Viral-host interactions:

from strain to natural planktonic communities

Eliana Ruiz Martínez

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Author: Eliana Ruiz Martínez

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"To stand at the edge of the sea, to sense the ebb and flow of the tides, to feel the breath of a mist moving over a great salt marsh, to watch the flight of shore birds that have swept up and down the surf lines of the continents for untold thousands of years, to see the running of the old eels and the young shad to the sea, is to have knowledge of things that are as nearly eternal as any earthly life can be."

Rachel Carson

Scientific environment

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Abstract

Being the most abundant and diverse entities in planet Earth, viruses are thought to play a relevant role in controlling the composition and diversity in phytoplanktonic microbial communities. Microbial communities sustain life in the oceans and even in terrestrial environments if we account for half of the oxygen in the atmosphere, which is produced by their photosynthetic members. Thus, understanding how viruses and their hosts interact at the vast oceanic scale, and the potential impact viruses might have on the development of marine microbial communities, remain of primary relevance.

To what extent do viruses exert a significant pressure on the microbial communities they infect? To what extent does that interaction lead to the existence of a variety of "virus-driven" trade-offs between host traits, such as resistance and growth capacity? Despite the progress that has been in this area, especially with prokaryotes, we still lack assertive answers to these questions. This thesis aims to increase the current knowledge on marine viral role and their potential action in shaping marine microbial communities.

To do so, cross-infectivity experiments were conducted and parameters such as growth rate (μ), resistance (R), and viral production (Vp), were investigated for two relevant eukaryotic phytoplankton systems: *Micromonas* / *Micromonas* Virus (MicV) (**Paper I**) and *Emiliania huxleyi* / *Emiliania huxleyi* Virus (EhV) (**Paper II**), respectively. Competition experiments between *Micromonas* strains with different resistance capacities and similar growth rate were also performed (Chapter 4.1). Viral impact was also measured at the broad level of complex natural marine microbial communities with six viral depletion microcosm experiments (**Paper III**).

The significant trends observed on single virus-host interactions demonstrated strong co-interactions at different levels between the tested phytoplankton strains and their viruses; however, a potential viral role as major drivers behind a growth-rate/resistance trade-off was not consistently observed in any of the studied systems

(**Paper I**, **Paper II**). In 4 out of 7 competition experiments was such trade-off possibly present, but even then not in an explicit manner. Surprisingly, higher viral production capacities were measured in generalist viral strains from both systems (**Paper I**, **Paper II**). For the viral depletion experiments (**Paper III**), the incubation period itself was sufficient to provoke significant changes in the composition of the microbial communities under study; however, viral impact was significant in half of the experiments, mostly in the prokaryotic community.

Overall, this work challenges the conception of viruses as main drivers of marine microbial diversity, emphasizing the need for more knowledge about virus-host interactions in the oceans.

List of publications

Paper I

Ruiz, E., Baudoux, A.-C., Simon, N., Sandaa, R.-A., Thingstad, T.F., and Pagarete, A. (2017b). Micromonas versus virus: New experimental insights challenge viral impact. *Environmental Microbiology* 19, 5: 2068–2076.

Paper II

Ruiz, E., Oosterhof, M., Sandaa, R.-A., Larsen, A., and Pagarete, A. (2017). Emerging Interaction Patterns in the Emiliania huxleyi-EhV System. Viruses 9, 3: 61.

Paper III

Ruiz, E., Lindivat, M. and Pagarete, A. (manuscript). Inconsistent viral impact on natural marine microbial communities.

Contents

List of abbreviations

ARISA – Automated rRNA intergenic spacer analysis

COR – Cost of Resistance

DOM – Dissolved organic matter

EhV- *Emiliania huxleyi* virus

GFG – Gene for gene

HGT – Horizontal gene transfer

KtW – Killing the Winner

MA – Matching alleles

MpV – *Micromonas* virus

NCLDV - Nucleo-Cytoplasmic Large DNA Virus

POM – Particulate organic matter

rRNA – Ribosomic RNA

TRFLP – Terminal restriction fragment length polymorphism

VHIN – Virus-host interaction network

1. Introduction

"Would you learn the secret of the sea?

Only those who brave its dangers comprehend its mystery!"

Henry Wedsworth Longfellow.

Oceans have always been surrounded by an aura of mystery and even today, lots of secrets wait to be discovered. Just in a drop of surface seawater, millions of imperceptible organisms can be found under the microscope. Among these, the most abundant ones are viruses (Bergh *et al.*, 1989). Despite being ubiquitous, half of the 10^{31} estimated viruses on Earth (Suttle, 2005) are found in the oceans and underlying sediments (Mitchell and Kirchman, 2008), averaging from 10^4 to 10^8 viruses per millilitre of seawater and 10^9 viruses per gram of soil or sediment (Wommack and Colwell, 2000). However, viral abundance fluctuates in time (Pagarete *et al.*, 2013a; Rozon and Short, 2013; Zingone *et al.*, 1999; Winget and Wommack, 2009) and space (Seymour *et al.*, 2006; Gustavsen *et al.*, 2014; Mojica *et al.*, 2016).

In the pelagic, high rates of viral production are reported, ranging from $2x10^3$ to $3x10^6$ viruses/mL/h (Weinbauer and Rassoulzadegan, 2004), indicating that viruses are effective predators. On average, viruses cause the mortality of about 10 to 40% of prokaryotes (Schwalbach *et al.*, 2004) and around 10% of phytoplankton on a daily basis (Kimmance *et al.*, 2007); however, the complete lysis of phytoplankton species can occur in bloom situations (Bratbak *et al.*, 1993; Brussaard *et al.*, 1996a; Brussaard *et al.*, 1996b). Unlike grazing by protists, which transfers carbon and nutrients to higher trophic levels (Sherr *et al.*, 1984; Fenchel and Fenchel, 1987), viral lysis fuels the microbial loop by releasing POM and DOM (with an average of 3-20 Gt of DOM per year, (Wilhelm and Suttle, 1999)) that can be up taken by heterotrophic and autotrophic organisms. The viral shunt then (Suttle, 2007; Jover *et al.*, 2014), is a viral-mediated recycling of organic matter, which increases microbial

respiration and production (Fuhrman, 1999; Jover *et al.*, 2014). It also decreases the prokaryote-mediated remineralisation of POM that sinks into the deep ocean (Osterberg *et al.*, 1963; Sarmiento and Gruber, 2013), interfering with the Biological Pump (Volk and Hoffert, 1985), the process of carbon sequestration from the atmosphere to the deep sea (Broecker, 1982).

Although viruses are commonly seen as predators that kill their hosts in order to reproduce, some of them act more like parasites introducing their genetic material into host's genome. It is known that both, lytic and lysogenic processes can lead to the movement of genetic information from one lineage of organism to another isolated lineage of organism; or what we call horizontal gene transfer (HGT) (Villarreal, 2005; Clokie *et al.*, 2003).

In general, the specificity of viral-host interaction happens at the strain or species level; the degree to which varies substantially in viruses. Some viruses are so specific that they are known to infect only a very reduced number of host microbial strains (Moebus, 1992; Moebus and Nattkemper, 1981), while others have been shown to have very broad host-ranges (Sullivan *et al.*, 2003; Wichels *et al.*, 1998; Holmfeldt *et al.*, 2007) (**Paper I, Paper II**). Inevitably, given the very probable existence of at least one viral type for every cellular form on the planet, viral evolution did require that some viruses have the capacity to cross species boundaries. This has also been observed in some phytoplankton systems (Johannessen *et al.*, 2015). Viral lysis is often positively correlated to host's abundance and physiological state (Middelboe, 2000) however, recent studies have shown that virus to microbial abundance relationship, is not linear (Wigington *et al.*, 2016). Particular species or strains should, at least in theory, become more exposed to infection as their density increases, in what would be a simple density-driven process. A consequence of that process would be that faster-growing/fitter/less resistant microbes would be under stronger viral pressure that would allow more resistant/slow-growers to have their ecological niche. Thus, viruses would not only influence the biogeochemical processes in the ocean, but also be fundamental for the unexpectedly high microbial diversity levels observed in marine planktonic communities; enunciated by

Hutchinson in the famous paradox that took his name (Hutchinson, 1961) (**Paper III**).

Due to methodological constrains like sampling, isolation, and maintenance of host-virus systems in the laboratory, less than 1% of the extant viral diversity has been explored so far (Mokili *et al.*, 2012). The fact that 1 out of 100 microbes can be cultivated, known as the great plate-count anomaly (Staley, 1985), significantly conditions what we know about virus-microbe interactions in the oceans. Moreover, there is a strong knowledge bias towards the prokaryotic realm. Infection patterns, lysis rates, viral production, host specificity, viral resistance, life strategies, and co-evolutive patterns have been more extensively studied in prokaryotes and their respective viruses than in eukaryotic systems. Hence viral-host interaction models, like "Killing the Winner" (KtW) (Thingstad, 2000; Thingstad and Lignell, 1997), have been mostly developed based on data on prokaryotic phages. Empirical evidence of the role that viruses might have shaping eukaryotic phytoplankton fitness is even scarcer (**Paper I**, **Paper II**); as it is also scarce the evidence for viruses being main drivers of microbial community diversity (**Paper III**).

Further knowledge on viral-host interactions will increase the insight on co-evolutive processes that help to create and maintain microbial diversity in the oceans. A better understanding of the functioning of the marine environment will ultimately improve current models, used to study and predict the dynamics of marine microbial populations, being a key role for future climate change prospective.

2. Aims of the project

The overall aim of this PhD project is to increase the knowledge on life-strategy patterns that emerge from microbe-virus interactions in the marine planktonic realm, along with potential viral-driven trade-offs on marine microbial forms. The study covers the different levels of virus-host system complexity by focusing on two specific eukaryotic algal hosts *Micromonas* (*Prasinophyceae*) and *Emiliania huxleyi* (*Primnesiophyceae*), and their respective viruses (*Phycodnaviridae*). Secondary objectives are:

- To obtain empirical values for key parameters like growth rate, resistance and viral production, in two ecologically-relevant marine phytoplanktonic groups, and compare these with existing theoretical hypotheses and experimental data in order to establish correlations of emerging patterns after viral infection (**Papers I** and **II**).

- To determine whether, or not, there is a cost of resistance to viral infection in marine eukaryotic phytoplankton (**Papers I** and **II**).

- To link the obtained interaction patterns from single host-viral pairs to complex populations (**Papers I, II** and **III**).

- To empirically test the hypothesis that viruses are fundamental drivers of diversity in natural marine microbial communities (**Paper III**).

3. Background: The virus- host systems.

"Viruses are capable of creating complex genes all by themselves. For the most part these are stitched together from bits and pieces, mainly from other viruses. The oceans are filled with viruses like these. What I am saying is that what we are whitnessing is genetic creativity on a very large scale, a kind of biological big bang." *Luis Villarreal.*

3.1 The *Phycodnaviridae*

Even if eukaryote-infecting marine viruses are less well known than phages, some of the most studied ones are the *Phycodnaviridae.* This family consists of a genetically diverse, but morphologically and structurally similar family of large (mean diameter of 160±60 nm) icosahedral viruses that infect marine or freshwater eukaryotic algae (Dunigan *et al.*, 2006). These lytic or lysogenic viruses are ubiquitous in nature and contain large linear, or circular, dsDNA genomes ranging from 160 to 560 kb (Wilson *et al.*, 2009).

The family is divided into six genera, named after the host groups they infect (*Chlorovirus*, *Coccolithovirus*, *Prasinovirus*, *Prymnesiovirus*, *Phaeovirus* and *Raphidovirus*), and has a monophyletic branching inside a wider group of Nucleo-Cytoplasmic Large DNA Viruses group (NCLDVs) (Iyer *et al.*, 2001; Wilson *et al.*, 2009; Iyer *et al.*, 2006) (**Fig. 1**). NCLDVs replicate, completely or partly, in the cytoplasm of their hosts (Iyer *et al.*, 2001). Recent studies, however, suggest a reclassification of the families that form the NCLDVs group, into the new order Megavirales (Colson *et al.*, 2012; Colson *et al.*, 2013). The readjustment also applies to the *Phycodnaviridae* family, as some of the members of this family are more similar to the Mimiviridae family in terms of genetic components, viral life cycle, and evolutionary relatedness (Maruyama and Ueki, 2016) (**Fig. 2**).

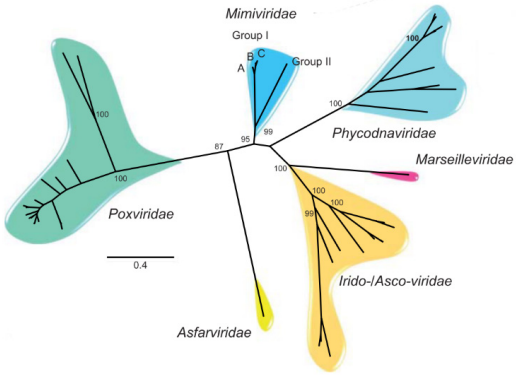


Figure 1: Phylogeny reconstruction from a cured concatenated alignment of universal NCVOGs [including primase-helicase (NCVOG0023), DNA polymerase (NCVOG0038), packaging ATPase (NCVOG0249), and A2L-like transcription factor (NCVOG0262)] for the giant viruses currently classified as NCLDVs. Adapted from Colson *et al.* (2012).

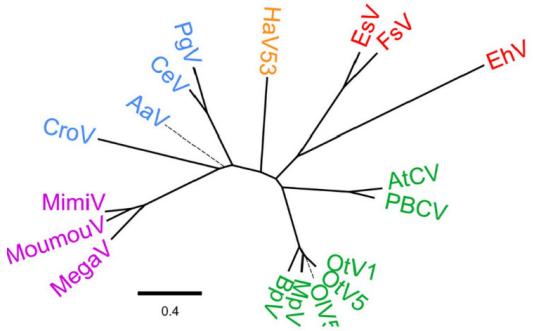


Figure 2: Phylogenetic relationships of *Phycodnaviridae* and Megaviridae. Phylogenetic tree based on the concatenated nine core gene-encoded protein sequences. Adapted from Maruyama and Ueki (2016).

The experimental work presented in this thesis focuses on two ecologically important genera of the *Phycodnaviridae* family: *Prasinoviruses* and *Coccolithoviruses* (**Papers I** and **II,** respectively).

3.1.1 Prasinoviruses

Prasinoviruses (**Paper I**) are abundant and widespread (Bellec *et al.*, 2010; Short and Short, 2008; Park *et al.*, 2011; Hingamp *et al.*, 2013; Zhong and Jacquet, 2014; Cottrell and Suttle, 1991). Their genomes size ranges 184-191 kb and the capsids are around 130-135 nm in dimeter (Mayer and Taylor, 1979; Martinez *et al.*, 2015). The latent period is about 7-14h and the average burst size of 72 (Waters and Chan, 1982).

Prasinoviruses infect the picoeukaryotic algal class Mamiellophyceae (Marin and Melkonian, 2010), which includes the three dominant genera *Bathycoccus*, *Micromonas* and *Ostreococcus*. The genera *Micromonas* dominates coastal picoeukaryotic communities in a wide range of marine environments (Knightjones and Walne, 1951; Thomsen and Buck, 1998a; Thomsen and Buck, 1998b; Not *et al.*, 2004) and represents an important contributor to global primary production (Marañón *et al.*, 2001; Worden *et al.*, 2004). Recent phylogenetic studies have demonstrated the existence of at least three major *Micromonas* clades, A, B and C (van Baren *et al.*, 2016). *Micromonas* cells are recurrently infected by species-, or even strain-specific, viruses (MicVs) as reported from several different marine ecosystems (Sahlsten, 1998).

It has been estimated that MicVs can lyse up to 25% of their host population on a daily basis (Baudoux *et al.*, 2015). MicVs also present a variable host specificity and life strategies (Baudoux and Brussaard, 2005; Baudoux *et al.*, 2015). These viral infection strategies appear to be related to the dynamics of their respective host clade. Viruses isolated from clade B (MicV-B) are the most virulent ones, with shorter latent periods and high number of viral progeny (Baudoux *et al.*, 2015). Viruses isolated from clade C (MicV-C) seem to be the least virulent ones, with longer latent periods and moderate to high burst size. Viruses isolated from clade A have an intermediate phenotype which falls between MicVs-B and MicVs-C (Baudoux *et al.*, 2015). Variation in infection and recovery time has been showed in host cultures after viral infection, even within the same strain (Zingone *et al.*, 2006). High growth rates

and diversity, along with high resistance persistence, may increase survival in the host species after bloom termination; allowing co-existence between virus and host (Zingone *et al.*, 2006; Zingone *et al.*, 1999; Brown *et al.*, 2007; Weynberg *et al.*, 2017).

3.1.2 Coccolithoviruses

Coccolithoviruses (**Paper II**) are lytic viruses that infect the most abundant and ubiquitous haptophyte in our oceans: *Emiliania huxleyi* (Haptophyta) (Bratbak *et al.*, 1993; Brussaard *et al.*, 1996b; Brown and Yoder, 1994). This ubiquity may be the result of the high intraspecific genetic variability found in this alga (Blanco-Ameijeiras *et al.*, 2016; Iglesias-Rodriguez *et al.*, 2006). This unicellular calcifying microalga has an extraordinary capacity to form immense blooms and is an important player in global geochemical cycles and climate (Broerse *et al.*, 2000; Burkill *et al.*, 2002; Westbroek *et al.*, 1993; Evans *et al.*, 2007; Bratbak *et al.*, 1993; Bratbak *et al.*, 1996; Brussaard *et al.*, 1996b).

The type species of these viruses, *Emiliania huxleyi* virus 86 (EhV-86), is 170-200 nm in diameter (Wilson *et al.*, 2002), has a latent period of 4-6 hours (Mackinder *et al.*, 2009) and a burst size of 400-1000 particles per cell (Castberg *et al.*, 2002). Its genome has a length of 407,339 bp containing 472 coding sequences (CDSs) (Wilson *et al.*, 2005). Among those we find six RNA polymerase genes, which contribute to their own viral-encoded transcription capacity (Pagarete *et al.*, 2013b); more curious is the almost complete *de novo* sphingolipid biosynthesis pathway that is encoded in this viral genome. The presence of this typical eukaryotic pathway in the EhV genome was the result of horizontal gene transfer, most certainly from its host (Wilson *et al.*, 2005; Monier *et al.*, 2009). These genes are highly expressed during infection (Pagarete *et al.*, 2009), and de novo viral glycosphingolipid (vGSL) production regulates host-virus interactions inducing host's programmed cell death (PCD) (Vardi *et al.*, 2012; Vardi *et al.*, 2009).

EhVs are highly species-specific, presumably because of their budding release mechanism, where the new progeny gains an envelope taken from their host's

membrane (Mackinder *et al.*, 2009); facilitated by this infection-induced GSL production (Rose *et al.*, 2014). Specific polar lipids involved in viral susceptibility have been identified in *E. huxleyi* membranes, since they are suspected to facilitate the viral attachment (Fulton *et al.*, 2014; Hunter, 2015). EhVs can increase in numbers, reaching $3.7x10^7$ viruses/mL, during the collapse of *E. huxleyi* blooms (Schroeder *et al.*, 2003), facilitating the release of dimethylsulfoniopropionate (DMSP) from the algal cells; a precursor of dimethyl sulphide (DMS) that may have implications on climate regulation (Evans *et al.*, 2007). The activity of the enzyme that boosts this reaction, DMSP-lyase, has been shown to be high in host strains resistant to infection, while low DMSP-lyase activity has been exhibited in sensitive algal strains; suggesting a possible anti-viral mechanism (Schroeder *et al.*, 2002; Evans *et al.*, 2006; Evans *et al.*, 2007). It has been also proposed that *E. huxleyi* would use a viral-triggered meiose-dependent phase change to scape viral infection, a process similar to a "Cheshire Cat" strategy (Frada *et al.*, 2008). However, there is a strong on-going debate on whether EhVs are unable to infect this haploid phase of *E. huxleyi* (Mordecai *et al.*, 2017), which is covered by tightly packed body scales but lacking coccoliths (Klaveness, 1971; Klaveness, 1972).

