Chapter 7

The viral shunt in a stratified Northeast Atlantic Ocean

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Abstract

The flux of photosynthetic carbon (C) through the viral shunt affects nutrient cycling, system respiration, and food web dynamics. Yet, little is known about large-scale biogeographical patterns in the functioning of the viral shunt in marine systems. In the summer of 2009, we examined both the production and loss rates (i.e., grazing and viral lysis) of autotrophic as well as prokaryotic microbial populations along a north-south latitudinal gradient in the Northeast Atlantic Ocean. The upper water column located between 30 and 63°N was characterized by a strong temperature-induced vertical stratification, with oligotrophic regions extending to 45°N. Here we present the flow of C through the different components of the microbial food web in order to consider how these latitudinal changes affected the overall role of the viral shunt. Our results demonstrate that 33 and 80% of the photosynthetically fixed C moved through the viral shunt into the dead particulate and dissolved matter pool in the north and south, respectively, indicating a more prominent role of viruses in marine nutrient cycles than theorized previously by Wilhelm and Suttle in 1999. The flux of C was reduced 2-fold in the north, as a consequence of lower viral-induced morality of both phytoplankton and bacteria. Our results suggest that future shifts in the regional climate of the ocean surface layer are likely to increase the role of the viral shunt in marine microbial food webs, which may reduce the transfer of matter and energy up the food chain and thus affect the capacity of the North Atlantic to act as a long-term sink for CO_2.

Introduction

Viruses are the smallest and most abundant biological entities on Earth. Perhaps nowhere is their importance better illustrated than in the world's oceans. A single milliliter of surface seawater contains on average 10^6 viruses and most of these viruses infect the numerically dominant hosts, i.e., microbial prokaryotes (bacteria and archaea) and photoautotrophic eukaryotes (Suttle 2007). Viral lysis of microbes diverts energy and biomass away from the classical food web towards microbial-mediated recycling and the dissolved organic matter (DOM) pool (Middelboe and Lyck 2002; Lønborg et al. 2013; Buchan et al. 2014). In this manner, the 'viral shunt' reduces the transfer of carbon and nutrients to higher trophic levels, while at the same time enhancing the recycling of potentially growth-limiting nutrients (Fuhrman 1999; Wilhelm and Suttle 1999). Through the use of theoretical models and poorly constrained rates, it was estimated that between 6 and 26% of the photosynthetically fixed carbon (PFC) is shunted to the DOM pool by the activity of viruses. However, until recently, our ability to confirm these figures and thus understand the true magnitude for the role of viruses in marine biogeochemical cycles has been restricted by a lack of quantitative measurements of viral lysis in marine phytoplankton populations (Weitz and Wilhelm 2012).

During the summer shipboard expedition of STRATIPHYT (Changes in vertical stratification and its impacts on phytoplankton communities) (Figure 1), the production and loss rates (i.e., grazing and viral lysis) of both autotrophic and prokaryotic microbial populations were examined along a latitudinal gradient in the Northeast Atlantic Ocean. This provided the possibility to model the flux of organic carbon (C) through the marine food web using measured values obtained from the same water. The Northeast Atlantic Ocean was characterized by a strong temperature-induced vertical stratification resulting in oligotrophic conditions in the upper 50 - 100 m at latitudes south of 45°N, whereas towards the north, nutrient limitation slightly relaxed in the upper 50 m surface layer (Mojica et al. 2015b). Dual measurements of viral lysis and grazing rates were obtained for all phytoplankton groups, except for *Prochlorococcus* HL which was largely absent from the sampled depths (Mojica et al. 2015a). Overall, rates of virus-induced mortality and grazing of phytoplankton were comparable. However, the relative share of viral lysis was highest at low and mid latitudes while phytoplankton mortality was dominated by microzooplankton grazing at higher latitudes (> 56°N). Total phytoplankton mortality (virus plus grazer-mediated) was comparable to the gross growth rates,

demonstrating high turnover rates of phytoplankton populations (Mojica et al. 2015a). For heterotrophic prokaryote populations, viral lysis was the dominate mortality factor (Mojica and Brussaard submitted).

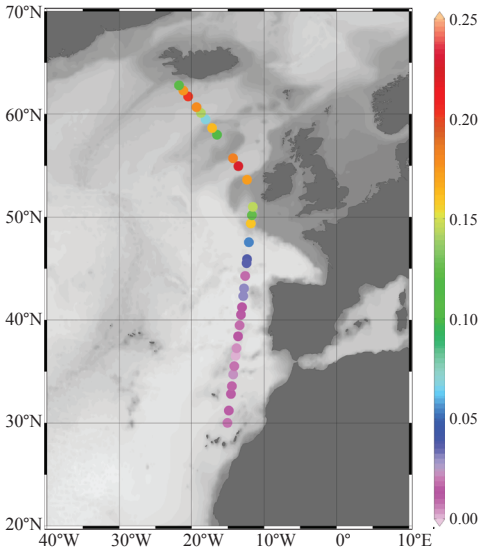

Figure 1. Bathymetric map of the Northeast Atlantic Ocean depicting station locations and PO_4 concentrations (μM) in the surface mixed layer during the summer 2009 (17 July – 9 August), using Ocean Data View (Schlitzer 2002).

