has occurred over the last 60 years has gone into warming the oceans (Barnett et al. 2005; Hallegraeff 2010; Hoegh-Guldberg and Bruno 2010). As global warming continues, the surface waters of the ocean are envisaged to further rise by 2-6°C over the next 100 years (Timmermann et al. 1999; Meehl et al. 2007). Ocean-climate models predict that this surface warming, in combination with changes in

fresh water input at high latitudes due to rises in precipitation and sea ice melt, will lead to increases vertical stratification processes (Sarmiento et al. 1998; Levitus et al. 2000; Sarmiento 2004; Toggweiler and Russell 2008). The oceans also provide one of the largest natural reservoirs of carbon and the flux and storage of CO₂ into the world's oceans is largely influenced by marine microorganisms living in the upper surface waters. Understanding the ecological and physiological mechanisms controlling changes in microbial community structure across gradients of vertical stability is therefore vital to predicting the response of ocean systems to global climate change. The North Atlantic Ocean provides a meridional gradient in vertical density stratification (Talley et al. 2011) and is an important sink for anthropogenic CO₂ (storing 23% of the global oceanic anthropogenic CO₂) (Sabine et al. 2004) and therefore provides a ideal model area to study the effects of vertical stratification on microbial community dynamics.

Stratification suppresses turbulence and reduces mixing depth, thereby exerting a fundamental control on phytoplankton resource availability, i.e., relaxing of potential light limitation and restricting the nutrient flux from depth (Huisman et al. 1999; Mahadevan et al. 2012). In temperate and high latitude regions, the annual establishment of seasonal stratification often triggers the highly productive phytoplankton spring bloom (Sverdrup 1953; Huisman et al. 1999). However, strong and prolonged stratification can lead to reductions in phytoplankton biomass and productivity in the surface layers as nutrients become depleted due to utilization, a process referred to as ocean oligotrophication. Changes in global climate and resulting alterations in stratification are believed to have led to the 15% increase in the size of the nutrient-poor oligotrophic regions of the Pacific and Atlantic Oceans which has occurred between 1998-2006 (Polovina et al. 2008). In addition, changes in vertical stratification have been linked to alterations in phytoplankton phenology, size, nutritional value, abundance, spatial distribution and community structure (Richardson and Schoeman 2004; Mitra and Flynn 2005; Behrenfeld et al. 2006; Finkel et al. 2010; Hilligsøe et al. 2011; Doney et al. 2012). Consequently, resulting changes in phytoplankton community composition are expected to affect the structure and functioning of marine food webs, as well as the potential for the ocean to act as a long term sink for carbon dioxide (Beaugrand 2009; Hoegh-Guldberg and Bruno 2010). Similarly, the physiological processes of heterotrophic prokaryotes are directly affected by temperature and by expected changes in the quality of DOM due to nutrient limitation (Ducklow et al. 2010; Sarmiento et al. 2010).

While it is becoming clear that global warming is directly affecting the production of primary and heterotrophic prokaryotic components of marine microbial food webs, these alterations are also expected to affect mortality processes (i.e., grazing rates, viral lysis rates, and sinking of phytoplankton). However, the effect of global warming on the mortality of microbes remains poorly understood. Alterations in prey populations will affect zooplankton grazing, whereby current evidence suggests that the absolute and relative importance of microzooplankton activities in plankton communities will increase in the future ocean due to increased dominance of small-sized algal prey (Sarmiento et al. 2010; Caron and Hutchins 2012). However, few studies have directly compared microzooplankton grazing to viral induced mortality in marine phytoplankton communities. The potential for stratification to regulate viral induced mortality of phytoplankton remains virtually unknown. As viruses rely upon their host to provide the machinery, energy and resources required for viral replication and assembly, factors regulating the physiology, production and removal of hosts are also important in governing viral dynamics (Moebus 1996; Wilson et al. 1996; Baudoux and Brussaard 2008; Maat et al. 2014). Therefore, future changes in stratification have the potential to affect the composition and distribution of viral assemblages associated with microbial communities. There is evidence that changes in inorganic nutrient availability can affect life strategy choice and production of viruses infecting prokaryotes (Wilhelm et al. 2002; Williamson et al. 2002; Bongiorno et al. 2005), however the effect of changes in the strength of vertical stratification is still largely unstudied.

Outline of thesis

The overall aim of this thesis is to investigate how changes in vertical stratification affect autotrophic and heterotrophic microbial communities along a meridional gradient in the Atlantic Ocean. The Northeast Atlantic Ocean is a key area in global ocean circulation and an important sink for atmospheric CO₂. In addition, stratification varies in the North Atlantic from strong permanent stratification in the (sub)tropics to weak seasonal stratification in the North and thus provides an ideal model system to investigate the role of vertical stratification in structuring microbial communities. In order to mechanistically understand the ecological relevance of stratification in structuring microbial populations this thesis specifically aims to (1) provide a comprehensive overview of what is currently

known regarding how environmental factors can regulated virus-host interactions in the marine environment, (2) determine the physicochemical mechanisms structuring phytoplankton communities over a large scale gradient in stratification, (3) determine the relative contribution of viral lysis and grazing to the mortality of phytoplankton and heterotrophic prokaryotes along a north-south gradient regulated by strong stratification and (4) place these finding in the context of implications for the flow of carbon through the marine food web in the present and future North Atlantic Ocean.