Open ocean studies in the North Atlantic suggest that the EhV community is dominated by several clones that are represented by a high abundance of conserved sequences, although they possess a significant genetic richness (Rowe *et al.*, 2011). This genetic diversity becomes more apparent during bloom conditions, where a viral succession takes place within the EhV community throughout the progression of the bloom, until one viral genotype dominates, and apparently terminates the bloom; as shown in diverse mesocosm experiments in the Norwegian fjords (Schroeder *et al.*, 2003; Martinez *et al.*, 2007; Sorensen *et al.*, 2009). Nonetheless, this dominant EhV genotype has not been found in open ocean studies in the English Channel, suggesting that changes in dominant viral strains could reflect modifications of the host's community due to environmental changes (Highfield *et al.*, 2014). Some of these studies have also exhibited a variable host-range among EhVs, being capable to

infect several cultured *E. huxleyi* strains from very distant places (Martinez *et al.*, 2007; Allen *et al.*, 2007; Schroeder *et al.*, 2003).

4. Interaction with their hosts – emerging patterns

"Simple laws can very well describe complex structures. The miracle is not the complexity of our world, but the simplicity of the equations describing that complexity." *Sander Bais.*

4.1 Cost of resistance to viral infection

Viruses are considered a top-down force shaping microbial populations; however, what type and level of impact viruses have on microbial communities, a central question to understand the global systemic role of viruses, is not completely resolved. In the 70's, and already in an attempt to shed light on the intricacies of virus-microbe interactions, Bruce Levin, together with Lin Chao and Frank Stewart, conducted some of the first experiments that integrated mathematical models to understand how viral infection change the dynamics of microbial populations (Levin *et al.*, 1977). Chemostat experiments demonstrated co-existence between host and phage at steady state, after bacteria developed resistance to viral infection; with hosts being more abundant than the viruses (Lenski, 1988a). The discovery of high virus/bacterial ratios in the environment (Bergh *et al.*, 1989) therefore seemed contradictory and has been termed "the infectivity paradox" (Weinbauer, 2004).

The existence of extremely high marine viral concentrations led to the idea that viruses could be the main drivers of microbial diversity in aquatic environments (**Paper III**). This idea has been progressively incorporated and became predominant in the efforts to model plankton dynamics. Most notably in the "Killing the Winner" hypothesis (Thingstad, 2000; Thingstad and Lignell, 1997), one of the most comprehensive and recognized model of microbe-host interactions in pelagic systems. KtW is based on Lotka-Volterra predator/prey type equations (Berryman, 1992) where microbial strain abundances are controlled by specific viral populations. In order to explain how different strains/species with different growth rates can co-exist in the same environment at steady state (Winter *et al.*, 2010), KtW assumes the existence of a trade-off between growth rate and resistance capacities where

competitive and highly abundant specialist strains are more strongly exposed to negative selection by viral infection than slow growing defence specialists.

Among other forms, resistance can arise from mutations that alter structurally, numerically or accessibly the host's membrane proteins or lipopolysaccharides, which serve as receptors for viral attachment. These viral attachment sites may also serve as nutrient receptors and resistance-driven adaptations are considered to have a fitness cost that can be measured as growth rate (Lenski and Levin, 1985; Schwartz, 1980; Thomas *et al.*, 2012). Empirical evidence for this cost of resistance (COR) has been demonstrated for some host-virus systems, but seem absent in others (**Tables 1** and **2**). COR has been explored extensively in prokaryotic host-virus systems (**Table 1**) but remains elusive in eukaryotic phytoplankton-virus systems (**Table 2**). Therefore, one of the main objectives of the current work was to measure COR in the *Micromonas* – MicV (**Paper I**) and *E. huxleyi*-EhV systems (**Paper II**) (**Table 2**). It was very interesting that we did not observe clear signs of this fundamental presumption in the two phytoplankton-virus systems here studied (**Papers I** and **II**). Despite the several hinders to the approach we used, these results are valid and have important consequences for the way we understand viral impact on phytoplanktonic fitness, and the way we model these virus-host interactions (**Table 3**).

Table 1. Experimental evidence for the presence/absence of COR to viral infection in prokaryotes. Note that the competitive disadvantage is referred when comparing the sensitive with the resistant strain/s.

Prokaryotes			
Reference	Phage	Host	Type of COR
(Chao *et al.*, 1977)	T7	*Escherichia coli*	Competitive disadvantage in phage-free competition.
(Lenski and Levin, 1985)	Several T-phage	*Escherichia coli B*	Competitive disadvantage in resource –limited conditions with one exception.
(Lenski, 1988b)	T4	*Escherichia coli B*	Competitive disadvantage.
(Waterbury and Valois, 1993)	*Synechococcus* viruses	*Synechococcus sp.*	Competitive disadvantage.
(Bohannan *et al.*, 1999)	T4 and λ	*Escherichia coli*	COR context-related.
(Middelboe, 2000)	*Pseudoalteromonas sp.* virus	*Pseudoalteromonas*	Competitive disadvantage.
(Bohannan and Lenski, 2000)	T2	*Escherichia coli*	COR context-related.
(Lythgoe and Chao, 2003)	ϕ6	*Pseudomonas syringae*	No COR observed.
(Mizoguchi *et al.*, 2003)	PP01	*Escherichia coli*	No COR observed.
(Brockhurst *et al.*, 2004)	SBW25ϕ2	*Pseudoalteromonas fluorescens* isolate SBW25	Competitive disadvantage in homogeneous environments.
(Brockhurst *et al.*, 2005)	PP7	*Pseudomonas aeruginosa*	Competitive disadvantage in phage-free competition.
(Lennon *et al.*, 2007)	*Synechococcus* viruses	*Synechococcus sp.*	Competitive disadvantage.
(Holmfeldt *et al.*,	*C. baltica* viruses	*Cellulophaga baltica*	Enhanced infection.

2007)			
(Lennon and Martiny, 2008)	S-RIM8	*Synechococcus* strain (WH7803)	Competitive disadvantage.
(Benmayor *et al.*, 2008)	ϕ2	*Pseudomonas fluorescens*	Competitive disadvantage.
(Middelboe *et al.*, 2009)	ϕS_M, ϕS_T	*Cellulophaga baltica*	Competitive disadvantage in terms of metabolization carbon compounds and enhanced resistance.
(Scanlan *et al.*, 2011)	ϕ2	*Pseudomonas fluorescens*	Enhanced infection.
(Avrani *et al.*, 2011)	Diverse Podoviruses	*Prochlorococcus*	Enhanced infection.
(Hall *et al.*, 2011)	SBW25ϕ2	*Pseudomonas fluorescens* SBW25	Competitive disadvantage.
(Koskella *et al.*, 2012)	*Pseudomonas viruses*	*Pseudomonas syringae*	Competitive disadvantage in heterogeneous parasite environments.
(Marston *et al.*, 2012)	S-RIM8	*Synechococcus sp.*	Enhanced infection.
(Castillo *et al.*, 2014)	*F. psychrophilum* viruses	*Flavobacterium psychrophilum*	Enhanced infection.
(Meaden *et al.*, 2015)	Diverse *P. syringae* viruses	*Pseudomonas syringae*	Competitive disadvantage in nature but no COR observed in nutrient-rich media.
(Avrani and Lindell, 2015)	T7-like cyanopodoviruses	*Prochlorococcus*	No COR observed.

Table 2. Experimental evidence for the presence/absence of COR to viral infection in phytoplanktonic eukaryotes. Note that the competitive disadvantage is referred when comparing the sensitive with the resistant strain/s.

Eukaryotes			
Reference	Phage	Host	Type of COR
(Thyrhaug *et al.*, 2003)	PpV-01	*Phaeocystis pouchetii*	Competitive disadvantage.
(Haaber and Middelboe, 2009)	PpV	*Phaeocystis pouchetii*	Competitive disadvantage.
(Thomas *et al.*, 2011)	OtV5	*Ostreococcus tauri*	Competitive disadvantage.
(Frickel *et al.*, 2016)	PBCV-1	*Chlorella variabilis*	Competitive disadvantage.
(Heath, 2016)	OtV5	*Ostreococcus tauri*	No COR observed.
(Heath, 2017)	OtV5	*Ostreococcus tauri*	No COR observed.
(Ruiz *et al.*, 2017a) (**Paper I**)	Diverse MicVs	*Micromonas*	No COR observed.
(Ruiz *et al.*, 2017b) (**Paper II**)	Diverse EhVs	*Emiliania huxleyi*	No COR observed.

Table 3: Tested hypotheses and observations from the cross-infectivity experiments for both, *Micromonas*-MicV and *E. huxleyi*-EhV, host-virus systems.

Number	**Hypothesis**	**Reference**	**Observed**	
			Micromonas-MicV (**Paper I**)	*E.huxleyi*-EhV (**Paper II**)
1.	Resistance is associated with reduced growth rates (COR)	See Tables 1 and 2	-	-
2.	Host strains with higher μ produce more viruses.	(Moebus, 1996b; Moebus, 1996a; Bratbak *et al.*, 1998; Parada *et al.*, 2006; Motegi and Nagata, 2007; Baudoux and Brussaard, 2008; Demory *et al.*, 2017; Maat *et al.*, 2014; Maat *et al.*, 2016)	+	-
3.	Host strains with higher μ are infected by more viral strains.	(Frickel *et al.*, 2016)	-	-
4.	Host strains with higher R produce fewer viruses.	(Thyrhaug *et al.*, 2003; Kendrick *et al.*, 2014)	-	+
5.	Specialist viruses have higher Vp than generalists.	(MacArthur, 1967; Winter *et al.*, 2010)	-	-

As mentioned above, the lack of correlation between growth rate and resistance has been observed before, including hosts with high growth rates and high resistance values (Avrani and Lindell, 2015; Avrani *et al.*, 2011), and this could have different explanations. One of them could be the impact of domestication. In **Paper II** we observed that the "oldest" strains that were isolated, meaning the ones that have spent the longest time free from viral pressure in the laboratory, produced significantly less viral progeny than the "youngest" ones. Here, the prolonged absence of contact with viruses and the conditions used in culture could have contributed to erase part of the COR potentially present in the wild (Lakeman *et al.*, 2009).

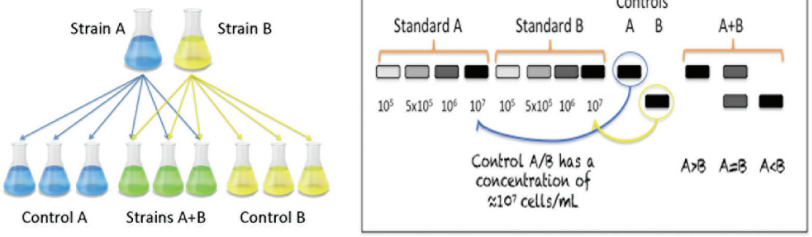
On the other hand, viruses are not the only factor determining diversity in marine phytoplanktonic communities. Effects from other selective factors that are antagonistic to the selection caused by viruses could also contribute to erase COR signal. Moreover, it has been demonstrated that COR can be context-related and, even if resistant and sensitive hosts can present similar fitness under the homogeneous laboratory conditions (Lythgoe and Chao, 2003), COR could arise in the natural habitat (Meaden *et al.*, 2015). For instance, in some studies COR has been proved only present under resource-limited conditions but not when resources are abundant (Lenski and Levin, 1985; Bohannan and Lenski, 2000) and, in others, CORs varied depending on the type of nutrients present in the media (Bohannan *et al.*, 1999; Middelboe *et al.*, 2009).

If the COR trade-off depends on more variables, it could not be evident when growing each strain separately. A different approach to test the existence/magnitude of COR would then be to put in competition strains with similar growth capacities and very different capacities to resist viral infection. Even if resistance to viral infection has been considered to be either present or absent, especially for practical purposes, intermediate values have been also observed and measured (Thomas et al 2011, Yan et al 2016) (**Papers I** and **II**). In theory, a very resistant strain would have hidden hinders to its growth leading it to be outcompeted by a strain with low resistance. We performed 7 competition experiments with *Micromonas* strains. The

protocol followed is briefly explained in **Box 1**, while a detailed methodology can be found in **Supplementary Information**.

BOX 1 | Brief description of the competition experiment setup

Pre-selected *Micromonas* strains were challenged for competitive capacities Discrimination between strains was possible using the 18S genetic marker, and restriction enzymes that would cut the 18S gene in different parts for each strain; respectively. The relative presence of each strain was quantified by comparing the profiles obtained with known standard concentrations.



Competition experiment setup (left) and possible enzymatic digestion results (right). Standards and controls for two hypothetical strains, A and B (respectively), are presented. 3 hypothetical competition outputs are also presented, after incubating together strains A and B. From left to right: strain A outcompetes B, co-existence of both strains, strain B outcompetes A.

In 4 out of 7 experiments, the outcome reflected the trade-off between growth rate and resistance (**Table 4**). This result is remarkable if we take into account that no trade-off was observed with *Micromonas* – MicV in **Paper I**. Yet, as discussed above, COR can be context-related as our results suggest. Moreover, trade-offs can occur in multiple dimensions (Edwards *et al.*, 2011), suggesting that their detection could be intricate if different factors have antagonistic selective effects.

Table 4: Results from the competition experiments. μ= growth rate (d-1), R= resistance measured previously in the infectivity experiments (Paper 1). Final concentrations are relative estimates explained in Fig. 3 (further details presented in the Supplementary information).

Pair No.	Strains	Infection experiment (Paper I)		Competition experiment				
		Control		μ±SD	Trade-off	Predicted winner	Winner	Final concentration
		μ	R					
1	844	0.34	0.81	0.64±0.01	-	1629	844	10^7
	1629	0.31	0.43	0.60±0.04				0
2	451	0.29	0.33	0.62±0.02	+	451	451	10^6
	570	0.30	0.71	0.62±0.01				10^4
3	573	0.44	0.99	0.41±0.04	+	829	829	10^5
	829	0.42	0.33	0.47±0.04				10^6
4	434	0.59	1.00	0.47±0.002	-	449	449	10^4
	449	0.58	0.36	0.51±0.001				10^6
5	692	0.60	0.64	0.71±0.02	-	692	844	10^4
	844	0.34	0.81	0.71±0.01				10^6
6	658	0.67	0.30	0.57±0.03	+	658	658	10^5
	1862	0.65	0.94	0.57±0.06				10^4
7	658	0.67	0.30	0.57±0.03	+	658	658	10^6
	1629	0.31	0.43	0.60±0.04				10^4

It should also be noted the difficulties encountered during this experiment. The first inconvenient was to find suitable molecular tools that would allow strain discrimination, which are not currently available in the case of these microalgae. Consequently, *E. huxleyi* had to be excluded from the experiment. Also, relative quantification was used, which does not allow a very accurate estimation of the concentrations of each strain (**Fig. 3**). That hinder was surpassed in the cases where growth between the two strains under competition was clearly different. Another problem was to obtain pairs of cells growing at the same rate. Growth rates were variable, not only from infectivity to competition experiments (separated in time by a year, approximately), but also within algal replicates, hampering comparisons. All this difficulties caused that most experiments had to be repeated several times, presenting here only the results where the pair of strains in the control samples grew consistently at the same rate.

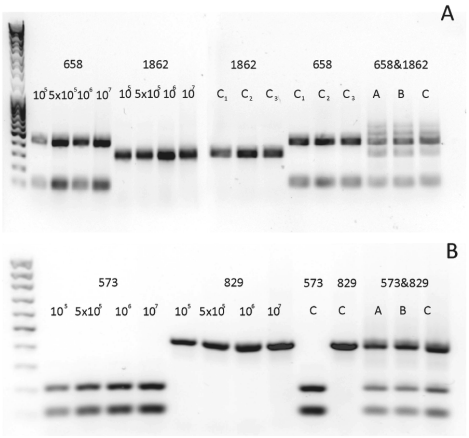


Figure 3: Enzymatic digestion products on agarose gel for the *Micromonas* pairs 658 and 1862 (A), and 573 and 829 (B). Known standard concentrations for each algal strain are presented (10^5, $5x10^5$, 10^6 and 10^7 cells/mL), followed by the control samples (C or C_1-C_3) for each algal strains and the competition samples (A,B,C).

Despite growth rate has been discussed as the antagonist of resistance, some other traits or variables can also affect COR. Taking into account that all metabolic and communication/detection processes in unicellular algae occur though their external membranes, it would not be a surprise that structural changes in these, due to resistance acquisition, will induce collateral effects. Among these effects, we find enhanced infection, a novel type of trade-off that results when resistance to one set of viruses causes susceptibility to other viruses (Avrani *et al.*, 2012; Avrani *et al.*, 2011; Scanlan *et al.*, 2011; Castillo *et al.*, 2014). Therefore, resistance can evolve in different ways, increasing the need to evaluate co-evolutive pleiotropic costs in multiple-predator environments (Koskella *et al.*, 2012), especially the possibility of a trade-off between resistance to grazers and viruses (Pasulka *et al.*, 2015). According to Middelboe *et al.* (2001), when a grazer was introduced into a phage-bacteria model, the grazer's presence allowed co-existence, since sensitive cells could recover by the grazer's non-selective predation. Nevertheless, sensitive cells were lysed consequently, maintaining the viral population and solving Weinbauer's paradox.

4.2 Is host's strategy connected to viral production?

Given that production of viruses is dependent on the metabolic state of the host (Middelboe, 2000), we expected a positive relationship between host growth rate and viral production (Parada *et al.*, 2006; Bratbak *et al.*, 1998; Baudoux and Brussaard, 2008). We found this tendency in the *Micromonas* – MicV system (**Paper I**), but not in the *E. huxleyi* - EhV system (**Paper II**). If a trade-off between growth rate and resistance does exist, then we would also expect the algal strains with higher resistance (R_1) to produce less viruses. This was only found in the *E. huxleyi* – EhV system (**Paper II**). Resistance was also measured based on the number of viral strains infecting (R_2) and, in this case, a positive correlation of R_2 and viral production was present in both virus-host systems (**Paper I**, **Paper II**).

An interesting observation, even if only in rare occasions, was an increase in algal growth when in the presence of viruses in the *Micromonas* - MicV system (**Paper I**). A similar observation has previously been found for *Ostreococcus tauri* (Thomas *et al.*, 2011; Heath and Collins, 2016), a close lineage to that of *Micromonas* inside the Prasinophytes. An empirically based explanation for this phenomenon is not possible at this stage, but enhanced cell division promoted by a cell signalling warning mechanism could be a hypothesis (Thomas *et al.*, 2012; Pagarete *et al.*, 2009). Moreover, motile species like *Micromonas* might have the capacity to actively avoid viruses and decrease the chance of being infected. In this case, if the capacity of the cell to move away from viral infection spots is higher than the viral diffusion rate, that could explain how sensitive cells with high growth rates can co-exist with the resistant ones (**Papers I**, **II** and **III**).

4.3 Viral strategies and trade-offs

Viral viability outside of their hosts depends on diverse environmental factors like temperature, pH and UV irradiation (Spencer, 1955; Børsheim, 1993; Rowe *et al.*, 2008; Silbert *et al.*, 1969; Jacquet and Bratbak, 2003; Suttle and Chen, 1992; Lytle and Sagripanti, 2005). In order to increase survival, viruses may optimize their

reproduction rates; which in turn are influenced by the adsorption rate, latent period and burst size (Ellis and Delbrück, 1939).