Here we present the flux of C through the different components of the microbial food web along a stratified latitudinal gradient in order to consider (1) the effect of trophic state, on the overall relevance of the viral shunt, and (2) compare our results to the more theorized steady state model by Wilhelm and Suttle (1999).

Methods

Phytoplankton (< 20 μm diameter), prokaryotes and viruses were enumerated using a Becton-Dickinson FACSCalibur flow cytometer (FCM) equipped with an air-cooled Argon laser with an excitation wavelength of 488 nm (15 mW). Phytoplankton were enumerated according to Marie et al. (2005) and bacteria and viruses according to Marie et al. (1999) and Brussaard *et al.* (2010), respectively, with modifications according to Mojica et al. (2014). Phytoplankton were

differentiated based on their auto-fluorescence properties using bivariate scatter plots of either orange (i.e., phycoerythrin, present in prokaryotic *Synechococcus* spp.) or red fluorescence (i.e., chlorophyll *a*, present in all phytoplankton) against side scatter. Average cell size for phytoplankton subpopulations were determined by size-fractionation of whole water by sequential gravity filtration. Viruses and prokaryotes were discriminated using the nucleic acid-specific SYBR Green I and side scatter characteristics. Phytoplankton community composition and abundances were described previously by Mojica et al. (2015b).

Phytoplankton growth and loss rates used to calculate organic C-flux originate from Mojica et al. (in review), with the exception of mesozooplankton grazing (this study). Briefly, growth, viral lysis and microzooplankton (< 200 µm) grazing rates of the different photoautotrophic groups were determined using the modified dilution assay (Kimmance and Brussaard 2010). Experiments were conducted onboard, using water samples obtained from those depths where Chl *a* autofluorescence was maximal. The microzooplankton grazing rate (M_G) was estimated from the regression coefficient of the apparent growth rate versus fraction of natural seawater for the 0.45 µm series (i.e., removing grazers). The combined rate of viral-induced lysis and microzooplankton grazing (M_{V+G}) was estimated from a similar regression of the 30 kDa series (i.e, viruses and grazer removed). Viral lysis rate (M_V) was then determined as the difference between the combined mortality rate and the microzooplankton grazing rate. Phytoplankton gross growth rate (μ_{gross}, in the absence of mortality) was derived from the y-intercept of the 30 kDa series regression, and phytoplankton gross production (P_{gross}, cells l^{-1} d^{-1}) was calculated as $P_{gross} = P_0 \times (e^{\mu t})$, where P_0 is phytoplankton abundance, t is time (d) and µ is μ_{gross}. Phytoplankton net growth rate (μ_{net}, d^{-1}) was defined as the difference between μ_{gross} and total mortality rate, and phytoplankton net production (P_{net}, cells l^{-1} d^{-1}) calculated according to the previous equation, where µ is μ_{net}. Total mortality rate in cells (TMM), was then calculated by subtracting P_{net} from P_{gross}. Virus mediated mortality (VMM) and grazing mediated mortality (GMM) was calculated as $VMM = (M_V/M_{V+G})*TMM$ and $GMM = (M_G/M_{V+G})*TMM$, respectively. To convert P_{gross} to gross primary production (C l^{-1} d^{-1}), cells were converted to C using conversion factors of 237 fg C μm^{-3} (Worden et al. 2004) and 196.5 fg C μm^{-3} (Garrison et al. 2000) for pico- and nano-sized phytoplankton, respectively, assuming spherical diameters equivalent to the average cell size determined from size fractionation. To obtain total photosynthetically fixed carbon (PFC), we added 20% for respiration (Langdon 1993, Lopez-Sandoval et al. 2014) and a standard 20% for excretion

(Teira et al. 2003). However, in order to maintain steady-state in the model with respect to net production in the north, excretion was increased to 28%.
Mesozooplankton were collected using a 300 µm vertical net with a 0.35 m^2 opening and grazing rates were determined using the gut fluorescence approach in combination with HPLC pigment analysis (Baars and Oosterhuis 1984). Mesozooplankton were dominated by copepods (95±7% of total counts), with *Acartia clausii* and *Calanus finmarchicus* comprising 49% and 26% of the community, respectively. Mesozooplankton grazing of phytoplankton was considered negligible (< 0.1%) in the south and on average 0.64±0.5% of Chl *a* in the north. To convert to C, a C:Chl *a* ratio of 50 was applied (Brown et al. 1999), this value was shown to give good agreement between carbon estimated from HPLC and FCM analysis (Mojica et al. 2015b). Due to dominance of pico-sized phytoplankton (95% of total), low net primary production and low mesozooplankton grazing rates, sinking was assumed negligible (Jackson 2001; Richardson and Jackson 2007) Although there is evidence of viral-induced mortality for marine zooplankton (Garza and Suttle 1995; Nagasaki et al. 1995; Drake and Dobbs 2005; Massana et al. 2007), actual rates of loss are still largely unknown (current estimates are < 5% of production for copepods) and therefore are not included in the model. The remaining mesozooplankton production was assumed to be ingested by higher trophic levels with 20% of the ingested carbon being transferred to the DOC pool (Jumars et al. 1989) (Table 1).
Heterotrophic prokaryotic abundance, viral mediated mortality and grazing data are reported in Mojica et al. (submitted). In short, heterotrophic prokaryotic production was determined from leucine incorporation rates according to Simon and Azam (1989) and corrected for loss due to viral lysis and grazing (Mojica et al. submitted; assuming the rate of mortality in the samples was equivalent to those measured by the mortality assays and 30% of carbon grazed was retained on filter). Protistian grazing rates of prokaryotes were determined using fluorescently labeled natural prokaryotes (FLP) according to the procedure described by Sherr and Sherr (1987). Protist mediated mortality (PMM) was calculated as $PMM = PA_0 \times (e^{rt}-1)$ where PA is prokaryote abundance, t is time (d), and r is the grazing rate obtained from FLP experiments. Viral production (VP) was determined according to Winget et al. (2005). Lytic viral mediated mortality (VMM) was calculated by dividing lytic virus production by a burst size of according to estimates for oligotrophic regions of the ocean (Parada et al. 2006). A conversion factor of 12.4 fg C $cell^{-1}$ was used for heterotrophic bacteria (Fukuda et al. 1998). In order to maintain steady-state in the model with respect to heterotrophic prokaryotic production, net C production was