Chapter 2 summarizes what is currently known about environmental factors that either directly (i.e., destruction or inactivation of free virus particles) or indirectly (i.e., affecting viral production via host) affect viruses-host interactions. At any spatio-temporal point in the ocean, viral abundance reflects the balance between rates of removal and production through host lysis. Once viral progeny are released from their hosts, they are present in the environment as free virus particles and are directly exposed to environmental factors which may reduce infectivity, degrade or remove virus particles, and adversely affect adsorption to host, thereby reducing the chance of a successful host encounter and infection. Moreover, as obligate parasites, viruses are reliant upon their host to provide not only the cellular machinery but also the necessary energy and resources required for viral replication and assembly. Consequently the factors regulating the physiology of the host, as well as its production and removal are also important in governing virus dynamics.

Chapter 3 presents data obtained from two research cruises conducted in the Northeast Atlantic across a N-S latitudinal gradient during two different seasons, i.e., spring during the onset of stratification and summer when stratification was maximum. The data provide a high-resolution mesoscale description of the phytoplankton community composition in relation to vertical mixing conditions and other key physiological parameters. Phytoplankton were assessed by a combination of flow cytometry and pigment fingerprinting (HPLC-CHEMTAX). Multivariate analysis identified water column stratification (based on depth-integrated Brunt-Väisälä frequency) as one of the key drivers for the distribution and separation of different phytoplankton taxa and size classes. The implications of the findings for the classification of phytoplankton functional types in biogeochemical and ecological ocean models are discussed.

Chapter 4 presents the biogeographical distribution of marine viruses and their contribution to phytoplankton group-specific mortality along a large-

scale gradient in the Northeast Atlantic Ocean (same gradient as in the summer Chapter 3). Virus composition changed with latitude, and was closely associated with the biogeographical distribution of different phytoplankton groups. Average virus-mediated lysis rates were higher for eukaryotic phytoplankton than for the prokaryotic cyanobacteria *Prochlorococcus* and *Synechococcus*. Phytoplankton viral lysis rates were of similar magnitude as microzooplankton grazing rates. Overall, the total phytoplankton mortality rate (viral lysis plus microzooplankton grazing) was comparable to phytoplankton gross growth rate, signifying high turnover rates of marine phytoplankton populations. Moreover, the data show a striking reduction in viral lysis rates of phytoplankton at higher latitudes in the North Atlantic. The importance of these results to future alterations in food web dynamics and biological carbon export in the Northeast Atlantic Ocean are further discussed. **Chapter 5** presents a simple and efficient method optimization for improving virus counts and optimal resolution of viruses populations when measured at low abundances. Flow cytometric enumeration has advanced our ability to analyze aquatic viruses samples and therefore our understanding of the ecological role that viruses play in the ocean (Brussaard et al. 2010). However, low virus abundances such as found in extreme oligotrophic waters, the deep ocean, or resulting from experimental design, require low dilutions in a buffer solution to obtain the optimal even rate (i.e., 200-800 events s^{-1}). This chapter shows that low dilution factors for viruses samples can lead to substantial underestimations in total virus abundances if not corrected by adjusting the buffering capacity of the diluent.

Chapter 6 presents virus induced mortality of prokaryotes relative to grazing and the proportion of lytic and lysogenic viral infection is assessed along a large-scale gradient in the surface waters of the Northeast Atlantic Ocean during summer (same gradient as in Chapter 4). The method applied in the study relies on the ability to quantify the reoccurrence of viruses after reducing *in situ* virus abundance to prevent new infection (Weinbauer et al. 2010). Consequently, to attain optimal virus counts using this approach, the method modification for FCM enumeration at low abundance was applied (Chapter 5). The results demonstrate that viruses were the dominant mortality factor regulating prokaryotic losses, with lytic infection being the favored life strategy in the upper surface layer.

Chapter 7 presents the flux of photosynthetic carbon (C) through the different components of the microbial food web in order to consider how latitudinal changes affected the overall role of the viral shunt. The simultaneous measurements of growth and loss rate rates for phytoplankton (Chapter 4) as well as heterotrophic

bacteria (Chapter 6) provides an ideal dataset to further substantiate the role of the viral shunt in marine systems. The results demonstrate a more prominent role of viral lysis than previously estimated for marine environments (i.e., 6-26%; Wilhelm and Suttle 1999). Our data show higher values for both phytoplankton and heterotrophic prokaryotes, with the strongest increase in flux of PFC from phytoplankton. Moreover, on average the flux of photosynthetically fixed carbon through the viral shunt was 2-fold higher in the southern oligotrophic region (80%) compared to the north (31%), as a consequence of relatively higher viral lysis of both phytoplankton and bacteria. These results have important implications for future shifts in the regional climate of the ocean surface layer of the North Atlantic. In **Chapter 8** the results presented in this thesis are discussed in the context of what is currently known and how these results can be used to increase our predictability of how the oceans will respond to climate change.

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Chapter 1

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