Theoretical predictions assume that specialist viruses have higher reproduction rates, which means to increase the adsorption rates and burst sizes, while decreasing the latent period (Keen, 2014). This would potentially compensate the lower probability of finding a host. However, the referred enhancements normally come at a cost, and the progeny usually experiences less stability, persistence and quality (De Paepe and Taddei, 2006; García-Villada and Drake, 2013); as well as a lower host range (Crill *et al.*, 2000; Duffy *et al.*, 2006; Ferris *et al.*, 2007; Holmfeldt *et al.*, 2014). On the other hand, generalist viruses, which are predicted to have lower replication rates, may possess higher stability, persistence, quality and host-range. It is interesting to note that for both the *Micromonas* - MicV (**Paper I**) and the *E. huxleyi* - EhV (**Paper II**) systems, we could demonstrate a niche for the existence of viruses that contradict these theoretical predictions. Notably, generalist viruses that have a significantly higher capacity to produce new progeny. Such absence of a cost to host-expansion can pose some interesting ecological questions like "what would be the advantage of having a narrow host-range?" "How could host-specific viruses compete with 'super' generalist viruses?" These questions have previously been addressed in Bedhomme *et al.* (2012) where a cost of host-range expansion was not verified in the *Tobacco etch potyvirus* (TEV) and four of its hosts.

During intra-genus competition experiments using EhV strains, EhV-207 not only outcompeted EhV-86, but also exhibited characteristics of both, generalist and specialists; meaning a high production potential and shorter latent period (Nissimov *et al.*, 2016). We identified these viral strains as generalist and specialist, respectively, with EhV-207 displaying a higher viral production than EhV-86 (**Paper II**) and suggest that these competitive interactions may explain the viral succession in the Norwegian fjords. In mesocosm experiments, it has been shown that the exponential phase of the bloom consists of diverse hosts and viruses until the termination phase, where one or few host-virus pairs dominate. We may assume that host strains with higher growth rates predominate during the exponential phase,

outcompeting the slow-growing ones. According to the theory, fast-growing competition strategists are infected by specialist viruses, while slow-growing resistant strategists are infected by generalists (Chao *et al.*, 1977). We therefore assume that specialist viruses like EhV-86 may increase in abundance and dominate the system in this phase of the bloom. As the bloom process moves forward, a shift in the host community may take place due to the highest survival of slow-growing resistant strains over the fast-growers, as trade-off theory suggests. Once reached the late stage of the bloom, the dominant hosts may predominantly be resistant strains infected by generalist viruses, like EhV-207. The mesocosm experiments in the Norwegian fjords reported that the viral strains that were allegedly found at the beginning of the bloom, were a mix of generalists and specialists (like EhV-207 and EhV-86 respectively); while at the end of the bloom generalist strains (like EhV-207) dominated (Schroeder *et al.*, 2003; Martinez *et al.*, 2007; Sorensen *et al.*, 2009) (**Paper II**). This result has, therefore, consequences regarding to the diversity of hosts and viruses and their co-evolutive relations.

In **Papers I** and **II** we argued if the generalist viral strains could hide a trade-off behind the measured high viral production. As said before, the progeny of some generalists can experience low survival rates. This is not the case of EhV-207 (reported as generalist in **Paper II**), however, which has found to present higher viral progeny infectivity rates than EhV-86, a specialist (Nissimov *et al*., *in press*.). It was incompressible for Nissimov though, to find by a molecular analysis higher presence of specialists than generalists (**Paper II),** in the North Atlantic; tentatively explained by enhanced removal of virulent EhVs due to aggregation into sinking particles, or increased UV damage and decay. Another conjecture to explain the co-existence of specialist and generalist viruses, through the lens of our results, could be the longer latent periods in the specialist ones. This means that viruses with longer latent periods might use their hosts as a protective vehicles, which may confer viruses more time to find new potential hosts (**Paper I**, **Paper II**).

Viral populations adapt to the hosts they encounter (Bedhomme *et al.*, 2012), and it is believed that specialists evolve faster than generalists in homogeneous environments

(Wilson and Yoshimura, 1994; Whitlock, 1996). This is supported by empirical evidence showing that organisms evolving in homogeneous environments/single host tend to be more specialized than those evolving in heterogeneous environments/multi-hosts (Nikolin *et al.*, 2012; Alto and Turner, 2010; Elena and Lenski, 2003; Elena *et al.*, 2009). Therefore, different strategies among viruses and host will depend on environmental conditions. If better competitor generalist viruses were the rule, it would contradict the fact that one finds organisms along a continuum spectrum of generalists-specialists coexisting in the ocean (Waterbury and Valois, 1993; Tarutani *et al.*, 2000).

5. Virus-host infection networks

"Linking models of co-evolutionary dynamics to specific virus-host systems is a current challenge and these interactions have fundamental effects on ecological dynamics." Joshua Weitz

5.1 From co-existence to co-evolution

Laboratory studies with single virus-host pairs do not represent interaction taking place in nature, but they represent a starting point for estimating those more complex networks. Moebus was among the first who performed an extended number of cross-infectivity experiments with bacteria and phage isolates from the North Sea (Moebus and Nattkemper, 1981).

Recently, the data from Moebus was re-analysed using a network-based analysis (Flores *et al.*, 2013), as the one applied for the analyses presented in **Papers I** and **II**. In these analyses, the interactions are expressed as a matrix; with rows representing host strains and columns viral strains. The cells within the matrix display how successful the infection was, normally represented as positive or negative (Flores et al 2011), and the resulting virus-host infection networks (VHINs), group and describe the interactions. A network lacking structure witnesses random interactions (**Figure 4a**). When one virus infects only one host, interactions are described as one-to-one (**Figure 4b**). When generalist viruses infect the most sensitive and resistant hosts, and specialists infect the most infected hosts, we are in the presence of a nested arrangement (**Figure 4c**). Finally, when a group of viruses infects a group of hosts but cross-group infections are not present, they are described as modular interactions (**Figure 4d**).

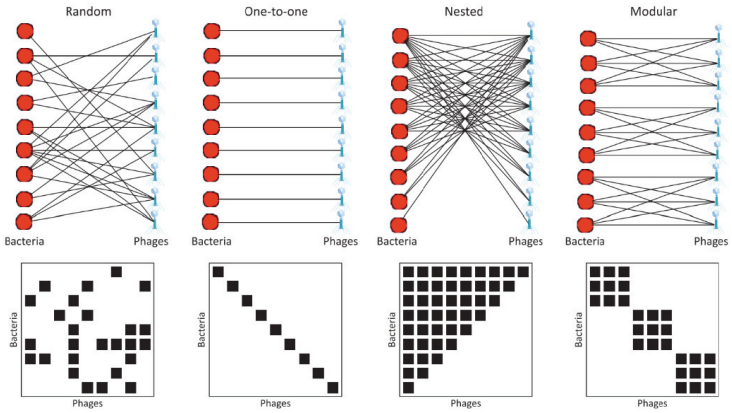


Figure 4: (a) random, (b) one-to-one, (c) nested and (d) modular PBINs. Network representation on top, matrix on the bottom. Adapted from Weitz et al. (2013).

In the *Micromonas* – MicV system we obtained a two module pattern, with each module corresponding to the phylogenetic division that exists between the two main *Micromonas* types (Baudoux *et al.*, 2015). Within each module the arrangement was nested (**Paper I**) as it was for the overall *E. huxleyi* – EhV interaction network (**Paper II**).

Nested patterns are hypothesized to result from gene for gene (GFG) processes (Flor, 1955; Lenski and Levin, 1985; Agrawal and Lively, 2003) (**Fig. 5a**), where hosts are resistant if they have alleles allowing for the recognition of a specific virulence allele in the virus. On the other hand, viruses are infective if their alleles are not specifically countered by a host's allele (Dennehy, 2012). Translated into cross-infectivity effect, new mutations arising in hosts/viruses will confer resistance/host-range expansion to recently evolved viruses/hosts while maintaining the resistance/infectivity to past viruses/hosts (Weitz

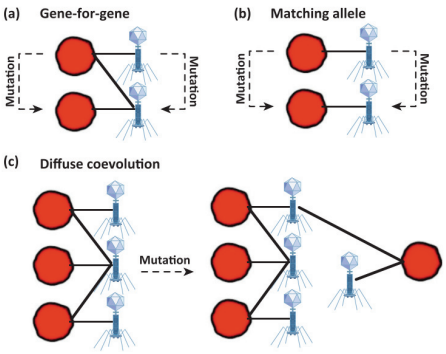


Figure 5: Genetic mechanisms of coevolution and their effect to cross-infection: (a) gene-for-gene, (b) matching allele and (c) diffuse coevolution. Black lines represent infections between phage and host types (circles). Adapted from Weitz *et al.* (2013).

et al., 2013). GFG processes may lead to arms races, meaning that when a host evolves resistance to its parasite, the parasite (a virus in this case) evolves new arms in response (Martiny *et al.*, 2014) (**Fig. 6a**) (**Paper I**, **Paper II**). In this model, COR or infectivity can vary and the selection is directional (**Fig. 6c).**

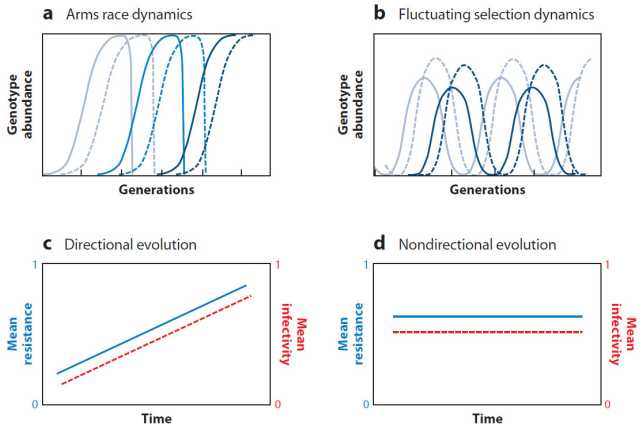


Figure 6: Models of co-evolutionary dynamics (a) Arms race dynamics and (b) Fluctuating selection dynamics, or Red Queen, and their respective modes of evolution, (c) directional evolution and (d) non-directional evolution. Solid lines are different hosts and dashed lines are viral genotypes invading the community. Adapted from Martiny *et al.* (2014).

Agrawal and Lively (2003) suggested that modular patterns in evolutionary VHINs (**Paper I**) are a result of matching alleles (MA) genetic processes **(Fig. 5b**), where viruses can avoid host resistance if their genotypes exactly match the host's genotype; leading to infection. In this case, hosts/viruses evolve resistance/infectivity to a single virus/host genotype, losing the resistance/infectivity to past ones (Weitz *et al.*, 2013). The fact that costs of resistance or infectivity are similar among all alleles, so there is no overall change in the average resistance/infectivity for host/virus genotypes over time (**Fig. 6d)**, leads to Red Queen dynamics. This mode of co-evolutionary dynamics is supposed to occur in presence of frequency-, or density dependent selection, allowing long-term persistence of both viral and host populations (**Fig. 6b)** (Dennehy, 2012), so that no single type can dominate over time (Weitz, 2016).

The modular matrix presented for *Micromonas* - MicV (**Paper I**) was the product of phylogenetic distances between hosts, since the two derived modules corresponded to the viral strains and the host clade they were isolated from (Baudoux *et al.*, 2015; Weitz, 2016). Virus-host interaction dynamics experiments have demonstrated how viruses and their hosts may undergo antagonistic co-evolution after several hundred generations (Forde *et al.*, 2008; Buckling and Rainey, 2002; Buckling *et al.*, 2006; Marston *et al.*, 2012), leading to arms races. Arms races are supposed to continue unless a trade-off changes the dynamics of the process as described by Hall *et al.* (2011); Frickel *et al.* (2016), where one isolated algal clone diversified after coevolving with its virus, changing from arms race to Red Queen dynamics as the different populations stabilized. Our results, then, may reflect the maturity degree of the co-evolutionary process in both systems. *Micromonas* (**Paper I**), which represent an older species complex than *E. huxleyi*, may have reached a higher diversification state than *E. huxleyi* (**Paper II**) (Falkowski *et al.*, 2004; De Vargas, 2007). In addition, the nestedness observed in both systems may be a demonstration of the on-going arms race between hosts and viruses, agreeing with the absence of a strong trade-off associated with development of resistance (**Paper I**, **Paper II**).

It has been thought that at large phylogenetic scales networks are modular (Flores *et al.*, 2011; Flores *et al.*, 2013), although small-scale intra-modular nestedness has also been found (**Paper I**) (Flores *et al.*, 2013). This phenomenon is hard to explain exclusively from the co-evolutive and molecular models presented above. Arms races and Red Queen operating at the same time (Weitz, 2016), or the existence of a single co-evolutionary mechanism that produces nested and modular patterns (Beckett and Williams, 2013), are possible explanations. Moreover, the molecular processes described in the GFG or MA models that drive these patterns, are theoretical scenarios and they are only applicable when analysing co-evolution between single pairs of species, which is qualitatively different from co-evolution in multispecies and/or multistrain interaction. A third genetic model, diffuse co-evolution (**Fig. 5c**), has been proposed to explain co-evolution at the community level (Inouye et al 2001). Therefore, the complexity found in nature will most likely lead to intermediate

or alternate situations (Agrawal and Lively, 2003; Frickel *et al.*, 2016), as seen in **Paper I**.

5.2 Linking ecological factors to VHINs

VHINs are related to the spatio-temporal scale over which the samples were collected (Weitz *et al.*, 2013). For instance, cross-infection studies have demonstrated that viruses infect hosts from the same site rather than those from similar but distant places (Koskella *et al.*, 2011). Communities' species composition has been proved more diverse when increasing the geographic and environmental distance, yet some experiments have reported successful infection of hosts from distant places, even from different biomes (Sano *et al.*, 2004), and isolated at different times (Holmfeldt *et al.*, 2007) (**Paper I**, **Paper II**).

In the *Micromonas*-MicV system, we saw that genetic similarity played an important role in the infection pattern, even if some viral strains could cross the phylogenetic distance between clades A and B (Baudoux *et al.*, 2015) (**Paper I**). *Micromonas* clades are generally concomitant in the oceans although species diversification, presumably due to ecological niche partitioning, is present (Lovejoy *et al.*, 2007; Foulon *et al.*, 2008; van Baren *et al.*, 2016). For *E. huxleyi*- EhV, however, different morphotypes and clades have been described, but clear morphological and physiological differentiation through environmental adaptation has not been recognized (Paasche, 2002; Hagino *et al.*, 2011; Cook *et al.*, 2011). Our nested VHIN (**Paper II**) reflects this "beyond space and time" cross-infectivity pattern, corroborating previous studies (Allen *et al.*, 2007; Pagarete, 2010). Apparently, neither viral pressure, nor the environmental conditions are enough to explain the absence of diversification in *E. huxleyi*. One possible explanation for this may be a high degree of dispersal in this species, keeping the different *E. huxleyi* populations in contact, and counterbalancing divergence as a product of environmental distance (Hanson *et al.*, 2012). Even if geographical barriers may not be an important drawback for marine phytoplankton to spread around (Hagino *et al.*, 2011), this migration process may compromise cell survival and take too long to maintain this

high identity among *E. huxleyi* populations. Other ubiquitous phytoplanktonic species, like *Micromonas* (**Paper I**), have diverged into different clades; thus, dispersal capacity alone seems improbable. Gene transfer within species is a more probable explanation since it decreases diversification, increasing the genetic similarity (Weinbauer and Rassoulzadegan, 2004). Another hypothesis, supported by our competition experiments (unpublished results), could be higher viral pressure on *Micromonas*, compared to *E. huxleyi*. Finally, *E. huxleyi* may exhibit a higher adaptability. *E. huxleyi* can be found in marine waters with temperatures ranging from 1 to 30 °C (Winter, 1994), and photosynthesis do not shown signs of photoinhibition at high irradiances (Paasche, 2002). This high tolerance to diverse and extreme environmental conditions, could partially explain the global coherence of the *E. huxleyi* system.

Temperature changes can inhibit viral infection, probably due to changes in the membrane receptor of the cells (Kendrick *et al.*, 2014; Demory *et al.*, 2017). Nutrient stress has also been shown to affect the interplay between viruses and their hosts. Recent studies have shown that nested matrices are supposed to emerge from environments with high resources, while modular matrices emerge from environments with low resources (Weitz *et al.*, 2013). That is, under low nutrient conditions, arms races that increase host ranges in viruses, and resistance in hosts, would be expected to carry higher pleiotropic costs. Instead, it has been hypothesized that more specialized interactions would emerge due to this costly trade-offs emerged from resource competition. The nestedness values observed in *Micromonas* (**Paper I**) and *E. huxleyi* (**Paper II**) were 0.77 for module 1 and 0.73 for module 2, and 0.60; respectively. An open question is if these high degrees of nestedness could be correlated with the ecological preferences of the algae, or if they may just be a laboratory effect; since a nutrient-rich media was used.

6. Viral impact on complex planktonic communities

"Why there are so many species is a question that might be answered by evolution science, and why their numbers of individuals are as they are, and why we are led to speak of diversity in the composition of communities, results from the workings in the frame of the biosphere."
Ramon Margalef.

In 1961 Hutchinson provided evidence for the huge diversity of microorganisms found in the oceans compared to those that were cultivated in the laboratory, in what we today call the paradox of the plankton (Hutchinson, 1961). This natural diversity can be divided into abundant taxa, which are the most active ones growing fast and experiencing higher predation pressure, and the rare taxa (or seed bank), which grow more slowly and experience less predation (Pedros-Alio, 2006; Sogin *et al.*, 2006; Gibbons *et al.*, 2013). It is thought that density-dependent viral predation may be the main solution to Hutchinson´s paradox; therefore, viruses are considered to have a potential effect on structuring microbial communities (**Paper III**) (Weinbauer and Rassoulzadegan, 2004; Thingstad *et al.*, 1993; Fuhrman, 1993).

Previous viral depletion experiments, in which replicates from the same water sample were incubated under high and low viral pressure, have reported changes in the abundance and growth rates of prokaryotes, as well as shifts in the microbial community diversity and composition (Peduzzi and Weinbauer, 1993; Weinbauer and Holfe, 1998; Hewson *et al.*, 2003; Meunier and Jacquet, 2015; Cram *et al.*, 2016). Some of these studies seem to support the rather counter-intuitive KtW hypothesis, which predicts that some bacterioplankton groups are normally rare because they are actually winners in the competition for nutrients that are on the other end more susceptible to viral infection. Once viruses are removed from the equation, those rare groups tend to become more abundant (Bouvier and del Giorgio, 2007; Cram *et al.*, 2016; Zhang *et al.*, 2007).

In our microcosm experiments, a common trend observed was the significant change in the prokaryote and unicellular eukaryote community profiles, probably caused by the incubation period itself (Schwalbach *et al.*, 2004) (**Paper III**). However, a significant community change associated with the viral depletion treatment was only found in 3 and 1 out of 6 experiments, for the prokaryotic and unicellular eukaryotic communities, respectively. The relatively modest impact viruses exerted on the unicellular eukaryotic community (compared to prokaryotes) has been observed before (Meunier and Jacquet, 2015; Hewson I, 2001; van Hannen *et al.*, 1999), and it is in accordance with the results from the cross-infectivity experiments presented in **Papers I** and **II**. We can, however not disregard the possibility that this effect is the cause of the filtration process, since all our samples were subjected to a pore diameter of 0.2 μm; a size diameter that would retain giant viruses, the ones that we consider to infect unicellular eukaryotes so far (Brussaard *et al.*, 2000; Sandaa, 2008).

BOX 2 | Richness, evenness and diversity definitions (Hamilton, 2005).

Richness: the number of species in a community.

Evenness or equitability: the distribution of abundance among the species.

Species diversity: is a measurement of species richness combined with evenness, meaning it takes into account not only how many species are present but also how evenly distributed the numbers of each species are.

Richness, evenness and diversity are concepts that can be misinterpreted sometimes. To avoid confusion, the terms used along this dissertation are defined in **Box 2**. As said before, viruses can induce significant changes on prokaryotic community richness. (Schwalbach *et al.*, 2004; Fuhrman and Schwalbach, 2003; Cram *et al.*, 2016). We compared the levels of OTU richness and the OTUs' progression for those experiments in which the reduction in viral load was associated with a prokaryotic (16S) community shift (**Paper III**). We only found one instance of higher richness level under "viral depletion" than in the control, and another one in which rare OTUs at the beginning of the experiment became more abundant in the control than in the viral deplete samples.