balanced as loss through excretion (Stoderegger and Herndl 1998, 2001), which were 4 and 16% for the south and north, respectively.

For the zooplankton, literature values were used for the growth efficiencies and fraction of C shunted to dead particulate and dissolved matter (P/DOC) pool (Table 1). Alternative modes of grazer-associated release include "sloppy feeding", excretion, and egestion and dissolution of fecal pellets. It is estimated that around 50% of the food ingested by microzooplankton is lost to respiration, which is consistent with a growth efficiency of around 30% (Calbet and Landry 2004). Twenty percent of the carbon intake by microzooplankton was considered to be partitioned to the P/DOC pool due to the combination of direct excretion (max 9%; Taylor et al. 1985) and rapid dissolution of small fecal pellets (Turner 2002; Buck et al. 2005), while sloppy feeding by microzooplankton was considered negligible (Møller et al. 2003; Møller 2005; Saba et al. 2011). We assume that all available microzooplankton biomass is grazed by mesozooplankton, with 24% of ingested C lost to DOC (3% by sloppy feeding, 12% excretion and 9% fecal pellet DOC released within in the euphotic zone; Møller et al. 2003; Urban-Rich et al. 2004; Møller 2005; Saba et al. 2011) and mesozooplankton gross growth efficiency was 0.25 (Straile 1997).

Table 1. Measured variables and model parameters applied in steady state C-flux model presented in Figure 2 and 3.

Variables			
α	Phytoplankton C production		
α_V	Phytoplankton C lysed		
α_{G1}	Phytoplankton C grazed by microzooplankton		
α_{G2}	Phytoplankton C grazed by mesozooplankton		
β	Bacterial C production		
β_V	Bacterial C lysed		
β_G	Bacterial C grazed by microzooplankton		
γ	Microzooplankton C production		
γ_G	Microzooplankton C grazed		
ζ	Mesozooplankton C production		
η	Higher trophic level C production		
P/DOC	Dissolved organic carbon from all sources and particulate cell debris C resulting from viral lysis		
Parameters			**References**
α_E	Phytoplankton C lost to excretion*	0.2	(Teira et al. 2003)
β_E	Bacterial C lost to excretion*	0.04	(Stoderegger and Herndl 1998; Stoderegger and Herndl 2001; Kawasaki and Benner 2006)
GGE_γ	Gross growth rate efficiency of microzooplankton	0.3	(Straile 1997; Calbet and Landry 2004)
φ_1	Fraction of C cycled to the P/DOC pool from microzooplankton activity	0.2	(Taylor et al. 1985; Turner 2002; Buck et al. 2005)
GGE_ζ	Gross growth rate efficiency of mesozooplankton	0.25	(Straile 1997)
φ_2	Fraction of C cycled to P/DOC pool from excretion, pellet dissolution and sloppy feeding of mesozooplankton	0.24	(Møller et al. 2003; Urban-Rich et al. 2004; Møller 2005; Saba et al. 2011)
GGE_η	Gross growth rate efficiency of higher trophic levels	0.15	(Houde 1989)
φ_3	Fraction of C cycled to P/DOC pool from higher trophic levels	0.2	(Jumars et al. 1989)

* to force steady state values differ in northern region

Results

The upper surface waters of the Northeast Atlantic Ocean along the meridional transect between 30 - 63°N were characterized by strong temperature-induced vertical stratification. The southern half of the transect (< 45°N) was distinguished by oligotrophic surface waters (i.e., Chl *a* < 0.7 µg l^{-1} and NO_3^- ≤ 0.13 and PO_4^{3-} ≤ 0.03 µM; Polovina et al. 2008; van de Poll et al. 2013) (Figure 1). In the northern half (46 - 63°N). In the north, inorganic nutrients and Chl *a* concentrations increased within the ML to average 1.30±0.60 µM, 0.14±0.04 µM and 1.1±0.3 µg $l^{-1}$for NO_3^-,

PO_4^{3-} and Chl *a*, respectively. Consequently, the distribution and composition of microbial communities varied between these two regions (Mojica et al. 2015b; Mojica and Brussaard submitted) and accordingly regions are presented separately here.

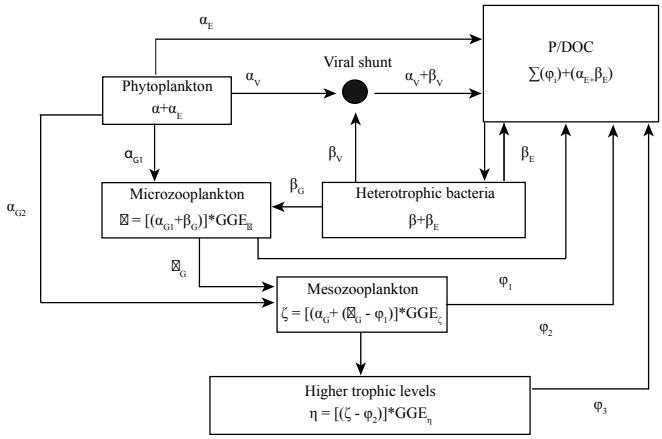

Figure 2. Flow diagram of steady state carbon flux model through a pelagic food web. See Table 1 for more explanation of variables, parameters and symbols. Carbon lost to respiration is included but not explicitly illustrated.

PFC in the oligotrophic south averaged 10.6 µg C l^{-1} d^{-1}, while in the north PFC was about 4-fold higher (47.2 µg C l^{-1} d^{-1}) as a result of 2-fold higher total phytoplankton biomass (larger contribution of nanoeukaryotic phytoplankton (Figure 2 and 3) (Mojica et al. 2015b). Viral lysis was the dominate loss factor for phytoplankton C in the south with an average 3.7 µg C l^{-1} d^{-1} being shunted into the P/DOM pool compared to 2.7 µg C l^{-1} d^{-1} being grazed (Table 1, Figure 3a). In the north, microzooplankton grazing C-flux was higher than viral lysis, accounting for 11.5 µg C l^{-1} d^{-1} of PFC loss compared to 9.9 µg C l^{-1} d^{-1} being lysed (Figure 3b). Heterotrophic prokaryotic production was 9.0 µg C l^{-1} d^{-1} in the south, with 4.8 µg C l^{-1} d^{-1} lost to viral lysis and 3.8 µg C l^{-1} d^{-1} being grazed by microzooplankton. In the north, production was only slightly higher at 10.4 µg C l^{-1} d^{-1} and viral lysis remained the dominate loss factor, with 5.7 µC l^{-1} d^{-1} being shunted to the P/DOC pool compared to 3.0 µg C l^{-1} d^{-1} being transferred to microzooplankton.

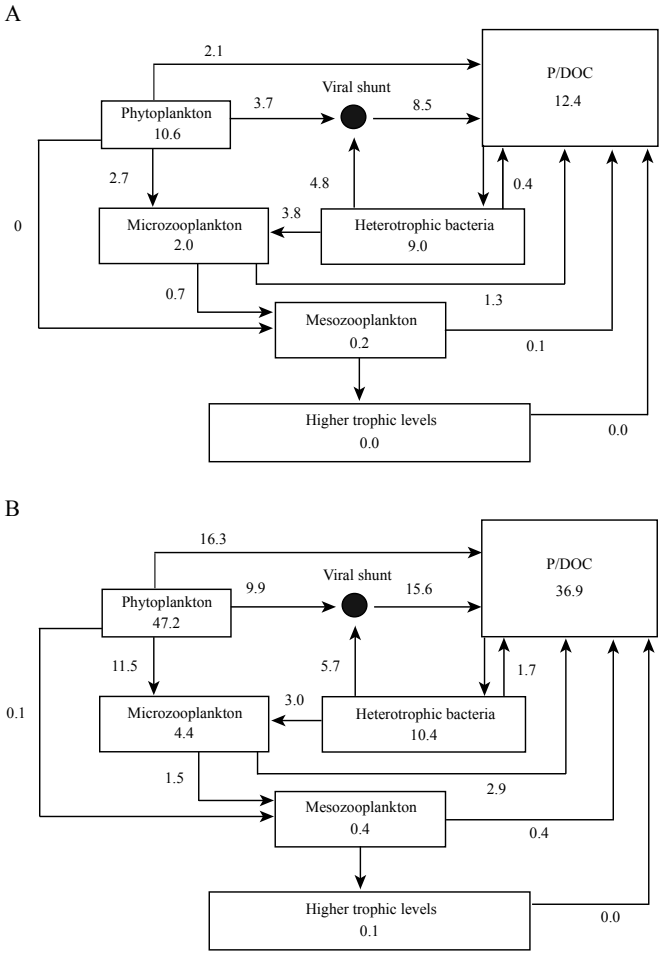

Figure 3. Carbon flow (μg C l^{-1} d^{-1}) through a pelagic food web in steady state for both the (**A**) southern (30 - 45°N) and (**B**) northern region (45 - 63°N) region of the Northeast Atlantic Ocean during the summer STRATIPHYT cruise. See Table 1 for explanation of variables, parameters and symbols, and Figure 2 for equations. Carbon lost to respiration is included but not explicitly illustrated.