In addition, significant viral impact on prokaryotic and eukaryotic diversity (Calculated as Shannon's H' index) was not observed. Similar to what Cram *et al.*

(2016) found, diversity between control and viral depleted samples diverged along the experimental period, to finally converge in the end, between both treatments. Therefore, we detected higher diversity in the control samples than the viral depleted ones at day 3, even if not significant, whereas at day 7 diversity was similar in both treatments (**Paper III**). We can argue if short experimental settings are enough to detect potential viral impact on microbial community structure, since Schwalbach *et al.* (2004) reported no viral impact on time-scales of 2 to 5 days. Viral-host interactions, however, have been shown to be more dynamic in time-scales of days, whereas more resilient over weeks or months; both, experimentally (Needham *et al.*, 2013; Middelboe *et al.*, 2001; Liu *et al.*, 2017), and theoretically (Cael, 2015).

Terminal restriction fragment length polymorphism (TRFLP) (Avaniss-Aghajani E, 1994) has successfully been applied to analyse rapid changes in microbial community composition (Moeseneder *et al.*, 1999; Balzano *et al.*, 2012; Lueders and Friedrich, 2003). However, the fact that Schwalbach *et al.* (2004) found that automated rRNA intergenic spacer analysis (ARISA) (Borneman and Triplett, 1997) fingerprints resolved differences between virus treatments better than TRFLP, opens up to the possibility that a more sensitive methodology might have performed better (Rodriguez-Brito *et al.*, 2010). The SSU rRNA gene region, which is targeted in TRFLP analysis, is highly conserved among species; however, it does not provide the phylogenetic resolution needed to resolve communities at the strain level. Fingerprinting analyses based on the ITS region of the rRNA gene like ARISA may be better for this purpose, since this marker gene is more variable due to insertions, deletions, and point mutations (Storesund *et al.*, 2015). Another possibility for our inconsistent results could have been the introduction of manipulation and incubation biases, which can have altered the microbial community fingerprint (Weinbauer *et al.*, 2007; Schwalbach *et al.*, 2004) (**Paper III**).

7. What if viruses are not the rulers of the ocean?

"Plankton is more than a diluted suspension of life." Ramon Margalef.

The work presented in this thesis provides insight of virus-host interactions in marine ecosystems. Overall, I have revealed that the strength of viral pressure on structuring marine microbial communities was found to be not as strong as previously theories suggested (Thingstad, 2000; Thingstad and Lignell, 1997; Winter *et al.*, 2010; Weitz and Wilhelm, 2012) (**Papers I, II and III**). Such findings raise questions about the way we conceive marine viruses and their inherent control of microbial composition, as well as how we model these interactions.

The marine environment is much more complex than any experimental approach that can be performed in the laboratory, even under semi-natural conditions, and manipulation errors are hardly avoided. It is difficult then to apply unequivocally the same patterns or models that work under controlled, and/or simplified, conditions in nature.

Viruses have been frequently placed as one of the main solutions for the plankton paradox, despite some other biotic and abiotic processes that contribute to the species shifts have also been described. Among the abiotic factors, grazers are the other top predators in microbial food webs, which effect structuring marine microbial communities has been found to be even higher than the one produced by viruses in some experiments (Cram *et al.*, 2016; Zhang *et al.*, 2007). However, when together, viruses and grazers have been found to act in a synergistic and antagonistic manner, suggesting variable and complicated interactions within microbial communities (Weinbauer *et al.*, 2007; Zhang *et al.*, 2007; Berdjeb *et al.*, 2011). Among the abiotic ones, we find turbulence, environmental heterogeneity, and disturbance (Károlyi *et al.*, 2000; Descamps-Julien and Gonzalez, 2005; Nes and Marten Scheffer, 2004; Ellner and Turchin, 1995). This "theory of chaos" has proven as well that species interactions themselves can generate fluctuations in species abundances, contributing

to the unexpected high diversity in the oceans over time (Beninca *et al.*, 2008; Huisman *et al.*, 2006; Huisman and Weissing, 1999).

Our results (**Papers I**, **II** and **III**) also shown that viruses are another factor influencing the structure of marine microbial communities. The course and magnitude of their selective force may however lead to different outcomes, depending on the stage and state of the affected community, along with the physicochemical properties of the surrounding environment over the time these interactions last.

8. Future perspectives

"Farewell, my brave Hobbits. My work is now finished. Here at last, on the shores of the sea... comes the end of our Fellowship." J.R.R. Tolkien

Virus-host interactions are not so straightforward to interpret, even in their simplified form, as evidenced in **Papers I** and **II**. Infection and defence mechanisms are still black boxes, especially at the molecular level. Therefore, of special interest is to dispose of high-throughput *in situ* methods for linking viruses to their hosts (Brum and Sullivan, 2015), studying interactions at the strain level (Thingstad *et al.*, 2015).

In turn, connecting this specific virus-host systems to co-evolutionary models is still a challenge in quantitative viral ecology (Weitz, 2016). Long-term observations would also help to resolve some of these co-evolutive aspects.

The competition experiments with *Micromonas* not only manifested this requirement to focus more at low taxonomic units (in this case improving again the molecular techniques that allow to discern between strains), but also the need to evaluate our results under different conditions. For instance, the re-evaluation of multiple correlated traits simultaneously and/or, the manipulation of the experimental physicochemical parameters may unveil possible trade-offs that could affect the observed patterns.

Increasing the number of species interactions (**Paper III)**, would further favour the comprehension of the dynamics in natural systems. However, this also requires the use of diverse and more precise techniques in order to capture and describe the complexity of marine microbial communities.

Unveiling the vast microbial diversity in the oceans will ultimately benefit not only virology, but also diverse scientific fields. This may be pretentious, but we should think macro in a micro world!

References

Agrawal, A. F. and Lively, C. M. (2003) Modelling infection as a two-step process combining gene-for-gene and matching-allele genetics. Proceedings of the Royal Society B-Biological Sciences, 270, 323-334.

Allen, M. J., Martinez-Martinez, J., Schroeder, D. C., Somerfield, P. J. and Wilson, W. H. (2007) Use of microarrays to assess viral diversity: from genotype to phenotype. Environmental Microbiology, 9, 971-982.

Alto, B. W. and Turner, P. E. (2010) Consequences of host adaptation for performance of vesicular stomatitis virus in novel thermal environments. Evolutionary Ecology, 24, 299-315.

Avaniss-Aghajani E, J. K., Chapman D, Brunk C. (1994) A molecular technique for identification of bacteria using small subunit ribosomal RNA sequences. Biotechniques, 17, 144-149.

Avrani, S. and Lindell, D. (2015) Convergent evolution toward an improved growth rate and a reduced resistance range in Prochlorococcus strains resistant to phage. Proceedings of the National Academy of Sciences of the United States of America, 112, E2191-E2200.

Avrani, S., Schwartz, D. A. and Lindell, D. (2012) Virus-host swinging party in the oceans: Incorporating biological complexity into paradigms of antagonistic coexistence. Mobile Genetic Elements, - 2, - 95.

Avrani, S., Wurtzel, O., Sharon, I., Sorek, R. and Lindell, D. (2011) Genomic island variability facilitates Prochlorococcus-virus coexistence. Nature, 474, 604-608.

Balzano, S., Marie, D., Gourvil, P. and Vaulot, D. (2012) Composition of the summer photosynthetic pico and nanoplankton communities in the Beaufort Sea assessed by T-RFLP and sequences of the 18S rRNA gene from flow cytometry sorted samples. ISME J, 6, 1480-1498.

Baudoux, A.-C. and Brussaard, C. P. D. (2008) Influence of irradiance on virus-algal host interactions. Journal of Phycology, 44, 902-908.

Baudoux, A. C. and Brussaard, C. P. D. (2005) Characterization of different viruses infecting the marine harmful algal bloom species Phaeocystis globosa. Virology, 341, 80-90.

Baudoux, A. C., Lebredonchel, H., Dehmer, H., Latimier, M., Edern, R., Rigaut-Jalabert, F., Ge, P., Guillou, L., Foulon, E., Bozec, Y., Cariou, T., Desdevises, Y., Derelle, E., Grimsley, N., Moreau, H. and Simon, N. (2015) Interplay between the genetic clades of Micromonas and their viruses in the Western English Channel. Environmental Microbiology Reports, 7, 765-773.

Beckett, S. J. and Williams, H. T. P. (2013) Coevolutionary diversification creates nested-modular structure in phage-bacteria interaction networks. Interface Focus, 3, 11.

Bedhomme, S., Lafforgue, G. and Elena, S. F. (2012) Multihost Experimental Evolution of a Plant RNA Virus Reveals Local Adaptation and Host-Specific Mutations. Molecular Biology and Evolution, 29, 1481-1492.

Bellec, L., Grimsley, N., Derelle, E., Moreau, H. and Desdevises, Y. (2010) Abundance, spatial distribution and genetic diversity of Ostreococcus tauri viruses in two different environments. Environmental Microbiology Reports, 2, 313-321.

Beninca, E., Huisman, J., Heerkloss, R., Johnk, K. D., Branco, P., Van Nes, E. H., Scheffer, M. and Ellner, S. P. (2008) Chaos in a long-term experiment with a plankton community. Nature, 451, 822-825.

Benmayor, R., Buckling, A., Bonsall, M. B., Brockhurst, M. A. and Hodgson, D. J. (2008) The interactive effects of parasitesf disturbance, and productivity on experimental adaptive radiations. Evolution, 62, 467-477.

Berdjeb, L., Pollet, T., Domaizon, I. and Jacquet, S. (2011) Effect of grazers and viruses on bacterial community structure and production in two contrasting trophic lakes. BMC Microbiology, 11, 88.

Bergh, O., Borsheim, K. Y., Bratbak, G. and Heldal, M. (1989) High abundance of viruses found in aquatic environments

Nature, 340, 467-468.

Berryman, A. A. (1992) The Orgins and Evolution of Predator-Prey Theory. Ecology, 73, 1530-1535.

Blanco-Ameijeiras, S., Lebrato, M., Stoll, H. M., Iglesias-Rodriguez, D., Muller, M. N., Mendez-Vicente, A. and Oschlies, A. (2016) Phenotypic Variability in the Coccolithophore Emiliania huxleyi. Plos One, 11, 17.

Bohannan, B. J. M. and Lenski, R. E. (2000) The relative importance of competition and predation varies with productivity in a model community. American Naturalist, 156, 329-340.

Bohannan, B. J. M., Travisano, M. and Lenski, R. E. (1999) Epistatic interactions can lower the cost of resistance to multiple consumers. Evolution, 53, 292-295.

Borneman, J. and Triplett, E. W. (1997) Molecular microbial diversity in soils from eastern Amazonia: evidence for unusual microorganisms and microbial population shifts associated with deforestation. Applied and Environmental Microbiology, 63, 2647-2653.

Bouvier, T. and del Giorgio, P. A. (2007) Key role of selective viral-induced mortality in determining marine bacterial community composition. Environmental Microbiology, 9, 287-297.

Bratbak, G., Egge, J. K. and Heldal, M. (1993) Viral mortality of the marine alga emiliania-huxleyi (haptophyceae) and termination of algal blooms. Marine Ecology Progress Series, 93, 39-48.

Bratbak, G., Jacobsen, A., Heldal, M., Nagasaki, K. and Thingstad, F. (1998) Virus production in Phaeocystis pouchetii and its relation to host cell growth and nutrition. Aquatic Microbial Ecology, 16, 1-9.

Bratbak, G., Wilson, W. and Heldal, M. (1996) Viral control of Emiliania huxleyi blooms? Journal of Marine Systems, 9, 75-81.

Brockhurst, M. A., Buckling, A. and Rainey, P. B. (2005) The effect of a bacteriophage on diversification of the opportunistic bacterial pathogen, Pseudomonas aeruginosa. Proceedings of the Royal Society B-Biological Sciences, 272, 1385-1391.

Brockhurst, M. A., Rainey, P. B. and Buckling, A. (2004) The effect of spatial heterogeneity and parasites on the evolution of host diversity. Proceedings of the Royal Society B-Biological Sciences, 271, 107-111.

Broecker, W. S. (1982) Glacial to interglacial changes in ocean chemistry. Progress in Oceanography, 11, 151-197.

Broerse, A. T. C., Ziveri, P., van Hinte, J. E. and Honjo, S. (2000) Coccolithophore export production, species composition, and coccolith-CaCO3 fluxes in the NE Atlantic (34 degrees N 21 degrees W and 48 degrees N 21 degrees W). Deep-Sea Research Part Ii-Topical Studies in Oceanography, 47, 1877-1905.

Brown, C. M., Campbell, D. A. and Lawrence, J. E. (2007) Resource dynamics during infection of Micromonas pusilla by virus MpV-Sp1. Environmental Microbiology, 9, 2720-2727.

Brown, C. W. and Yoder, J. A. (1994) Coccolithophorid blooms in the global ocean. Journal of Geophysical Research-Oceans, 99, 7467-7482.

Brum, J. R. and Sullivan, M. B. (2015) Rising to the challenge: accelerated pace of discovery transforms marine virology. Nat Rev Micro, 13, 147-159.

Brussaard, C. P. D., Gast, G. J., vanDuyl, F. C. and Riegman, R. (1996a) Impact of phytoplankton bloom magnitude on a pelagic microbial food web. Marine Ecology Progress Series, 144, 211-221.

Brussaard, C. P. D., Kempers, R. S., Kop, A. J., Riegman, R. and Heldal, M. (1996b) Virus-like particles in a summer bloom of Emiliania huxleyi in the North Sea. Aquatic Microbial Ecology, 10, 105-113.

Brussaard, C. P. D., Marie, D. and Bratbak, G. (2000) Flow cytometric detection of viruses. Journal of Virological Methods, 85, 175-182.

Buckling, A. and Rainey, P. B. (2002) Antagonistic coevolution between a bacterium and a bacteriophage. Proceedings of the Royal Society B-Biological Sciences, 269, 931-936.

Buckling, A., Wei, Y., Massey, R. C., Brockhurst, M. A. and Hochberg, M. E. (2006) Antagonistic coevolution with parasites increases the cost of host deleterious mutations. Proc R Soc Lond B Biol Sci, 273.

Burkill, P. H., Archer, S. D., Robinson, C., Nightingale, P. D., Groom, S. B., Tarran, G. A. and Zubkov, M. V. (2002) Dimethyl sulphide biogeochemistry within a coccolithophore bloom (DISCO): an overview. Deep-Sea Research Part Ii-Topical Studies in Oceanography, 49, 2863-2885.

Børsheim, K. Y. (1993) NATIVE MARINE BACTERIOPHAGES. Fems Microbiology Ecology, 102, 141-159.

Cael, B. B. (2015) The Good, the Bad, and the Tiny: A Simple, Mechanistic-Probabilistic Model of Virus-Nutrient Colimitation in Microbes. PLoS ONE, 10, e0143299.

Castberg, T., Thyrhaug, R., Larsen, A., Sandaa, R. A., Heldal, M., Van Etten, J. L. and Bratbak, G. (2002) Isolation and characterization of a virus that infects Emiliania huxleyi (Haptophyta). Journal of Phycology, 38, 767-774.

Castillo, D., Christiansen, R. H., Espejo, R. and Middelboe, M. (2014) Diversity and Geographical Distribution of Flavobacterium psychrophilum Isolates and Their Phages: Patterns of Susceptibility to Phage Infection and Phage Host Range. Microbial Ecology, 67, 748-757.

Chao, L., Levin, B. R. and Stewart, F. M. (1977) A Complex Community in a Simple Habitat: An Experimental Study with Bacteria and Phage. Ecology, 58, 369-378.

Clokie, M. R. J., Millard, A. D., Wilson, W. H. and Mann, N. H. (2003) Encapsidation of host DNA by bacteriophages infecting marine Synechococcus strains. Fems Microbiology Ecology, 46, 349-352.

Colson, P., de Lamballerie, X., Fournous, G. and Raoult, D. (2012) Reclassification of Giant Viruses Composing a Fourth Domain of Life in the New Order Megavirales. Intervirology, 55, 321-332.

Colson, P., De Lamballerie, X., Yutin, N., Asgari, S., Bigot, Y., Bideshi, D. K., Cheng, X. W., Federici, B. A., Van Etten, J. L., Koonin, E. V., La Scola, B. and Raoult, D. (2013) "Megavirales", a proposed new order for eukaryotic nucleocytoplasmic large DNA viruses. Archives of Virology, 158, 2517-2521.

Cook, S. S., Whitlock, L., Wright, S. W. and Hallegraeff, G. M. (2011) Photosynthetic pigment and genetic differences between two southern ocean morphotypes of emiliania huxleyi (haptophyta). Journal of Phycology, 47, 615-626.

Cottrell, M. T. and Suttle, C. A. (1991) Wide-spread occurrence and clonal variation in viruses which cause lysis of a cosmopolitan, eukaryotic marine phytoplankter, micromonas-pusilla. Marine Ecology Progress Series, 78, 1-9.

Cram, J. A., Parada, A. E. and Fuhrman, J. A. (2016) Dilution reveals how viral lysis and grazing shape microbial communities. Limnology and Oceanography, 61, 889-905.

Crill, W. D., Wichman, H. A. and Bull, J. J. (2000) Evolutionary reversals during viral adaptation to alternating hosts. Genetics, 154, 27-37.

De Paepe, M. and Taddei, F. (2006) Viruses' life history: Towards a mechanistic basis of a trade-off between survival and reproduction among phages. Plos Biology, 4, 1248-1256.

De Vargas, C. A., Marie-Pierre; Probert, Ian and Young, Jeremy (2007) Origin and Evolution of Coccolithophores: From Coastal Hunters to Oceanic Farmers

In Evolution of primary producers in the sea (Ed, Andrew H. Knoll, a. P. F.) Elsevier Science, Amsterdam, The Neederlands, pp. 251-285.

Demory, D., Arsenieff, L., Simon, N., Six, C., Rigaut-Jalabert, F., Marie, D., Ge, P., Bigeard, E., Jacquet, S., Sciandra, A., Bernard, O., Rabouille, S. and Baudoux, A.-C. (2017) Temperature is a key factor in Micromonas-virus interactions. ISME J, 11, 601-612.

Dennehy, J. J. (2012) What Can Phages Tell Us about Host-Pathogen Coevolution? International journal of evolutionary biology, 2012, 396165-396165.

Descamps-Julien, B. and Gonzalez, A. (2005) Stable Coexistence in a Fluctuating Environment: An Experimental Demonstration. Ecology, 86, 2815-2824.

Duffy, S., Turner, P. E. and Burch, C. L. (2006) Pleiotropic costs of niche expansion in the RNA bacteriophage Phi 6. Genetics, 172, 751-757.

Dunigan, D. D., Fitzgerald, L. A. and Van Etten, J. L. (2006) Phycodnaviruses: A peek at genetic diversity. Virus Research, 117, 119-132.

Edwards, K. F., Klausmeier, C. A. and Litchman, E. (2011) Evidence for a three-way trade-off between nitrogen and phosphorus competitive abilities and cell size in phytoplankton. Ecology, 92, 2085-2095.

Elena, S. F., Agudelo-Romero, P. and Lalic, J. (2009) The evolution of viruses in multi-host fitness landscapes. The open virology journal, 3, 1-6.

Elena, S. F. and Lenski, R. E. (2003) Evolution experiments with microorganisms: The dynamics and genetic bases of adaptation. Nature Reviews Genetics, 4, 457-469.

Ellis, E. L. and Delbrück, M. (1939) The growth of bacteriophage. The Journal of General Physiology, 22, 365-384.

Ellner, S. and Turchin, P. (1995) Chaos in a Noisy World: New Methods and Evidence from Time-Series Analysis. The American Naturalist, 145, 343-375.

Evans, C., Kadner, S. V., Darroch, L. J., Wilson, W. H., Liss, P. S. and Malin, G. (2007) The relative significance of viral lysis and microzooplankton grazing as pathways of dimethylsulfoniopropionate (DMSP) cleavage: An Emiliania huxleyi culture study. Limnology and Oceanography, 52, 1036-1045.