Total microzooplankton production and the contribution by microzooplankton to the P/DOM pool was ~ 2-fold higher in the south compared to the north (i.e., 2.0 and 1.3 μg C l^{-1} d^{-1} compared to 4.4 and 2.9 μg C l^{-1} d^{-1}) (Figure 3a and b). DOC release by mesozooplankton was 0.1 and 0.4 μg C l^{-1} d^{-1} for the south and north, respectively. The P/DOC contribution from higher trophic levels was then 0.04 (taken as zero) and 0.06 (taken as 0.1) μg C l^{-1} d^{-1} for the south and north respectively, assuming all zooplankton production was consumed.

Discussion

The steady state assumption of our model was supported in the southern region, with 100% and 95% of the phytoplankton and prokaryotic heterotrophic cellular production being lost through the combined mortality of grazing and viral lysis (Figure 3a; excluding respiration and excretion). However, the north demonstrated a net production of 14% and 16% for phytoplankton and bacteria, respectively, when steady state was not enforced via increasing excretion. Nevertheless, values of excretion remained within the range reported in literature (Stoderegger and Herndl 1998; Stoderegger and Herndl 2001; Kawasaki and Benner 2006) and confer with evidence that rates increase with productivity (Baines and Pace 1991). The ratios of prokaryotic heterotrophic production to primary production (HP:PP) in our study (0.85 and 0.22 for south and north, respectively) are relatively high (Ducklow 1999). However, they are comparable to HP:PP reported for the North Atlantic by Hoppe et al. (2002) (0.01 - 0.83), who found also the highest values in subtropical regions. HP:PP is dependent upon the conversion efficiency and the degree of recycling and therefore can theoretically exceed 1.0 when recycling is intense (Ducklow et al. 2002). The source of dissolved and dead particulate dead matter, whether through passive diffusion across phytoplankton cell membranes, actively excreted, released from sloppy feeding, diffused from fecal pellets or released from viral lysis, affects both the chemical composition and bioavailability (Middelboe and Jorgensen 2006; Kirchman et al. 2013; Lønborg et al. 2013). Taking into considering dominance of viral lysis as a loss factor for both heterotrophic and autotrophic production within the oligotrophic region (thus prokaryotic C-demand is not restricted to excretion and DOC released from grazing activity) and recent evidence for a diverse array of enzymatic capabilities within bacteria in subtrophic regions (Arnosti et al. 2011), together may help explain why open ocean areas can have bacterial carbon demands that exceed primary production estimates. Furthermore, the steady-state model does not account for excretion by standing stock populations which would also be utilized to support the bacterial carbon demand.

The recognition for the importance of the 'viral shunt' to nutrient cycles and energy flow in the ocean was supported through the use of (mostly) theoretical models (Fuhrman 1992; Wilhelm and Suttle 1999). Simultaneous measurements of growth and loss rate rates for phytoplankton as well as heterotrophic bacteria provide an ideal dataset to further substantiate the role of the viral shunt in marine systems.

Our study confirms the relevance of the viral shunt for diverting energy and biomass away from the classical grazer-mediated food web towards microbial-mediated recycling and the dissolved organic matter pool (Figure 4). More importantly, our data show that the percentage of PFC in stratified waters which was recycled back to the P/DOC pool by viral lysis, 33 and 80% (Figure 4), was substantially higher than the previous estimates of 6 - 26% (Wilhelm and Suttle 1999). These former estimates consisted of a 2 - 10% contribution of PFC from the viral-induced mortality of phytoplankton and 3 - 15% from heterotrophic bacteria. Our data show higher values for both groups, i.e., 35 and 45% for the southern and 21 and 12% for the northern phytoplankton and heterotrophic prokaryotes, respectively (Figure 4), with the strongest increase in flux of PFC from phytoplankton lysis. Considering that the mortality of zooplankton due to viral infection was not accounted for here (Garza and Suttle 1995; Nagasaki et al. 1995; Drake and Dobbs 2005; Massana et al. 2007), these values likely still represent an underestimate. Overall, our results indicate that during summer stratification in the northeastern Atlantic Ocean the viral shunt plays a significant role in marine food web dynamics, particularly in the oligotrophic region.

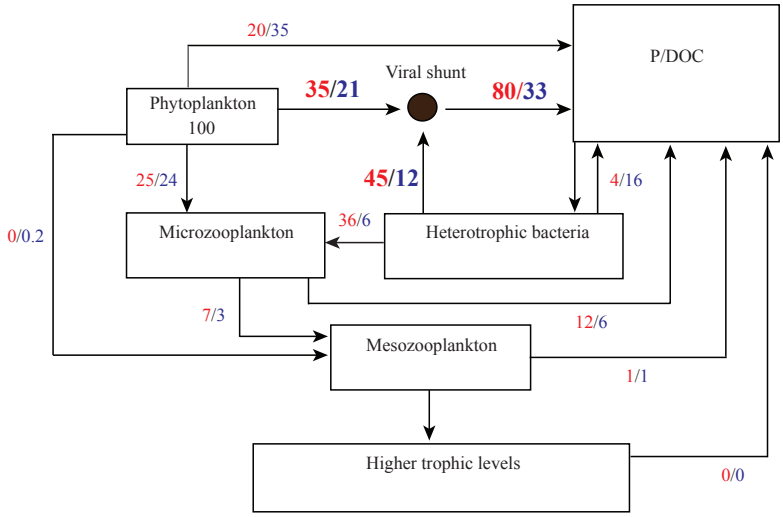

Figure 4. The C-flux for the pelagic food web for both the oligotrophic south (red) and the northern region (blue) of the Northeast Atlantic Ocean cruise transect. Fluxes are indicated as percentage of total photosynthetically fixed carbon (100%). The percentage of photosynthetically fixed carbon flowing through the viral shunt is indicated in large bold print. All carbon is assumed to be eventually respired, with negligible loss due to export. This steady state model also assumes that all carbon in the P/DOC pool is bioavailable to heterotrophic prokaryotes.

Due to deep water formation, the North Atlantic is key to ocean circulation and global climate (Sabine et al. 2004). Several studies predict that global warming will result in a stronger temperature-induced vertical stratification and subsequent oligotrophication in the North Atlantic Ocean (Sarmiento 2004; Polovina et al. 2008). Consequently, changes in phytoplankton community structure are anticipated, e.g. enhanced dominance of smaller-sized phytoplankton (Mojica et al. in press) and northward expansion of (sub)tropical photoautotrophs such as the cyanobacterium *Prochlorococcus* spp. (expanding up to 50°N in the year 2100; Flombaum et al. 2013). The C-flux model presented here indicates that this will enhance the role of the microbial loop due to an amplified viral shunt, increased microzooplankton grazing on heterotrophic prokaryotes and tighter coupling between P/DOC and heterotrophic production. The partitioning of photosynthetic C through the different pathways (i.e., grazing versus cell lysis) has important implications for ecosystem function as each pathway differentially affects the structure and functioning of pelagic microbial food webs. Grazing transfers matter to higher trophic levels, thereby increasing the overall efficiency and carrying capacity of the ecosystem. In addition, the production of fecal pellets by mesozooplankton in the open ocean is responsible for much of the carbon transported out of the euphotic zone into the deeper ocean (Ducklow et al. 2001). A more prominent role of the viral shunt in the northern North Atlantic Ocean would thus markedly reduce biological C-export into the ocean's interior in one of the key areas of global C-sequestration, and reduce the potential for it to function as a long-term sink for anthropogenic carbon dioxide.

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References

Arnosti C, Steen AD, Ziervogel K, Ghobrial S, Jeffrey WH (2011) Latitudinal gradients in degradation of marine dissolved organic carbon. PLOS One 6: e28900. doi: 10.1371/journal.pone.0028900

Baars MA, Oosterhuis SS (1984) Diurnal feeding rhythms in North Sea copepods measured by gut fluorescence, digestive enzyme activity and grazing on labeled food. Netherlands Journal of Sea Research 18:97-119

Baines SB, Pace ML (1991) The production of dissolved organic-matter by phytoplankton and its importance to bacteria: patterns across marine and fresh water systems. Limnology and Oceanography 36:1078-1090

Brown SL, Landry MR, Barber RT, Campbell L, Garrison DL, Gowing MM (1999) Picophytoplankton dynamics and production in the Arabian Sea during the 1995 Southwest Monsoon. Deep-Sea Research, Part II 46:1745-1768

Brussaard CPD, Payet JP, Winter C, Weinbauer M (2010) Quantification of aquatic viruses by flow cytometry. In: Wilhelm SW, Weinbauer MG, Suttle CA (eds) Manual of Aquatic Viral Ecology. ASLO

Buchan A, LeCleir GR, Gulvik CA, Gonzalez JM (2014) Master recyclers: features and functions of bacteria associated with phytoplankton blooms. Nature Reviews Microbiololgy 12:686-698

Buck KR, Marin R, Chavez FP (2005) Heterotrophic dinoflagellate fecal pellet production: grazing of large, chain-forming diatoms during upwelling events in Monterey Bay, California. Aquatic Microbial Ecology 40:293-298

Calbet A, Landry MR (2004) Phytoplankton growth, microzooplankton grazing, and carbon cycling in marine systems. Limnology and Oceanography 49:51-57

Drake LA, Dobbs FC (2005) Do viruses affect fecundity and survival of the copepod *Acartia tonsa* Dana? Journal of Plankton Research 27:167-174

Ducklow HW (1999) The bacterial component of the oceanic euphotic zone. FEMS Microbiology Ecology 30:1-10

Ducklow HW, Kirchman DL, Anderson TR (2002) The magnitude of spring bacterial production in the North Atlantic Ocean. Limnology and Oceanography 47:1684-1693

Ducklow HW, Steinberg DK, Buesseler KO (2001) Upper ocean carbon export and the biological pump. Oceanography 14:50-58