Evans, C., Malin, G., Wilson, W. H. and Liss, P. S. (2006) Infectious titers of Emiliania huxleyi virus 86 are reduced by exposure to millimolar dimethyl sulfide and acrylic acid. Limnology and Oceanography, 51, 2468-2471.

Falkowski, P. G., Katz, M. E., Knoll, A. H., Quigg, A., Raven, J. A., Schofield, O. and Taylor, F. J. R. (2004) The Evolution of Modern Eukaryotic Phytoplankton. Science, 305, 354-360.

Fenchel, T. and Fenchel, T. (1987) Ecology of Protozoa. The biology of free-living phagotrophic protists.

Ferris, M. T., Joyce, P. and Burch, C. L. (2007) High frequency of mutations that expand the host range of an RNA virus. Genetics, 176, 1013-1022.

Flor, H. H. (1955) Host-parasite interaction in flax rust - its genetics and other implications. Phytopathology, 45, 680-685.

Flores, C. O., Meyer, J. R., Valverde, S., Farr, L. and Weitz, J. S. (2011) Statistical structure of host-phage interactions. Proceedings of the National Academy of Sciences of the United States of America, 108, E288-E297.

Flores, C. O., Valverde, S. and Weitz, J. S. (2013) Multi-scale structure and geographic drivers of cross-infection within marine bacteria and phages. Isme Journal, 7, 520-532.

Forde, S. E., Beardmore, R. E., Gudelj, I., Arkin, S. S., Thompson, J. N. and Hurst, L. D. (2008) Understanding the limits to generalizability of experimental evolutionary models. Nature, 455, 220-U244.

Foulon, E., Not, F., Jalabert, F., Cariou, T., Massana, R. and Simon, N. (2008) Ecological niche partitioning in the picoplanktonic green alga Micromonas pusilla: evidence from environmental surveys using phylogenetic probes. Environmental Microbiology, 10, 2433-2443.

Frada, M., Probert, I., Allen, M. J., Wilson, W. H. and de Vargas, C. (2008) The "Cheshire Cat" escape strategy of the coccolithophore Emiliania huxleyi in response to viral infection. Proceedings of the National Academy of Sciences of the United States of America, 105, 15944-15949.

Frickel, J., Sieber, M. and Becks, L. (2016) Eco-evolutionary dynamics in a coevolving host-virus system. Ecology Letters, 19, 450-459.

Fuhrman, J. A. (1999) Marine viruses and their biogeochemical and ecological effects. Nature, 399, 541-548.

Fuhrman, J. A. and Schwalbach, M. (2003) Viral Influence on Aquatic Bacterial Communities. The Biological Bulletin, 204, 192-195.

Fuhrman, J. A. S., C. A. (1993) Viruses in Marine Planktonic Systems Oceanography, 6.

Fulton, J. M., Fredricks, H. F., Bidle, K. D., Vardi, A., Kendrick, B. J., DiTullio, G. R. and Van Mooy, B. A. S. (2014) Novel molecular determinants of viral susceptibility and resistance in the lipidome of Emiliania huxleyi. Environmental Microbiology, 16, 1137-1149.

García-Villada, L. and Drake, J. W. (2013) Experimental selection reveals a trade-off between fecundity and lifespan in the coliphage Qß. Open Biology, 3, 130043.

Gibbons, S. M., Caporaso, J. G., Pirrung, M., Field, D., Knight, R. and Gilbert, J. A. (2013) Evidence for a persistent microbial seed bank throughout the global ocean. Proceedings of the National Academy of Sciences of the United States of America, 110, 4651-4655.

Gustavsen, J. A., Winget, D. M., Tian, X. and Suttle, C. A. (2014) High temporal and spatial diversity in marine RNA viruses implies that they have an important role in mortality and structuring plankton communities. Frontiers in Microbiology, 5, 13.

Haaber, J. and Middelboe, M. (2009) Viral lysis of Phaeocystis pouchetii: Implications for algal population dynamics and heterotrophic C, N and P cycling. Isme Journal, 3, 430-441.

Hagino, K., Bendif, E., Young, J. R., Kogame, K., Probert, I., Takano, Y., Horiguchi, T., de Vargas, C. and Okada, H. (2011) New evidence for morphological and genetic variation in the cosmopolitan coccolithophore emiliania huxleyi (prymnesiophyceae) from the cox1b-atp4 genes. Journal of Phycology, 47, 1164-1176.

Hall, A. R., Scanlan, P. D., Morgan, A. D. and Buckling, A. (2011) Host–parasite coevolutionary arms races give way to fluctuating selection. Ecology Letters, 14, 635-642.

Hamilton, A. J. (2005) Species diversity or biodiversity? Journal of Environmental Management, 75, 89-92.

Hanson, C. A., Fuhrman, J. A., Horner-Devine, M. C. and Martiny, J. B. H. (2012) Beyond biogeographic patterns: processes shaping the microbial landscape. Nat Rev Micro, 10, 497-506.

Heath, S. E. and Collins, S. (2016) Mode of resistance to viral lysis affects host growth across multiple environments in the marine picoeukaryote Ostreococcus tauri. Environmental Microbiology, 18, 4628-4639.

Heath, S. E. C., Sinead (2016) Mode of resistance to viral lysis affects host growth across multiple. D - 100883692, T - aheadofprint.

Heath, S. E. K., Kirsten; Vale, Pedro F.; Collins, Sinead. (2017) Virus resistance is not costly in a marine alga evolving under multiple environmental stressors. Viruses 9.

Hewson I, O. N. J., Heil CA, Bratbak G, Dennison WC (2001) Effects of concentrated viral communities on photosynthesis and community composition of cooccurring benthic microalgae and phytoplankton. Aquatic Microbial Ecology, 25, 1-10.

Hewson, I., Vargo, G. A. and Fuhrman, J. A. (2003) Bacterial diversity in shallow oligotrophic marine benthos and overlying waters: Effects of virus infection, containment, and nutrient enrichment. Microbial Ecology, 46, 322-336.

Highfield, A., Evans, C., Walne, A., Miller, P. I. and Schroeder, D. C. (2014) How many Coccolithovirus genotypes does it take to terminate an Emiliania huxleyi bloom? Virology, 466, 138-145.

Hingamp, P., Grimsley, N., Acinas, S. G., Clerissi, C., Subirana, L., Poulain, J., Ferrera, I., Sarmento, H., Villar, E., Lima-Mendez, G., Faust, K., Sunagawa, S., Claverie, J. M., Moreau, H., Desdevises, Y., Bork, P., Raes, J., de Vargas, C., Karsenti, E., Kandels-Lewis, S., Jaillon, O., Not, F., Pesant, S., Wincker, P. and Ogata, H. (2013) Exploring nucleo-cytoplasmic large DNA viruses in Tara Oceans microbial metagenomes. Isme Journal, 7, 1678-1695.

Holmfeldt, K., Howard-Varona, C., Solonenko, N. and Sullivan, M. B. (2014) Contrasting genomic patterns and infection strategies of two co-existing Bacteroidetes podovirus genera. Environmental Microbiology, 16, 2501-2513.

Holmfeldt, K., Middelboe, M., Nybroe, O. and Riemann, L. (2007) Large Variabilities in host strain susceptibility and phage host range govern interactions between lytic marine phages and their Flavobactetium hosts. Applied and Environmental Microbiology, 73, 6730-6739.

Huisman, J., Pham Thi, N. N., Karl, D. M. and Sommeijer, B. (2006) Reduced mixing generates oscillations and chaos in the oceanic deep chlorophyll maximum. Nature, 439, 322-325.

Huisman, J. and Weissing, F. J. (1999) Biodiversity of plankton by species oscillations and chaos. Nature, 402, 407-410.

Hunter, J. E., Frada, Miguel J., Fredricks, Helen F., Vardi, Assaf, Mooy, Benjamin A. S. Van (2015) Targeted and untargeted lipidomics of Emiliania huxleyi viral infection and life cycle phases highlights molecular biomarkers of infection, susceptibility, and ploidy. Frontiers in Marine Science, 2.

Hutchinson, G. E. (1961) The paradox of the plankton. American Naturalist, 95, 137-145.

Iglesias-Rodriguez, M. D., Schofield, O. M., Batley, J., Medlin, L. K. and Hayes, P. K. (2006) Intraspecific genetic diversity in the marine coccolithophore Emiliania

huxleyi (Prymnesiophyceae): The use of microsatellite analysis in marine phytoplankton population studies. Journal of Phycology, 42, 526-536.
Iyer, L. A., Balaji, S., Koonin, E. V. and Aravind, L. (2006) Evolutionary genomics of nucleo-cytoplasmic large DNA viruses. Virus Research, 117, 156-184.
Iyer, L. M., Aravind, L. and Koonin, E. V. (2001) Common origin of four diverse families of large eukaryotic DNA viruses. Journal of Virology, 75, 11720-11734.
Jacquet, S. and Bratbak, G. (2003) Effects of ultraviolet radiation on marine virus-phytoplankton interactions. FEMS Microbiology Ecology, 44, 279-289.
Johannessen, T. V., Bratbak, G., Larsen, A., Ogata, H., Egge, E. S., Edvardsen, B., Eikrem, W. and Sandaa, R.-A. (2015) Characterisation of three novel giant viruses reveals huge diversity among viruses infecting Prymnesiales (Haptophyta). Virology, 476, 180-188.
Jover, L. F., Effler, T. C., Buchan, A., Wilhelm, S. W. and Weitz, J. S. (2014) The elemental composition of virus particles: implications for marine biogeochemical cycles. Nat Rev Micro, 12, 519-528.
Károlyi, G., Péntek, Á., Scheuring, I., Tél, T. and Toroczkai, Z. (2000) Chaotic flow: The physics of species coexistence. Proceedings of the National Academy of Sciences, 97, 13661-13665.
Keen, E. C. (2014) Tradeoffs in bacteriophage life histories. Bacteriophage, 4, e28365.
Kendrick, B. J., DiTullio, G. R., Cyronak, T. J., Fulton, J. M., Van Mooy, B. A. S. and Bidle, K. D. (2014) Temperature-Induced Viral Resistance in Emiliania huxleyi (Prymnesiophyceae). Plos One, 9.
Kimmance, S. A., Wilson, W. H. and Archer, S. D. (2007) Modified dilution technique to estimate viral versus grazing mortality of phytoplankton: limitations associated with method sensitivity in natural waters. Aquatic Microbial Ecology, 49, 207-222.
Klaveness, D. (1972) Coccolithus huxleyi (Lohm.) Kamptn II. The flagellate cell, aberrant cell types, vegetative propagation and life cycles. British Phycological Journal, 7, 309-318.
Klaveness, D. P., E. (1971) Two different Coccolithus huxleyi cell types incapable of coccolith formation. Arch. Microbiol., 75, 382-385.
Knightjones, E. W. and Walne, P. R. (1951) Chromulina-pusilla butcher, a dominant member of the ultraplankton. Nature, 167, 445-446.
Koskella, B., Lin, D. M., Buckling, A. and Thompson, J. N. (2012) The costs of evolving resistance in heterogeneous parasite environments. Proceedings of the Royal Society B-Biological Sciences, 279, 1896-1903.
Koskella, B., Thompson, J. N., Preston, G. M. and Buckling, A. (2011) Local Biotic Environment Shapes the Spatial Scale of Bacteriophage Adaptation to Bacteria. The American Naturalist, 177, 440-451.
Lakeman, M. B., von Dassow, P. and Cattolico, R. A. (2009) The strain concept in phytoplankton ecology. Harmful Algae, 8, 746-758.
Lennon, J. T., Khatana, S. A. M., Marston, M. F. and Martiny, J. B. H. (2007) Is there a cost of virus resistance in marine cyanobacteria? Isme Journal, 1, 300-312.
Lennon, J. T. and Martiny, J. B. H. (2008) Rapid evolution buffers ecosystem impacts of viruses in a microbial food web. Ecology Letters, 11, 1178-1188.
Lenski, R. E. (1988a) Dynamics of interactions between bacteria and virulent bacteriophage. Advances in Microbial Ecology, 10, 1-44.
Lenski, R. E. (1988b) Experimental studies of pleiotropy and epistasis in escherichia-coli .1. Variation in competitive fitness among mutants resistant to virus-t4. Evolution, 42, 425-432.

Lenski, R. E. and Levin, B. R. (1985) Constraints on the Coevolution of Bacteria and Virulent Phage: A Model, Some Experiments, and Predictions for Natural Communities. The American Naturalist, 125, 585-602.

Levin, B. R., Stewart, F. M. and Chao, L. (1977) Resource-limited growth, competition, and predation - a model and experimental studies with bacteria and bacteriophage. American Naturalist, 111, 3-24.

Liu, H., Tan, S., Xu, J., Guo, W., Xia, X. and Yan Cheung, S. (2017) Interactive regulations by viruses and dissolved organic matter on the bacterial community. Limnology and Oceanography, n/a-n/a.

Lovejoy, C., Vincent, W. F., Bonilla, S., Roy, S., Martineau, M. J., Terrado, R., Potvin, M., Massana, R. and Pedros-Alio, C. (2007) Distribution, phylogeny, and growth of cold-adapted picoprasinophytes in arctic seas. Journal of Phycology, 43, 78-89.

Lueders, T. and Friedrich, M. W. (2003) Evaluation of PCR Amplification Bias by Terminal Restriction Fragment Length Polymorphism Analysis of Small-Subunit rRNA and mcrA Genes by Using Defined Template Mixtures of Methanogenic Pure Cultures and Soil DNA Extracts. Applied and Environmental Microbiology, 69, 320-326.

Lythgoe, K. A. and Chao, L. (2003) Mechanisms of coexistence of a bacteria and a bacteriophage in a spatially homogeneous environment. Ecology Letters, 6, 326-334.

Lytle, C. D. and Sagripanti, J. L. (2005) Predicted inactivation of viruses of relevance to biodefense by solar radiation. Journal of Virology, 79, 14244-14252.

Maat, D. S., Crawfurd, K. J., Timmermans, K. R. and Brussaard, C. P. D. (2014) Elevated CO2 and Phosphate Limitation Favor Micromonas pusilla through Stimulated Growth and Reduced Viral Impact. Applied and Environmental Microbiology, 80, 3119-3127.

Maat, D. S., de Blok, R. and Brussaard, C. P. D. (2016) Combined Phosphorus Limitation and Light Stress Prevent Viral Proliferation in the Phytoplankton Species Phaeocystis globosa, but Not in Micromonas pusilla. Frontiers in Marine Science, 3.

MacArthur, R. H., and E. O. Wilson (1967) The theory of island biogeography. Princeton University Press, Princeton, NJ, USA, 203.

Mackinder, L. C. M., Worthy, C. A., Biggi, G., Hall, M., Ryan, K. P., Varsani, A., Harper, G. M., Wilson, W. H., Brownlee, C. and Schroeder, D. C. (2009) A unicellular algal virus, Emiliania huxleyi virus 86, exploits an animal-like infection strategy. Journal of General Virology, 90, 2306-2316.

Marañón, E., Holligan, P. M., Barciela, R., Gonzalez, N., Mourino, B., Pazo, M. J. and Varela, M. (2001) Patterns of phytoplankton size structure and productivity in contrasting open-ocean environments. Marine Ecology Progress Series, 43-56.

Marin, B. and Melkonian, M. (2010) Molecular Phylogeny and Classification of the Mamiellophyceae class. nov (Chlorophyta) based on Sequence Comparisons of the Nuclear- and Plastid-encoded rRNA Operons. Protist, 161, 304-336.

Marston, M. F., Pierciey, F. J., Shepard, A., Gearin, G., Qi, J., Yandava, C., Schuster, S. C., Henn, M. R. and Martiny, J. B. H. (2012) Rapid diversification of coevolving marine Synechococcus and a virus. Proceedings of the National Academy of Sciences of the United States of America, 109, 4544-4549.

Martinez, J. M., Boere, A., Gilg, L., van Lent, J. W. M., Witte, H. J., van Bleijswijk, J. D. L. and Brussaard, C. P. D. (2015) New lipid envelope-containing dsDNA virus isolates infecting Micromonas pusilla reveal a separate phylogenetic group. Aquatic Microbial Ecology, 74, 17-28.

Martinez, J. M., Schroeder, D. C., Larsen, A., Bratbak, G. and Wilson, W. H. (2007) Molecular dynamics of Emiliania huxleyi and cooccurring viruses during two separate mesocosm studies. Applied and Environmental Microbiology, 73, 554-562.

Martiny, J. B. H., Riemann, L., Marston, M. F. and Middelboe, M. (2014) Antagonistic Coevolution of Marine Planktonic Viruses and Their Hosts. In Annual Review of Marine Science, Vol 6, Vol. 6 (Eds, Carlson, C. A. and Giovannoni, S. J.), pp. 393-414.

Maruyama, F. and Ueki, S. (2016) Evolution and Phylogeny of Large DNA Viruses, Mimiviridae and Phycodnaviridae Including Newly Characterized Heterosigma akashiwo Virus. Frontiers in Microbiology, 7.

Mayer, J. A. and Taylor, F. J. R. (1979) Virus which lyses the marine nanoflagellate micromonas-pusilla. Nature, 281, 299-301.

Meaden, S., Paszkiewicz, K. and Koskella, B. (2015) The cost of phage resistance in a plant pathogenic bacterium is context-dependent. Evolution, 69, 1321-1328.

Meunier, A. and Jacquet, S. (2015) Do phages impact microbial dynamics, prokaryotic community structure and nutrient dynamics in Lake Bourget? Biology Open, 4, 1528-1537.

Middelboe, M. (2000) Bacterial growth rate and marine virus-host dynamics. Microbial Ecology, 40, 114-124.

Middelboe, M., Hagstrom, A., Blackburn, N., Sinn, B., Fischer, U., Borch, N. H., Pinhassi, J., Simu, K. and Lorenz, M. G. (2001) Effects of bacteriophages on the population dynamics of four strains of pelagic marine bacteria. Microbial Ecology, 42, 395-406.

Middelboe, M., Holmfeldt, K., Riemann, L., Nybroe, O. and Haaber, J. (2009) Bacteriophages drive strain diversification in a marine Flavobacterium: implications for phage resistance and physiological properties. Environmental Microbiology, 11, 1971-1982.

Mitchell, R. and Kirchman, D. L. (2008) Microbial Ecology of the Oceans, Wiley, Hoboken, United States.

Mizoguchi, K., Morita, M., Fischer, C. R., Yoichi, M., Tanji, Y. and Unno, H. (2003) Coevolution of bacteriophage PP01 and Escherichia coli O157 : H7 in continuous culture. Applied and Environmental Microbiology, 69, 170-176.

Moebus, K. (1992) Further investigations on the concentration of marine bacteriophages in the water around helgoland, with reference to the phage-host systems encountered. Helgolander Meeresuntersuchungen, 46, 275-292.

Moebus, K. (1996a) Marine bacteriophage reproduction under nutrient-limited growth of host bacteria .1. Investigations with six phage-host systems. Marine Ecology Progress Series, 144, 1-12.

Moebus, K. (1996b) Marine bacteriophage reproduction under nutrient-limited growth of host bacteria .2. Investigations with phage-host system H3:H3/1. Marine Ecology Progress Series, 144, 13-22.

Moebus, K. and Nattkemper, H. (1981) Bacteriophage sensitivity patterns among bacteria isolated from marine waters. Helgolander Meeresuntersuchungen, 34, 375-&.

Moeseneder, M. M., Arrieta, J. M., Muyzer, G., Winter, C. and Herndl, G. J. (1999) Optimization of Terminal-Restriction Fragment Length Polymorphism Analysis for Complex Marine Bacterioplankton Communities and Comparison with Denaturing Gradient Gel Electrophoresis. Applied and Environmental Microbiology, 65, 3518-3525.

Mojica, K. D. A., Huisman, J., Wilhelm, S. W. and Brussaard, C. P. D. (2016) Latitudinal variation in virus-induced mortality of phytoplankton across the North Atlantic Ocean. Isme Journal, 10, 500-513.

Mokili, J. L., Rohwer, F. and Dutilh, B. E. (2012) Metagenomics and future perspectives in virus discovery. Current Opinion in Virology, 2, 63-77.

Monier, A., Pagarete, A., de Vargas, C., Allen, M. J., Read, B., Claverie, J. M. and Ogata, H. (2009) Horizontal gene transfer of an entire metabolic pathway between a eukaryotic alga and its DNA virus. Genome Research, 19, 1441-1449.

Mordecai, G. J., Verret, F., Highfield, A. and Schroeder, D. C. (2017) Schrödinger's Cheshire Cat: Are Haploid Emiliania huxleyi Cells Resistant to Viral Infection or Not? Viruses, 9, 51.

Motegi, C. and Nagata, T. (2007) Enhancement of viral production by addition of nitrogen or nitrogen plus carbon in subtropical surface waters of the South Pacific. Aquatic Microbial Ecology, 48, 27-34.

Needham, D. M., Chow, C. E. T., Cram, J. A., Sachdeva, R., Parada, A. and Fuhrman, J. A. (2013) Short-term observations of marine bacterial and viral communities: patterns, connections and resilience. Isme Journal, 7, 1274-1285.

Nes, Egbert H. v. and Marten Scheffer (2004) Large Species Shifts Triggered by Small Forces. The American Naturalist, 164, 255-266.

Nikolin, V. M., Osterrieder, K., von Messling, V., Hofer, H., Anderson, D., Dubovi, E., Brunner, E. and East, M. L. (2012) Antagonistic Pleiotropy and Fitness Trade-Offs Reveal Specialist and Generalist Traits in Strains of Canine Distemper Virus. Plos One, 7, 9.

Nissimov, J. I., Napier, J. A., Allen, M. J. and Kimmance, S. A. (2016) Intragenus competition between coccolithoviruses: an insight on how a select few can come to dominate many. Environmental Microbiology, 18, 133-145.

Not, F., Latasa, M., Marie, D., Cariou, T., Vaulot, D. and Simon, N. (2004) A Single Species, Micromonas pusilla (Prasinophyceae), Dominates the Eukaryotic Picoplankton in the Western English Channel. Applied and Environmental Microbiology, 70, 4064-4072.

Osterberg, C., Carey, A. G. and Curl, H. (1963) Acceleration of Sinking Rates of Radionuclides in the Ocean. Nature, 200, 1276-1277.

Paasche, E. (2002) A review of the coccolithophorid Emiliania huxleyi (Prymnesiophyceae), with particular reference to growth, coccolith formation, and calcification-photosynthesis interactions. Phycologia, 40, 503-529.

Pagarete, A., Allen, M. J., Wilson, W. H., Kimmance, S. A. and de Vargas, C. (2009) Host-virus shift of the sphingolipid pathway along an Emiliania huxleyi bloom: survival of the fattest. Environmental Microbiology, 11, 2840-2848.

Pagarete, A., Chow, C. E. T., Johannessen, T., Fuhrman, J. A., Thingstad, T. F. and Sandaa, R. A. (2013a) Strong Seasonality and Interannual Recurrence in Marine Myovirus Communities. Applied and Environmental Microbiology, 79, 6253-6259.

Pagarete, A., Lanzen, A., Puntervoll, P., Sandaa, R. A., Larsen, A., Larsen, J. B., Allen, M. J. and Bratbak, G. (2013b) Genomic Sequence and Analysis of EhV-99B1, a New Coccolithovirus from the Norwegian Fjords. Intervirology, 56, 60-66.

Pagarete, A. J. (2010) Functional genomics of coccolithophore viruses. Paris 6, Paris, France, 222.

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

Park, Y., Lee, K., Lee, Y. S., Kim, S. W. and Choi, T. J. (2011) Detection of diverse marine algal viruses in the South Sea regions of Korea by PCR amplification of the DNA polymerase and major capsid protein genes. Virus Research, 159, 43-50.

Pasulka, A. L., Samo, T. J. and Landry, M. R. (2015) Grazer and viral impacts on microbial growth and mortality in the southern California Current Ecosystem. Journal of Plankton Research, 37, 320-336.

Pedros-Alio, C. (2006) Marine microbial diversity: can it be determined? Trends in Microbiology, 14, 257-263.

Peduzzi, P. and Weinbauer, M. G. (1993) The submicron size fraction of seawater containing high numbers of virus particles as bioactive agent in unicellular plankton community successions. Journal of Plankton Research, 15, 1375-1386.

Rodriguez-Brito, B., Li, L., Wegley, L., Furlan, M., Angly, F., Breitbart, M., Buchanan, J., Desnues, C., Dinsdale, E., Edwards, R., Felts, B., Haynes, M., Liu, H., Lipson, D., Mahaffy, J., Martin-Cuadrado, A. B., Mira, A., Nulton, J., Pasic, L., Rayhawk, S., Rodriguez-Mueller, J., Rodriguez-Valera, F., Salamon, P., Srinagesh, S., Thingstad, T. F., Tran, T., Thurber, R. V., Willner, D., Youle, M. and Rohwer, F. (2010) Viral and microbial community dynamics in four aquatic environments. ISME J, 4, 739-751.

Rose, S. L., Fulton, J. M., Brown, C. M., Natale, F., Van Mooy, B. A. S. and Bidle, K. D. (2014) Isolation and characterization of lipid rafts in Emiliania huxleyi: a role for membrane microdomains in host–virus interactions. Environmental Microbiology, 16, 1150-1166.

Rowe, J. M., Fabre, M. F., Gobena, D., Wilson, W. H. and Wilhelm, S. W. (2011) Application of the major capsid protein as a marker of the phylogenetic diversity of Emiliania huxleyi viruses. Fems Microbiology Ecology, 76, 373-380.

Rowe, J. M., Saxton, M. A., Cottrell, M. T., DeBruyn, J. M., Berg, G. M., Kirchman, D. L., Hutchins, D. A. and Wilhelm, S. W. (2008) Constraints on viral production in the Sargasso Sea and North Atlantic. Aquatic Microbial Ecology, 52, 233-244.

Rozon, R. M. and Short, S. M. (2013) Complex seasonality observed amongst diverse phytoplankton viruses in the Bay of Quinte, an embayment of Lake Ontario. Freshwater Biology, 58, 2648-2663.

Ruiz, E., Baudoux, A.-C., Simon, N., Sandaa, R.-A., Thingstad, T. F. and Pagarete, A. (2017a) Micromonas versus virus: New experimental insights challenge viral impact. Environmental Microbiology, 19, 2068–2076.

Ruiz, E., Oosterhof, M., Sandaa, R.-A., Larsen, A. and Pagarete, A. (2017b) Emerging Interaction Patterns in the Emiliania huxleyi-EhV System. Viruses, 9, 61.

Sahlsten, E. (1998) Seasonal abundance in Skagerrak-Kattegat coastal waters and host specificity of viruses infecting the marine photosynthetic flagellate Micromonas pusilla. Aquatic Microbial Ecology, 16, 103-108.

Sandaa, R. A. (2008) Burden or benefit? Virus-host interactions in the marine environment. Research in Microbiology, 159, 374-381.

Sano, E., Carlson, S., Wegley, L. and Rohwer, F. (2004) Movement of Viruses between Biomes. Applied and Environmental Microbiology, 70, 5842-5846.

Sarmiento, J. L. and Gruber, N. (2013) Ocean Biogeochemical Dynamics, Princeton University Press, Princeton, UNITED STATES.

Scanlan, P. D., Hall, A. R., Lopez-Pascua, L. D. C. and Buckling, A. (2011) Genetic basis of infectivity evolution in a bacteriophage. Molecular Ecology, 20, 981-989.

Schroeder, D. C., Oke, J., Hall, M., Malin, G. and Wilson, W. H. (2003) Virus succession observed during an Emiliania huxleyi bloom. Applied and Environmental Microbiology, 69, 2484-2490.

Schroeder, D. C., Oke, J., Malin, G. and Wilson, W. H. (2002) Coccolithovirus (Phycodnaviridae): Characterisation of a new large dsDNA algal virus that infects Emiliania huxleyi. Archives of Virology, 147, 1685-1698.

Schwalbach, M. S., Hewson, I. and Fuhrman, J. A. (2004) Viral effects on bacterial community composition in marine plankton microcosms. Aquatic Microbial Ecology, 34, 117-127.

Schwartz, M. (1980) Interaction of Phages with their Receptor Proteins. In Virus Receptors: Part 1 Bacterial Viruses (Eds, Randall, L. L. and Philipson, L.) Springer Netherlands, Dordrecht, pp. 59-94.

Seymour, J. R., Seuront, L., Doubell, M., Waters, R. L. and Mitchell, J. G. (2006) Microscale patchiness of virioplankton. Journal of the Marine Biological Association of the United Kingdom, 86, 551-561.

Sherr, B. F., Sherr, E. B., Klug, M. J. and Reddy, C. A. (1984) Role of heterotrophic Protozoa in carbon and energy flow in aquatic ecosystems.

Short, S. M. and Short, C. M. (2008) Diversity of algal viruses in various North American freshwater environments. Aquatic Microbial Ecology, 51, 13-21.

Silbert, J. A., Salditt, M. and Franklin, R. M. (1969) Structure and synthesis of a lipid-containing bacteriophage .3. Purification of bacteriophage-pm2 and some structural studies on virion. Virology, 39, 666-&.

Sogin, M. L., Morrison, H. G., Huber, J. A., Mark Welch, D., Huse, S. M., Neal, P. R., Arrieta, J. M. and Herndl, G. J. (2006) Microbial diversity in the deep sea and the underexplored "rare biosphere". Proceedings of the National Academy of Sciences of the United States of America, 103, 12115-12120.

Sorensen, G., Baker, A. C., Hall, M. J., Munn, C. B. and Schroeder, D. C. (2009) Novel virus dynamics in an Emiliania huxleyi bloom. Journal of Plankton Research, 31, 787-791.

Spencer, R. (1955) A Marine Bacteriophage. Nature, 175, 690-691.

Staley, J. T., and Konopka, A. (1985) Measurement of in Situ Activities of Nonphotosynthetic Microorganisms in Aquatic and Terrestrial Habitats. Annual Review Microbiology, 39, 321-346.

Storesund, J. E., Erga, S. R., Ray, J. L., Thingstad, T. F. and Sandaa, R.-A. (2015) Top-down and bottom-up control on bacterial diversity in a western Norwegian deep-silled fjord. FEMS Microbiology Ecology, 91, fiv076-fiv076.

Sullivan, M. B., Waterbury, J. B. and Chisholm, S. W. (2003) Cyanophages infecting the oceanic cyanobacterium Prochlorococcus. Nature, 424, 1047-1051.

Suttle, C. A. (2005) Viruses in the sea. Nature, 437, 356-361.

Suttle, C. A. (2007) Marine viruses - major players in the global ecosystem. Nature Reviews Microbiology, 5, 801-812.

Suttle, C. A. and Chen, F. (1992) Mechanisms and Rates of Decay of Marine Viruses in Seawater. Applied and Environmental Microbiology, 58, 3721-3729.

Tarutani, K., Nagasaki, K. and Yamaguchi, M. (2000) Viral impacts on total abundance and clonal composition of the harmful bloom-forming phytoplankton Heterosigma akashiwo. Applied and Environmental Microbiology, 66, 4916-+.

Thingstad, T. F. (2000) Elements of a theory for the mechanisms controlling abundance, diversity, and biogeochemical role of lytic bacterial viruses in aquatic systems. Limnology and Oceanography, 45, 1320-1328.

Thingstad, T. F., Heldal, M., Bratbak, G. and Dundas, I. (1993) Are viruses important partners in pelagic fend webs? Trends in Ecology & Evolution, 8, 209-213.

Thingstad, T. F. and Lignell, R. (1997) Theoretical models for the control of bacterial growth rate, abundance, diversity and carbon demand. Aquatic Microbial Ecology, 13, 19-27.

Thingstad, T. F., Pree, B., Giske, J. and Våge, S. (2015) What difference does it make if viruses are strain-, rather than species-specific? Frontiers in Microbiology, 6, 320.

Thomas, R., Grimsley, N., Escande, M. L., Subirana, L., Derelle, E. and Moreau, H. (2011) Acquisition and maintenance of resistance to viruses in eukaryotic phytoplankton populations. Environmental Microbiology, 13, 1412-1420.

Thomas, R., Jacquet, S., Grimsley, N. and Moreau, H. (2012) Strategies and mechanisms of resistance to viruses in photosynthetic aquatic microorganisms. 2012, 3, 15.

Thomsen, H. A. and Buck, K. R. (1998a) Nanoflagellates of East Pacific coastal waters: Morphology, taxonomy, and biogeography of weakly calcified coccolithophorids (Prymnesiophyceae). Cryptogamie Algologie, 19, 29-48.

Thomsen, H. A. and Buck, K. R. (1998b) Nanoflagellates of the central California waters: taxonomy, biogeography and abundance of primitive, green flagellates (Pedinophyceae, Prasinophyceae). Deep-Sea Research Part Ii-Topical Studies in Oceanography, 45, 1687-1707.

Thyrhaug, R., Larsen, A., Thingstad, T. F. and Bratbak, G. (2003) Stable coexistence in marine algal host-virus systems. Marine Ecology Progress Series, 254, 27-35.

van Baren, M. J., Bachy, C., Reistetter, E. N., Purvine, S. O., Grimwood, J., Sudek, S., Yu, H., Poirier, C., Deerinck, T. J., Kuo, A., Grigoriev, I. V., Wong, C.-H., Smith, R. D., Callister, S. J., Wei, C.-L., Schmutz, J. and Worden, A. Z. (2016) Evidence-based green algal genomics reveals marine diversity and ancestral characteristics of land plants. Bmc Genomics, 17.

van Hannen, E. J., Zwart, G., van Agterveld, M. P., Gons, H. J., Ebert, J. and Laanbroek, H. J. (1999) Changes in Bacterial and Eukaryotic Community Structure after Mass Lysis of Filamentous Cyanobacteria Associated with Viruses. Applied and Environmental Microbiology, 65, 795-801.

Vardi, A., Haramaty, L., Van Mooy, B. A. S., Fredricks, H. F., Kimmance, S. A., Larsen, A. and Bidle, K. D. (2012) Host-virus dynamics and subcellular controls of cell fate in a natural coccolithophore population. Proceedings of the National Academy of Sciences of the United States of America, 109, 19327-19332.

Vardi, A., Van Mooy, B. A. S., Fredricks, H. F., Popendorf, K. J., Ossolinski, J. E., Haramaty, L. and Bidle, K. D. (2009) Viral Glycosphingolipids Induce Lytic Infection and Cell Death in Marine Phytoplankton. Science, 326, 861-865.

Villarreal, L. P. (2005) Viruses and the Evolution of Life, American Society of Microbiology.

Volk, T. and Hoffert, M. I. (1985) Ocean Carbon Pumps: Analysis of Relative Strengths and Efficiencies in Ocean-Driven Atmospheric CO2 Changes. In The Carbon Cycle and Atmospheric CO2: Natural Variations Archean to Present American Geophysical Union, pp. 99-110.

Waterbury, J. B. and Valois, F. W. (1993) Resistance to cooccurring phages enables marine synechococcus communities to coexist with cyanophages abundant in seawater. Applied and Environmental Microbiology, 59, 3393-3399.

Waters, R. E. and Chan, A. T. (1982) Micromonas-pusilla virus - the virus growth-cycle and associated physiological events within the host-cells - host range mutation. Journal of General Virology, 63, 199-206.

Weinbauer, M. G. (2004) Ecology of prokaryotic viruses. Fems Microbiology Reviews, 28, 127-181.

Weinbauer, M. G. and Holfe, M. G. (1998) Distribution and life strategies of two bacterial populations in a eutrophic lake. Appl Environ Microbiol, 64.

Weinbauer, M. G., Hornak, K., Jezbera, J., Nedoma, J., Dolan, J. R. and Simek, K. (2007) Synergistic and antagonistic effects of viral lysis and protistan grazing on bacterial biomass, production and diversity. Environmental Microbiology, 9, 777-788.

Weinbauer, M. G. and Rassoulzadegan, F. (2004) Are viruses driving microbial diversification and diversity? Environmental Microbiology, 6, 1-11.

Weitz, J. S. (2016) Quantitative Viral Ecology: Dynamics of Viruses and Their Microbial Hosts, Princeton University Press, Princeton, NJ.

Weitz, J. S., Poisot, T., Meyer, J. R., Flores, C. O., Valverde, S., Sullivan, M. B. and Hochberg, M. E. (2013) Phage-bacteria infection networks. Trends in Microbiology, 21, 82-91.

Weitz, J. S. and Wilhelm, S. W. (2012) Ocean viruses and their effects on microbial communities and biogeochemical cycles. F1000 Biology Reports, - 4.

Westbroek, P., Brown, C. W., Vanbleijswijk, J., Brownlee, C., Brummer, G. J., Conte, M., Egge, J., Fernandez, E., Jordan, R., Knappertsbusch, M., Stefels, J., Veldhuis, M., Vanderwal, P. and Young, J. (1993) A model system approach to biological climate forcing - the example of emiliania-huxleyi. Global and Planetary Change, 8, 27-46.

Weynberg, K., Allen, M. and Wilson, W. (2017) Marine Prasinoviruses and Their Tiny Plankton Hosts: A Review. Viruses, 9, 43.

Whitlock, M. C. (1996) The red queen beats the jack-of-all-trades: The limitations on the evolution of phenotypic plasticity and niche breadth. American Naturalist, 148, S65-S77.

Wichels, A., Biel, S. S., Gelderblom, H. R., Brinkhoff, T., Muyzer, G. and Schutt, C. (1998) Bacteriophage diversity in the North Sea. Applied and Environmental Microbiology, 64, 4128-4133.

Wigington, C. H., Sonderegger, D., Brussaard, C. P. D., Buchan, A., Finke, J. F., Fuhrman, J. A., Lennon, J. T., Middelboe, M., Suttle, C. A., Stock, C., Wilson, W. H., Wommack, E. K., Wilhelm, S. W. and Weitz, J. S. (2016) Re-examination of the relationship between marine virus and microbial cell abundances. Nature Microbiology, 1.

Wilhelm, S. W. and Suttle, C. A. (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.

Wilson, D. S. and Yoshimura, J. (1994) On the coexistence of specialists and generalists. American Naturalist, 144, 692-707.

Wilson, W. H., Schroeder, D. C., Allen, M. J., Holden, M. T. G., Parkhill, J., Barrell, B. G., Churcher, C., Harnlin, N., Mungall, K., Norbertczak, H., Quail, M. A., Price, C., Rabbinowitsch, E., Walker, D., Craigon, M., Roy, D. and Ghazal, P. (2005) Complete genome sequence and lytic phase transcription profile of a Coccolithovirus. Science, 309, 1090-1092.

Wilson, W. H., Tarran, G. A., Schroeder, D., Cox, M., Oke, J. and Malin, G. (2002) Isolation of viruses responsible for the demise of an Emiliania huxleyi bloom in the English Channel. Journal of the Marine Biological Association of the United Kingdom, 82, 369-377.

Wilson, W. H., Van Etten, J. L. and Allen, M. J. (2009) The Phycodnaviridae: The Story of How Tiny Giants Rule the World. In Lesser Known Large Dsdna Viruses, Vol. 328 (Ed, VanEtten, J. L.), pp. 1-42.

Winget, D. M. and Wommack, K. E. (2009) Diel and daily fluctuations in virioplankton production in coastal ecosystems. Environmental Microbiology, 11, 2904-2914.

Winter, A. J., R.W.; Roth, P.H. (1994) Biogeography of living coccolithophores in ocean waters. In Coccolithophores (Ed, Siesser., A. W. a. W. G.) Cambridge University Press., Cambridge, pp. 161-177.

Winter, C., Bouvier, T., Weinbauer, M. G. and Thingstad, T. F. (2010) Trade-Offs between Competition and Defense Specialists among Unicellular Planktonic Organisms: the "Killing the Winner" Hypothesis Revisited. Microbiology and Molecular Biology Reviews, 74, 42-57.