Flombaum P, Gallegos JL, Gordillo RA, Rincon J, Zabala LL, Jiao N, Karl DM, Li WKW, Lomas MW, Veneziano D, Vera CS, Vrugt JA, Martiny AC (2013) Present and future global distributions of the marine cyanobacteria *Prochlorococcus* and *Synechococcus*. Proceedings of the National Academy of Sciences of the United States of America 110:9824-9829

Fuhrman JA (1992) Bacterioplankton roles in cyling of organic matter: the microbial food web. In: Falkowski PG, Woodhead AD (eds) Primary Productivity and Biogeochemical Cycles in the Sea. Plenum Press, New York, NY

Fuhrman JA (1999) Marine viruses and their biogeochemical and ecological effects. Nature 399:541-548

Fukuda R, Ogawa H, Nagata T, Koike I (1998) Direct determination of carbon and nitrogen contents of natural bacterial assemblages in marine environments. Applied and Environmental Microbiology 64:3352-3358

Garrison DL, Gowing MM, Hughes MP, Campbell L, Caron DA, Dennett MR, Shalapyonok A, Olson RJ, Landry MR, Brown SL, Liu HB, Azam F, Steward GF, Ducklow HW, Smith DC (2000) Microbial food web structure in the Arabian Sea: a US JGOFS study. Deep-Sea Research, Part II 47:1387-1422

Garza DR, Suttle CA (1995) Large double-stranded DNA viruses which cause the lysis of a marine heterotrophic nanoflagellate (*Bodo* sp) occur in natural marine viral communities. Aquatic Microbial Ecology 9:203-210

Hoppe HG, Gocke K, Koppe R, Begler C (2002) Bacterial growth and primary production along a north-south transect of the Atlantic Ocean. Nature 416:168-171

Houde ED (1989) Comparative growth, mortality, and energetics of marine fish larvae: temperature and implied latitudinal effects. Fishery Bulletin 87:471-495

Jackson GA (2001) Effect of coagulation on a model planktonic food web. Deep-Sea Research Part I 48:95-123

Jumars PA, Penry DL, Baross JA, Perry MJ, Frost BW (1989) Closing the microbial loop: dissolved carbon pathway to heterotrophic bacteria from incomplete ingestion, digestion and absorption in animals. Deep-Sea Research 36:483-495

Kawasaki N, Benner R (2006) Bacterial release of dissolved organic matter during cell growth and decline: molecular origin and composition. Limnology and Oceanography 51:2170-2180

Kimmance SA, Brussaard CPD (2010) Estimation of viral-induced phytoplankton mortality using the modified dilution method. In: Wilhelm SW, Weinbauer M, Suttle CA (eds) Manual of Aquatic Viral Ecology. ASLO

Kirchman DL, Lancelot C, Fasham M, Legendre L, Radach G, Scott M (2013) Dissolved organic matter in biogeochemical models of the ocean. In: Evans GT, Fasham MJR (eds) Towards a Model of Ocean Biogeochemical Processes. Springer Berlin Heidelberg

Langdon C (1993) The significance of respiration in production measurements based on oxygen. ICES Marine Science Symposium 197:69-78

Lønborg C, Middelboe M, Brussaard CPD (2013) Viral lysis of *Micromonas pusilla*: impacts on dissolved organic matter production and composition. Biogeochemistry 116:231-240

López-Sandoval DC, Rodríguez-Ramos T, Cermeño P, Sobrino C, Marañón E (2014) Photosynthesis and respiration in marine phytoplankton: Relationship with cell size, taxonomic affiliation, and growth phase. Journal of Experimental Marine Biology and Ecology 457:151-159

Marie D, Brussaard CPD, Thyrhaug R, Bratbak G, Vaulot D (1999) Enumeration of marine viruses in culture and natural samples by flow cytometry. Applied and Environmental Microbiology 65:45-52

Marie D, Simon N, Vaulot D (2005) Phytoplankton cell counting by flow cytometry. In: Andersen RA (ed) Algal Culturing Techniques. Elsevier Academic Press: Burlington, MA

Massana R, del Campo J, Dinter C, Sommaruga R (2007) Crash of a population of the marine heterotrophic flagellate *Cafeteria roenbergensis* by viral infection. Environmental Microbiology 9:2660-2669

Middelboe M, Jørgensen NOG (2006) Viral lysis of bacteria: an important source of dissolved amino acids and cell wall compounds. Journal of the Marine Biological Association of the United Kingdom 86:605-612

Middelboe M, Lyck PG (2002) Regeneration of dissolved organic matter by viral lysis in marine microbial communities. Aquatic Microbial Ecology 27:187-194

Mojica KDA, Evans C, Brussaard CPD (2014) Flow cytometric enumeration of marine viral populations at low abundances. Aquatic Microbial Ecology 71:203-209

Mojica KDA, Huisman J, Wilhelm SW, Brussaard CPD (2015a) Latitudinal variation in virus-induced mortality of phytoplankton across the North Atlantic Ocean. The ISME Journal, *in press*

Mojica KDA, van de Poll WH, Kehoe M, Huisman J, Timmermans KR, Buma AGJ, van der Woerd HJ, Hahn-Woernle L, Dijkstra HA, Brussaard CPD (2015b). Phytoplankton community structure in relation to vertical stratification along a north-south gradient in the Northeast Atlantic Ocean. Limnology and Oceanography (*in press*). doi: 10.1002/lno.10113