Wommack and Colwell (2000) Virioplankton: Viruses in aquatic ecosystems. Microbiology and Molecular Biology Reviews, 64, 69-+.

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

Zhang, R., Weinbauer, M. G. and Qian, P. Y. (2007) Viruses and flagellates sustain apparent richness and reduce biomass accumulation of bacterioplankton in coastal marine waters. Environmental Microbiology, 9, 3008-3018.

Zhong, X. and Jacquet, S. (2014) Contrasting diversity of phycodnavirus signature genes in two large and deep western European lakes. Environmental Microbiology, 16, 759-773.

Zingone, A., Natale, F., Biffali, E., Borra, M., Forlani, G. and Sarno, D. (2006) Diversity in morphology, infectivity, molecular characteristics and induced host resistance between two viruses infecting Micromonas pusilla. Aquatic Microbial Ecology, 45, 1-14.

Zingone, A., Sarno, D. and Forlani, G. (1999) Seasonal dynamics in the abundance of Micromonas pusilla (Prasinophyceae) and its viruses in the Gulf of Naples (Mediterranean Sea). Journal of Plankton Research, 21, 2143-2159.

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Micromonas versus virus: New experimental insights challenge viral impact

Eliana Ruiz,[1]* Anne-Claire Baudoux,[2] Nathalie Simon,[2] Ruth-Anne Sandaa,[1] T. Frede Thingstad[1] and António Pagarete[1]

[1]*Department of Biology, University of Bergen, Bergen, Norway.*

[2]*CNRS, UMR 7144 (Adaptation et Diversité en Milieu Marin), Station Biologique de Roscoff, Sorbonne Universités, UPMC Univ Paris 06, Roscoff, 29680, France.*

Summary

Viruses have recurrently been hypothesized as instrumental in driving microbial population diversity. Nonetheless, viral mediated co-existence of r/k-strategists, predicted in the Killing-the-Winner (KtW) hypothesis, remains controversial and demands empirical evidence. Therefore, we measured the life strategy parameters that characterize the relevant system *Micromonas*-Micromonas Virus (MicV). A large number of host and viral strains (37 and 17, respectively) were used in a total of 629 cross-infectivity tests. Algal and viral abundances were monitored by flow cytometry and used to calculate values of growth rate, resistance capacity, and viral production. Two main assumptions of the KtW model, namely (1) a resistance-associated cost on growth and (2) a negative correlation between resistance and viral production capacity, were mildly observed and lacked statistical significance. *Micromonas* strains infected by more MicV strains presented higher lysis and viral production rates as the number of infectious virus strains increased, suggesting a 'one-gate' regulation of infection in this system. MicV strains demonstrated a vast range of virion production capacity, which unexpectedly grew with increasing host-range. Overall, the significant trends observed in here demonstrate strong co-interactions at different levels between *Micromonas* and MicV populations, however, the role of viruses as major driving force in phytoplankton fitness wasn't explicitly observed.

Received 4 July, 2016; accepted 13 March, 2017. *For correspondence. E-mail Eliana.Martinez@uib.no; Tel. +47 55 58 4400; Fax +47 55 58 4450.

Introduction

It is now well acknowledged that marine viruses interact with their cellular counterparts in the microbial kingdom on an unforeseen scale (Fuhrman, 1999; Brussaard, 2004; Suttle, 2007; Breitbart, 2012). An estimated 10^{23} new infections occur every second in the ocean, and induce the mortality through cell lysis of an important fraction of marine microbes, which include bacteria, phytoplankton and zooplankton (Suttle and Chan, 1994; Suttle, 2005; Baudoux and Brussaard, 2008; Mojica *et al.*, 2015). The extent of viral mediated mortality can be remarkably high during phytoplankton blooms where it can reach near 100% cell mortality (Bratbak *et al.*, 1993; Brussaard *et al.*, 1996a,b; Baudoux *et al.*, 2006). Viral lysis is of fundamental importance to global biogeochemical cycling and ecosystem function (Fuhrman, 1999). Understanding viral impact on marine microbes is hence an indispensable part in our efforts to model marine ecosystem functioning. Although it has become evident that viruses can contribute to the structure and diversity of microbial communities (Waterbury and Valois, 1993; Suttle, 1994; Brussaard, 2004), the extent to which they impact their eukaryotic hosts remains unknown. These relentless viral interactions with their cellular hosts represent, at least theoretically, an important factor regulating phytoplankton fitness (Thingstad and Lignell, 1997).

In the pelagic realm, where microorganisms have a life expectancy of hours to days before they are either consumed by a predator or lysed by a virus (Våge *et al.*, 2013), selection pressure for efficient life strategies including competition for nutrients and defence against predation or parasitism is likely to be high. In theory at least, if viruses are major drivers of phytoplankton fitness, then the expectation would be to observe the existence of r and k-selected cells, demonstrating a clear trade-off between their capacity to grow or to fight viral infection (resistance) (Thingstad, 2000; Suttle, 2007). Several studies suggested that viral infection induces morphological and metabolic changes in host population and, in most cases, the development of resistance is associated with a fitness penalty

for the resistant microbes (Brockhurst *et al.*, 2004; Brockhurst *et al.*, 2005; Benmayor *et al.*, 2008; Riemann and Grossart, 2008; Middelboe *et al.*, 2009). For example, in the marine cyanobacteria *Synechococcus*, such cost of resistance (COR) was manifested by a 20% reduction in fitness, in terms of growth rate and competitive capacity, compared to ancestral susceptible strains (Lennon *et al.*, 2007). In the case of *Prochlorococcus*, another major cyanobacterial group, fitness costs have been associated with resistance-rendering mutations in genomic island regions (Avrani *et al.*, 2011). If the existence of a COR seems to be well acknowledged for some bacterial strains, the question remains open regarding eukaryotic phytoplankton.

Dynamic models of viral-host interactions in marine environments have, at their core, the same set of assumptions as were made for dynamics of *Escherichia coli* and associated phage in chemostats (Levin *et al.*, 1977). These 'box' models consider how abundances of hosts, viruses and resources change with time due to the effects of resource uptake, cell division, viral-induced mortality of hosts, and viral reproduction via lysis. Extensions of these models to the ocean have taken into account a greater complexity of factors, including the possible covariation of bacterial and viral life history traits (Middelboe, 2000; Middelboe *et al.*, 2001; Weitz, 2008). The most prominent one is the Killing the Winner model (KtW), which predicts that viruses represent a balancing factor that allows the co-existence of host species with different growth rates (Thingstad and Lignell, 1997). Viruses that specifically kill (or control) fast-growing host cells provide niches for the development of slower-growing cells. This would be the *sine qua non* condition that allows the maintenance of highly dynamic and diverse microbial communities (Thingstad, 2000).

In this study, we present the first extensive estimation of viral resistance-associated fitness costs, in terms of growth rate, in the relevant marine phytoplankton *Micromonas*. This prominent phytoplankton recurrently dominates coastal picoeukaryotic communities in a wide range of marine environments (Knightjones and Walne, 1951; Thomsen and Buck, 1998a,b) and represents an important contributor to global primary production (Marañón *et al.*, 2001; Worden *et al.*, 2004). Recent phylogenetic studies have demonstrated the existence of at least three major *Micromonas* clades (van Baren *et al.*, 2016). *Micromonas* cells are recurrently infected by viruses in contrasted marine ecosystems. The vast majority of *Micromonas* viruses (hereafter referred to as MicV) belong to the genus *Prasinovirus*, within the *Phycodnaviridae* family (double stranded DNA viruses that infect marine or freshwater eukaryotic algae) (Dunigan *et al.*, 2006; Bellec *et al.*, 2009). These lytic viruses are wide-spread (Cottrell and Suttle, 1991), abundant, genetically diverse (Cottrell and Suttle, 1995), and exhibit variable host specificity and life strategies (Baudoux and Brussaard, 2005). Taking advantage of the large number of different but closely related host and virus strains for this host-virus system, an extensive array of cross-infectivity experiments was conducted in order to investigate the existence of a COR and the applicability of the KtW model in this eukaryotic phytoplankton-virus system.

Results

Host-based parameters

Micromonas average growth rate (μ) varied among strains from 0.09 (SD $\pm$ 0.11) to 0.98 (SD $\pm$ 0.02) d^{-1}. Variability in growth rate was not correlated to phylogenetic clade affiliation (F (2, 34) = 0.095, $p = 0.909$) (Supporting Information Fig. S1).

In order to investigate the existence of a trade-off between growth and resistance capacities, we measured the level of resistance of each *Micromonas* strain to viral infection in two manners: (1) percentage of cells that were not lysed after incubation with viruses (Fig. 1) and (2) the number of MicV strains that successfully produced progeny on that host (Supporting Information Fig. S2). Using either type of resistance measurement we did not identify a significant correlation between growth rate and resistance (Pearson's $r = -0.174$, $p = 0.302$, and Pearson's $r = 0.143$, $p = 0.397$, respectively). Yet, a tendency for resistance to decrease as growth rate increased was observed, but the level of variation was high and the trend not statistically significant (Fig. 1). Curiously, in three cases out of 201 successful infections the algae grew faster with viral inoculum than in the control.

Micromonas strains with lower levels of viral-provoked cell lysis were infected by a significantly lower number of viral strains (Pearson's $r = -0.737$, $p < 0.01$) (Fig. 2). Also

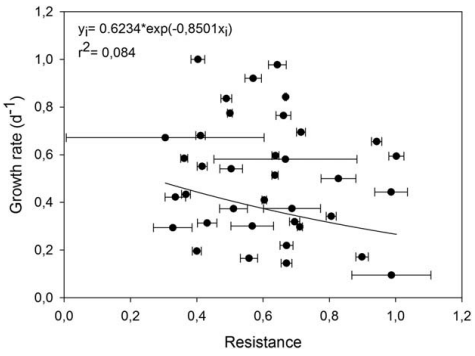

Fig. 1. Growth rates of different *Micromonas* strains as a function of resistance to viral infection (as % of surviving cells). Resistance tended to increase as growth rate (μ) decreased but the correlation was not statistically significant.

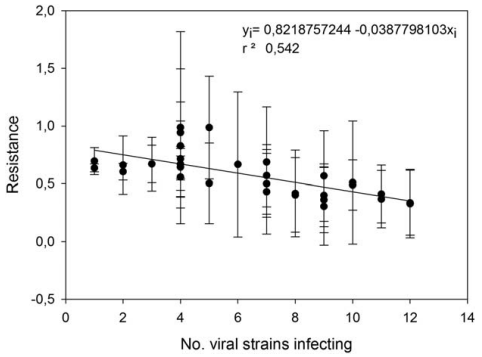

Fig. 2. Resistance (as % of surviving cells) of different *Micromonas* strains as a function of the number of viral strains that induced cell lysis. The number of viruses infecting each *Micromonas* strain clearly decreased as their capacity to survive infection increased.

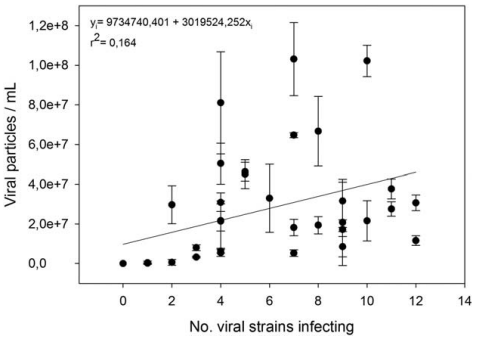

Fig. 4. Correlation between number of viral strains infecting each *Micromonas* strain and maximum viral production. Note that when an algal strain is infected by more viruses, the maximum viral production is also higher.

significant (Pearson's $r = 0.697$, $p < 0.01$) was the correlation between growth rate and viral production, with the fastest growing cells being capable of producing more virions (Fig. 3). Maximum viral production was positively correlated with the number of viral strains infecting each algal strain (Pearson's $r = 0.406$, $p = 0.0128$) (Fig. 4).

Virus-based parameters

We observed an important variation in virion production capacity among the different MicV strains, which was clearly reflected in the 'Maximal Viral Production' (Supporting Information Fig. S3b). On the other hand, 'Average Viral Production' (Supporting Information Fig. S3a) indicated that while some viral strains tend to be consistent regarding the amount of progeny they produce on each different host (small error bars), other MicV strains displayed variable virion production depending on the host (large error bars). There were no significant relationships in average and maximal viral production associated with clade affiliation of host strains as determined by one-way ANOVA (F (1, 15) = 0.042, $p = 0.839$; F (1, 15) = 0.253, $p = 0.621$, respectively). Among the 17 MicV strains used in this study only 2, RCC4240 and RCC4245 were strictly clade-specific (both isolated in *Micromonas* clade A) (Guillou *et al.*, 2004; Baudoux *et al.*, 2015). The remaining MicV isolates could infect hosts from clades A and B (Fig. 5). Host range among MicV strains was very variable, from generalists who could infect up to 23 host strains (e.g., RCC4256 and RCC4247) to specialists that could infect only two strains (e.g. RCC4240). Viruses isolated from *Micromonas* clade A strains tended to be more clade-specific than those isolated from clade B **(**Fig. 5**)** (F (1, 15) = 12.21, $p = 0.0033$).

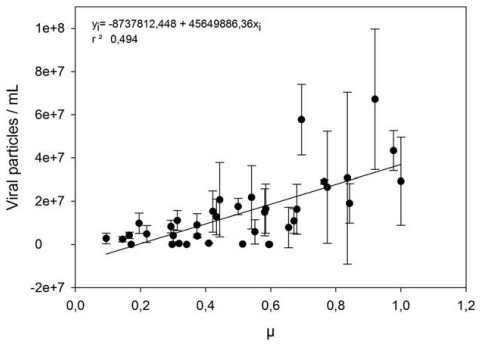

Fig. 3. A positive correlation was observed between growth rate (μ) and viral production capacity among the *Micromonas* strains under study.

We observed an unexpected tendency for more generalist viruses to display higher viral production of new progeny than viruses with narrow host ranges (Fig. 6). This was supported by significant positive correlations observed between average/maximum viral production and the number of different algal strains that a virus can infect (Pearson's $r = 0.680$, $p = 0.00268$ and Pearson's $r = 0.842$, $p < 0.01$, respectively).

Modularity and nestedness analysis

The bipartite network analysis applied to the whole host-range matrix displayed a combination of nestedness and modularity levels. Two modules were clearly discriminated, and comprised viruses isolated from hosts that belong to clades A and B, respectively (Fig. 5). At the same time, each module showed an intra-modular nested structure. In

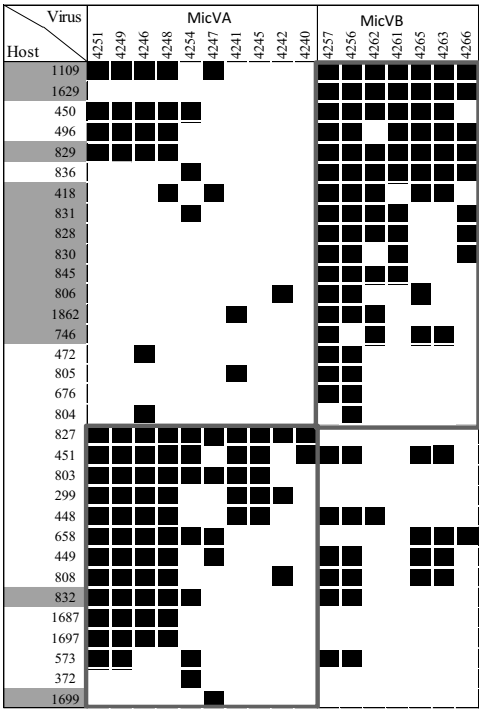

Fig. 5. Host range of the 17 MicV strains. Both, viral and algal strains, are organized according to the modular structure derived from the modularity analysis (see Methods for details). Black squares indicate successful infections. White and grey colours in the host column correspond to the phylogenetic clades A and B, respectively. The only *Micromonas* strain from clade C that was used was not infected by any of the viruses and hence is not represented.

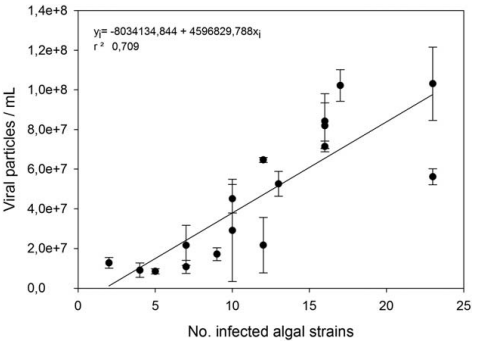

Fig. 6. Maximal viral production increased significantly with expanding host-range. We can appreciate the increasing of the viral production as the virus increases its host range. The same results were obtained with average viral production (**Fig. S4**).

that nested pattern specialist viruses tended to infect the most susceptible hosts, while the viruses with broader host-range infected hosts with higher resistance (Fig. 5).

Discussion

In this study, we performed an extensive array of cross-infectivity experiments between *Micromonas* and MicV to investigate viral impact on phytoplankton fitness and the applicability of the KtW model to this eukaryotic phytoplankton-virus system. To our knowledge, this is the first study where KtW has been empirically tested using a large collection of eukaryotic phytoplankton strains and viruses. One of the most consensual hypotheses about viral impact on aquatic microbial organisms (including phytoplankton) is the co-existence of fast-growers along with others that specialized in resisting viral infection with incurring costs on their growth capacity. This trade-off, often called Cost of Resistance, is also one of the pillars of the KtW model (Thingstad, 2000; Short, 2012; Våge *et al.*, 2013). The existence/magnitude of this trade-off has not yet been demonstrated in eukaryotic phytoplankton (Heath and Collins, 2016). It was reasonable to expect that a trend should emerge when analysing resistance capacity among a large number of hosts with variable growth rates as attempted here. In our study we observed a tendency for growth rate to decrease with increasing resistance (Fig. 1), which would point towards the existence of a trade-off between these two parameters; as the KtW model predicts. However, and most noticeable, that tendency was not significant. Hence, our observations contribute with scepticism on the prevalence of COR in this phytoplankton-virus system. Nonetheless, and despite the lack of statistical significance, the mild decreasing slope observed between growth and resistance capacities would agree rather with a lower than a high COR value (Våge *et al.*, 2013). The identification of a COR in phytoplankton-virus systems is a demanding challenge. Phytoplankton cell fitness results from different selective pressures, such as: nutrient harvesting capacity, resistance to grazing, or tolerance to abiotic factors (Lythgoe and Chao, 2003; Brockhurst *et al.*, 2004; Meaden *et al.*, 2015). Viruses should hence be only another selective force amongst a pool of vectors that are not all pulling cell fitness in the same direction. This can, and most probably will, cloud the trace of COR in any phytoplankton-virus system. The impact of other selective forces on *Micromonas* could explain the pronounced levels of variation measured, and why the correlation between growth rate and resistance observed in this study was not significant. In this eukaryotic phytoplankton system, viruses do appear to have a measurable impact on host fitness, but they are probably not the only or main source of selection. If they were, that

'growth rate versus resistance' decreasing trend would have been significant.

In rare occasions (3 out of 201) we observed increased algal growth when in the presence of viruses. This interesting phenomenon has also been previously reported in the related phytoplankton *Ostreococcus tauri* (Thomas *et al.*, 2011). We can speculate that this could be linked to a group response to viral presence, where infected cells could use cell signalling warning mechanisms that would speed up cell division in other cells. However, to these days we do not have an answer to this question.

In the *Micromonas* - MicV system the capacity of a cell to produce virions was strongly linked to its growth rate (μ) (Fig. 3). This was theoretically expected and reported previously for other host/virus systems (Moebus, 1996a,b; Middelboe, 2000; Parada *et al.*, 2006; Motegi and Nagata, 2007). Cell growth rate is generally related to the number of ribosomes, which can vary significantly between rapid and slow-growing cells (Knoll et al., 1999). The number of ribosomes is responsible for the protein synthesizing capacity at the time of infection, which can then condition the rate of virus production (Hadas *et al.*, 1997). Less intuitive is the KtW prediction that resistance should be correlated with viral production. Namely, that the higher the resistance the smaller the number of virions produced (Våge *et al.*, 2013). We did not observe (with statistical significance) such correlation in our data.