Møller EF (2005) Sloppy feeding in marine copepods: prey-size-dependent production of dissolved organic carbon. Journal of Plankton Research 27:27-35

Møller EF, Thor P, Nielsen TG (2003) Production of DOC by *Calanus finmarchicus*, *C. glacialis* and *C. hyperboreus* through sloppy feeding and leakage from fecal pellets. Marine Ecology Progress Series 262:185-191

Nagasaki K, Ando M, Imai I, Itakura S, Ishida Y (1995) Virus-like particles in unicellular apochlorotic microorganisms in the coastal water of Japan. Fisheries Science 61:235-239

Parada V, Herndl GJ, Weinbauer MG (2006) Viral burst size of heterotrophic prokaryotes in aquatic systems. Journal of the Marine Biological Association of the United Kingdom 86:613-621

Polovina JJ, Howell EA, Abecassis M (2008) Ocean's least productive waters are expanding. Geophysical Research Letters 35:L03618. doi: 10.1029/2007GL031745

Richardson TL, Jackson GA (2007) Small phytoplankton and carbon export from the surface ocean. Science 315:838-840

Saba GK, Steinberg DK, Bronk DA (2011) The relative importance of sloppy feeding, excretion, and fecal pellet leaching in the release of dissolved carbon and nitrogen by *Acartia tonsa* copepods. Journal of Experimental Marine Biology and Ecology 404:47-56

Sabine CL, Feely RA, Gruber N, Key RM, Lee K, Bullister JL, Wanninkhof R, Wong CS, Wallace DWR, Tilbrook B, Millero FJ, Peng TH, Kozyr A, Ono T, Rios AF (2004) The oceanic sink for anthropogenic CO_2. Science 305:367-371

Sarmiento JL (2004) Response of ocean ecosystems to climate warming. Global Biogeochemical Cycles 18:GB3003. doi: 10.1029/2003GB002134

Schlitzer R (2002) Interactive analysis and visualization of geoscience data with Ocean Data View. Computers and Geosciences 28:1211-1218

Sherr BF, Sherr EB, Fallon RD (1987) Use of monodispersed, fluorescently labeled bacteria to estimate *in situ* protozoan bacterivory. Applied and Environmental Microbiology 53:958-965

Simon M, Azam F (1989) Protein content and protein synthesis rates of planktonic marine bacteria. Marine Ecology Progress Series 51:201-213

Stoderegger K, Herndl GJ (1998) Production and release of bacterial capsular material and its subsequent utilization by marine bacterioplankton. Limnology and Oceanography 43:877-884

Stoderegger KE, Herndl GJ (2001) Visualization of the exopolysaccharide bacterial capsule and its distribution in oceanic environments. Aquatic Microbial Ecology 26:195-199

Straile D (1997) Gross growth efficiencies of protozoan and metazoan zooplankton and their dependence on food concentration, predator-prey weight ratio, and taxonomic group. Limnology and Oceanography 42:1375-1385

Suttle CA (2007) Marine viruses - major players in the global ecosystem. Nature Reviews 5:801-812

Taylor GT, Iturriaga R, Sullivan CW (1985) Interactions of bactivorous grazers and heterotrophic bacteria with dissolved organic matter. Marine Ecology Progress Series 23:129-141

Teira E, Pazo MJ, Quevedo M, Fuentes MV, Niell FX, Fernandez E (2003) Rates of dissolved organic carbon production and bacterial activity in the eastern North Atlantic Subtropical Gyre during summer. Marine Ecology Progress Series 249:53-67

Turner JT (2002) Zooplankton fecal pellets, marine snow and sinking phytoplankton blooms. Aquatic Microbial Ecology 27:57-102

Urban-Rich J, McCarty JT, Shailer M (2004) Effects of food concentration and diet on chromophoric dissolved organic matter accumulation and fluorescent composition during grazing experiments with the copepod *Calanus finmarchicus*. ICES Journal of Marine Science 61:542-551

van de Poll WH, Kulk G, Timmermans KR, Brussaard CPD, van der Woerd HJ, Kehoe MJ, Mojica KDA, Visser RJW, Rozeman PD, Buma AGJ (2013) Phytoplankton chlorophyll *a* biomass, composition, and productivity along a temperature and stratification gradient in the northeast Atlantic Ocean. Biogeosciences 10:4227-4240

Weitz JS, Wilhelm SW (2012) Ocean viruses and their effects on microbial communities and biogeochemical cycles. F1000 Biology Reports 4: 17

Wilhelm SW, Suttle CA (1999) Viruses and nutrient cycles in the sea - Viruses play critical roles in the structure and function of aquatic food webs. Bioscience 49:781-788

Winget DM, Williamson KE, Helton RR, Wommack KE (2005) Tangential flow diafiltration: and improved technique for estimation of virioplankton production. Aquatic Microbial Ecology 41:221-232

Worden AZ, Nolan JK, Palenik B (2004) Assessing the dynamics and ecology of marine picophytoplankton: The importance of the eukaryotic component. Limnology and Oceanography 49:168-179