Resistance (percentage of surviving cells) did significantly decrease with the number of viral strains capable of infecting the host (Fig. 2). This means that, in strains that are susceptible to fewer viruses, the few viruses that infect them do not provoke severe cell lysis. On the other extreme, we have strains that not only are incapable of keeping viruses outside their gates, but also provide a viral-friendly intracellular environment that will lead to significantly increased cell lysis. In some co-evolution experiments with marine bacteria, it has been proven that the gain of resistance to a virus after infection leads to a broader resistance to many other viruses (Middelboe *et al.*, 2009; Avrani *et al.*, 2011; Flores *et al.*, 2011; Marston *et al.*, 2012). In those cases, immunity seems to be primarily regulated in one (or few) main gate(s). If one viral strain is capable of breeching that gate, then the probably that another viral strain does too increases. This is what we observed in *Micromonas* too. One should expect, if viruses were the main selective force driving *Micromonas* fitness, that those extremely immune-diminished strains would compensate with significantly higher growth rates. Our results show that that is not always the case, suggesting that other factors are probably as important trimming the adaptive space of this phytoplankton species.

From the virus perspective, our results show that there are hosts that offer better conditions for viral replication, allowing a broader range of viral strains to maximize their viral production. We also observed increased viral production with increasing number of possible hosts. This is curious for it means that generalist viruses produce more progeny than specialist viruses. This apparently is a paradox. How can then specialist viruses compete with viruses that have a bigger host pool and can produce more virions? And if being a generalist virus is such an advantage, why did we register the existence of so many specialized MicV strains? In several cases a generalist strategy has been reported to have inherent disadvantages, especially due to antagonistic pleiotropy (Duffy *et al.*, 2006; Elena *et al.*, 2009; Nikolin *et al.*, 2012; Keen, 2014), accumulation of neutral mutations that are deleterious in alternative niches (Kawecki, 1994), and slower evolutionary rates compared with specialists (Whitlock, 1996). In the case of *Micromonas*, a recent study indicates that the thermal stability of a generalist virus (RCC4265) was significantly lower when compared to the one displayed by a specialist virus (RCC4229) (Demory *et al.*, 2017), suggesting structural differences. These type of trade-offs would explain why a great variation in host-ranges is observed in nature, instead of a predominance of generalist viruses. It should also be mentioned that in recent studies with *Tobacco etch potyvirus* (TEV), Bedhomme *et al.* (2012) questioned this classical theoretical need for a trade-off. They have done this by presenting 'un-costly' strong adaptive potential to new hosts.

The MicV strains demonstrated capacity to infect hosts isolated from contrasted marine ecosystems and different oceans. This reveals the maturity of this co-evolutionary interaction (Thingstad *et al.*, 2014) and possibly implies strong stabilizing selection of host defence mechanisms and/or the loss of resistance mechanisms (Waterbury and Valois, 1993). In the virus-host interaction network (VHIN) obtained in our study (Fig. 5), there was a significant modularity match between viral strains and the host clade they were isolated from. This modularity is mostly likely related to the phylogenetic distances between hosts (Baudoux *et al.*, 2015; Weitz, 2016). A high degree of intra-modular nestedness was also revealed in the *Micromonas* - MicV system. Such nested structure is characteristic of VHINs that form ordered subsets of each other (Flores *et al.*, 2016). Two popular models attempt to explain the process that would lead to such co-evolutionary pattern: sequential gene-for-gene adaptations (GFG) or the appearance of viral alleles that facilitate infection against specific host defensive alleles (Matching Allele, MA) (Flor, 1955; Agrawal and Lively, 2002). These two models lead to different outcomes. The MA model implies that COR is similar among all alleles, predicting local adaptation and specialization, and resulting in a Red Queen scenario where frequency-dependent selections favour rare genotypes. The GFG model would result in an Arms Race dynamics where one genotype replaces another leading to continual

improvements in both populations. Weitz *et al.* (2013) debate why this last scenario is more suitable to explain nestedness in planktonic VHINs. The intra-modular nestedness observed is hence consistent with the KtW hypothesis where co-infection is taken into consideration, leading to nested interaction matrices and viruses with broader host ranges (Thingstad *et al.*, 2014). Finally, it should be noted that the VHIN analysis presented here would potentially benefit from the use of quantitative data, instead of its binary form that not only loses information as it introduces bias that will accentuate some features and mask others (Beckett and Williams, 2013).

Conclusions

Overall, the major findings in this study are in agreement with the trends expected theoretically, notably those supporting the KtW model. However, the incapacity to unequivocally demonstrate the existence of a strong resistance-associated trade-off or shows that there is large space for improvement not only of this model, but also of our theories on phytoplankton-virus interactions in the oceans. The unexpected higher viral production registered for generalist viruses also adds in that need to better understand the parameters that regulate phytoplankton-virus relationships. Those efforts should also consider the incorporation of increased complexity and relevant ecological context, namely in situations where cells have to compete for limited resources.

Materials and methods

Micromonas and MicV strains

Algal and viral strains were obtained from the Roscoff Culture Collection, France. A total of 37 *Micromonas* strains were maintained in 30 mL crystal flasks with IMR $^1/_2$ medium (Klochkova *et al.*, 2006) at 16°C and a 14:10 h light:dark illumination cycle at 155 μmol photon m^{-2} s^{-1} irradiance. The strains belonged to the three major phylogenetic clades previously identified for this species (22 from clade A, 14 from clade B and 1 from clade C) (Guillou *et al.*, 2004) and have been isolated from a wide geographic range (Supporting Information Table S1). MicV viral strains have been previously assigned to two main groups, MicV-A and MicV-B, according to the clade affiliation of the host from which they were isolated (Baudoux *et al.*, 2015). We selected 17 MicV strains, with 10 and 7 belonging to groups A and B, respectively (Supporting Information Table S2). For all viral isolates, viral stocks were produced by infection of exponentially growing *Micromonas* RCC827, RCC451 and RCC829 strains (Supporting Information Table S2). Viral lysates were centrifuged at 12 000 $\times$ *g* for 20 min and the supernatant was filtered through a 0.45 μm syringe filter (Whatman plc, GE Healthcare Life Sciences, Kent, England) to remove cellular debris. Stocks were kept at 4°C in the dark and were renewed so often as to never be more than 2 weeks old before inoculation.

Cross-infectivity experiments

Cross-infectivity experiments were performed between all the *Micromonas* and MicV strains and represented a total of 629 crossings. Prior to the experiment, *Micromonas* cultures were maintained in exponential growth phase with cell concentrations ranging from 10^5 to 10^6 cells mL^{-1}. The experiments were performed in 24 well culture plates under the same temperature and light conditions mentioned above. Two mL of each algal culture at $1x10^5$ cells mL^{-1}, were inoculated in triplicate with each of the 17 viral strains at a concentration of $1x10^6$ viral particles mL^{-1}, resulting in multiplicity of infection (MOI) of 10. Three replicates of uninfected culture were also used as a control for each *Micromonas* strain. Cultures were incubated for 72h.

Enumeration of algae and viruses

At times 0 h and 72 h, samples (500 μl) were taken for algal and viral counting using a FACSCalibur BC flow cytometer (Becton–Dickinson, Biosciences, NJ, USA) provided with an air-cooled laser procuring an excitation beam of 15 mW at 488 nm. Viral samples were fixed with 20 μl of glutaraldehyde (final concentration 1%) for 30 min at 4°C, and frozen at −80°C until further use. For flow cytometry analysis, samples were thawed, diluted 500-fold in TE buffer (10:1 mM Tris:EDTA, pH 8, filtered through 0,2μm), and stained with SYBR Green I 10000x diluted (Invitrogen, 1600 Faraday Avenue, PO Box 6482, Carlsbad CA, 92008 United States) for 10 min at 80°C before analysis. Algal enumeration was conducted on fresh samples and cell population were discriminated using chlorophyll auto-fluorescence (670 LP) and SSC signals. Viruses were discriminated using the green fluorescence (530/30) and SSC signals.

Growth rate, resistance, viral production

Growth rates (μ) were calculated for each *Micromonas* strain using the control non-inoculated incubations according to the following formula (Levasseur *et al.*, 1993):

$$\mu = \mathrm{Ln}\ (N2/N1)/t$$

Where N1 and N2 were the cell concentrations at the beginning and end of the experiment, respectively, and t was the incubation time, in days. Growth rate values were normalized to values between 0 and 1.

The level of resistance of each *Micromonas* strain to viral infection was measured in two manners. First manner was the percentage of cells that were not lysed after incubation with viruses, by comparison with the non-inoculated controls. For each *Micromonas* strain a resistance value was hence calculated against each of the 17 MicV strains. Those 17 resistance values were then averaged to obtain an overall resistance capacity for each alga strain. Resistance was also estimated as the number of MicV strains that successfully produced progeny on that host.

Viral production was estimated as the capacity of each viral strain to produce new progeny in a specific host. This was calculated as the difference between final and initial viral concentrations. These values were averaged to obtain an

average viral production capacity for each viral strain. The maximum amount of viruses that each MicV strain could produce was also registered as 'Maximum viral production.'

Potential correlations between the different parameters (growth rate, resistance, and viral production) were investigated with regression slopes and statistical probability analyses, using either Anova (*F*) or Pearson analysis.

Host-virus network analysis

In order to test the structure of the infection network, we used the BiMat package for Matlab (Flores *et al.*, 2016). This network-based analysis was applied on a binary matrix where 0 referred to no lysis and 1 to lysis. The unique *Micromonas* strain from clade c was not included in this analysis. The NODF algorithm was used to measure nestedness and is based on overlap and decreasing fill (Almeida-Neto *et al.*, 2008). It returns a score between 0 and 1, where 1 corresponds to a perfectly nested structure. Modularity (Qb) was calculated using the Leading-Eigenvector algorithm (Newman, 2006). The value Qb, introduced by Barber (2007), is calculated using the standard bipartite modularity function. To quantify the statistical significance of the nestedness (NODF) and modularity (Qb), 100 null random matrices (for each) were created with the null model Equiprobable (a random matrix in which all the interactions are uniformly permuted).

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References

Agrawal, A., and Lively, C.M. (2002) Infection genetics: gene-for-gene versus matching-alleles models and all points in between. *Evol Ecol Res* **4:** 79–90.

Almeida-Neto, M., Guimaraes, P., Guimaraes, P.R., Loyola, R.D., and Ulrich, W. (2008) A consistent metric for nestedness analysis in ecological systems: reconciling concept and measurement. *Oikos* **117:** 1227–1239.

Avrani, S., Wurtzel, O., Sharon, I., Sorek, R., and Lindell, D. (2011) Genomic island variability facilitates Prochlorococcus-virus coexistence. *Nature* **474:** 604–608.

Barber, M.J. (2007) Modularity and community detection in bipartite networks. *Phys Rev E* **76:** 9.

van Baren, M.J., Bachy, C., Reistetter, E.N., Purvine, S.O., Grimwood, J., Sudek, S., *et al.* (2016) Evidence-based green algal genomics reveals marine diversity and ancestral characteristics of land plants. *BMC Genomics* **17:** 267.

Baudoux, A.C., and Brussaard, C.P.D. (2005) Characterization of different viruses infecting the marine harmful algal bloom species Phaeocystis globosa. *Virology* **341:** 80–90.

Baudoux, A.-C., and Brussaard, C.P.D. (2008) Influence of irradiance on virus-algal host interactions. *J Phycol* **44:** 902–908.

Baudoux, A.C., Noordeloos, A.A.M., Veldhuis, M.J.W., and Brussaard, C.P.D. (2006) Virally induced mortality of Phaeocystis globosa during two spring blooms in temperate coastal waters. *Aquat Microbial Ecol* **44:** 207–217.

Baudoux, A.C., Lebredonchel, H., Dehmer, H., Latimier, M., Edern, R., Rigaut-Jalabert, F., *et al.* (2015) Interplay between the genetic clades of Micromonas and their viruses in the Western English Channel. *Environ Microbiol Rep* **7:** 765–773.

Beckett, S.J., and Williams, H.T.P. (2013) Coevolutionary diversification creates nested-modular structure in phage-bacteria interaction networks. *Interface Focus* **3:** 1–11.

Bedhomme, S., Lafforgue, G., and Elena, S.F. (2012) Multihost experimental evolution of a plant RNA virus reveals local adaptation and host-specific mutations. *Mol Biol Evol* **29:** 1481–1492.

Bellec, L., Grimsley, N., Moreau, H., and Desdevises, Y. (2009) Phylogenetic analysis of new Prasinoviruses (Phycodnaviridae) that infect the green unicellular algae Ostreococcus, Bathycoccus and Micromonas. *Environ Microbiol Rep* **1:** 114–123.

Benmayor, R., Buckling, A., Bonsall, M.B., Brockhurst, M.A., and Hodgson, D.J. (2008) The interactive effects of parasitesf disturbance, and productivity on experimental adaptive radiations. *Evolution* **62:** 467–477.

Bratbak, G., Egge, J.K., and Heldal, M. (1993) Viral mortality of the marine alga emiliania-huxleyi (Haptophyceae) and termination of algal blooms. *Mar Ecol Prog Ser* **93:** 39–48.

Breitbart, M. (2012) Marine viruses: Truth or dare. *Annual Review of Marine Science* **4:** 425–448.

Brockhurst, M.A., Rainey, P.B., and Buckling, A. (2004) The effect of spatial heterogeneity and parasites on the evolution of host diversity. *Proc R Soc B-Biol Sci* **271:** 107–111.

Brockhurst, M.A., Buckling, A., and Rainey, P.B. (2005) The effect of a bacteriophage on diversification of the opportunistic bacterial pathogen, Pseudomonas aeruginosa. *Proc R Soc B-Biol Sci* **272:** 1385–1391.

Brussaard, C.P.D. (2004) Viral control of phytoplankton populations - a review. *J Eukaryot Microbiol* **51:** 125–138.

Brussaard, C.P.D., Gast, G.J., vanDuyl, F.C., and Riegman, R. (1996a) Impact of phytoplankton bloom magnitude on a pelagic microbial food web. *Mar Ecol Prog Ser* **144:** 211–221.

Brussaard, C.P.D., Kempers, R.S., Kop, A.J., Riegman, R., and Heldal, M. (1996b) Virus-like particles in a summer bloom of Emiliania huxleyi in the North Sea. *Aquat Microbial Ecol* **10:** 105–113.

Cottrell, M.T., and Suttle, C.A. (1991) Wide-spread occurrence and clonal variation in viruses which cause lysis of a cosmopolitan, eukaryotic marine phytoplankter, Micromonas-pusilla. *Mar Ecol Prog Ser* **78:** 1–9.

Cottrell, M.T., and Suttle, C.A. (1995) Genetic diversity of algal viruses which lyse the photosynthetic picoflagellate micromonas-pusilla (prasinophyceae). *Appl Environ Microbiol* **61:** 3088–3091.

Demory, L.A., Simon, S.N., Rigaut-Jalabert, C., Marie, F., Gei, D., Bigeard, P., *et al.* (2017) Temperature is a key factor in Micromonas–virus interactions. *ISME J* **11:** 601–612.

Duffy, S., Turner, P.E., and Burch, C.L. (2006) Pleiotropic costs of niche expansion in the RNA bacteriophage Phi 6. *Genetics* **172:** 751–757.

Dunigan, D.D., Fitzgerald, L.A., and Van Etten, J.L. (2006) Phycodnaviruses: A peek at genetic diversity. *Virus Res* **117:** 119–132.

Elena, S.F., Agudelo-Romero, P., and Lalic, J. (2009) The evolution of viruses in multi-host fitness landscapes. *Open Virol J* **3:** 1–6.

Flor, H.H. (1955) Host-parasite interaction in flax rust - its genetics and other implications. *Phytopathology* **45:** 680–685.

Flores, C.O., Poisot, T., Valverde, S., and Weitz, J.S. (2016) BiMat: a MATLAB package to facilitate the analysis of bipartite networks. *Methods Ecol Evol* **7:** 127–132.

Flores, C.O., Meyer, J.R., Valverde, S., Farr, L., and Weitz, J.S. (2011) Statistical structure of host-phage interactions. *Proc Natl Acad Sci U S A* **108:** E288–E297.

Fuhrman, J.A. (1999) Marine viruses and their biogeochemical and ecological effects. *Nature* **399:** 541–548.

Guillou, L., Eikrem, W., Chretiennot-Dinet, M.J., Le Gall, F., Massana, R., Romari, K., *et al.* (2004) Diversity of picoplanktonic prasinophytes assessed by direct nuclear SSU rDNA sequencing of environmental samples and novel isolates retrieved from oceanic and coastal marine ecosystems. *Protist* **155:** 193–214.

Hadas, H., Einav, M., Fishov, I., and Zaritsky, A. (1997) Bacteriophage T4 development depends on the physiology of its host Escherichia coli. *Microbiology* **143:** 179–185.

Heath, S.E., and Collins, S. (2016) Mode of resistance to viral lysis affects host growth across multiple. *Environ Microbiol* **18:** 4628–4639.

Kawecki, T.J. (1994) Accumulation of deleterious metations and the evolutionary cost of being a generalist. *Am Nat* **144:** 833–838.

Keen, E.C. (2014) Tradeoffs in bacteriophage life histories. *Bacteriophage* **4:** e28365.

Klochkova, T.A., Kang, S.H., Cho, G.Y., Pueschel, C.M., West, J.A., and Kim, G.H. (2006) Biology of a terrestrial green alga, Chlorococcum sp (Chlorococcales, Chlorophyta), collected from the Miruksazi stupa in Korea. *Phycologia* **45:** 349–358.

Knightjones, E.W., and Walne, P.R. (1951) Chromulina-pusilla butcher, a dominant member of the ultraplankton. *Nature* **167:** 445–446.

Knoll, A., Osborn, M.J., Baross, J., Berg, H.C., Pace, N.R., and Sogin, M. (1999) *Size Limits of Very Small Microorganisms: proceedings of a Workshop*. Washington, DC: National Academy Press.

Lennon, J.T., Khatana, S.A.M., Marston, M.F., and Martiny, J.B.H. (2007) Is there a cost of virus resistance in marine cyanobacteria? *ISME J* **1:** 300–312.

Levasseur, M., Thompson, P.A., and Harrison, P.J. (1993) Physiological acclimation of marine-phytoplankton to different nitrogen-sources. *J Phycol* **29:** 587–595.

Levin, B.R., Stewart, F.M., and Chao, L. (1977) Resource-limited growth, competition, and predation - a model and experimental studies with bacteria and bacteriophage. *Am Nat* **111:** 3–24.

Lovejoy, C., Vincent, W.F., Bonilla, S., Roy, S., Martineau, M.J., Terrado, R., *et al.* (2007) Distribution, phylogeny, and growth of cold-adapted picoprasinophytes in arctic seas. *J Phycol* **43:** 78–89.

Lythgoe, K.A., and Chao, L. (2003) Mechanisms of coexistence of a bacteria and a bacteriophage in a spatially homogeneous environment. *Ecol Lett* **6:** 326–334.

Marañón, E., Holligan, P.M., Barciela, R., Gonzalez, N., Mourino, B., Pazo, M.J., and Varela, M. (2001) Patterns of phytoplankton size structure and productivity in contrasting open-ocean environments. *Mar Ecol Prog Ser* **216:** 43–56.

Marston, M.F., Pierciey, F.J., Shepard, A., Gearin, G., Qi, J., Yandava, C., *et al.* (2012) Rapid diversification of coevolving marine Synechococcus and a virus. *Proc Natl Acad Sci U S A* **109:** 4544–4549.

Meaden, S., Paszkiewicz, K., and Koskella, B. (2015) The cost of phage resistance in a plant pathogenic bacterium is context-dependent. *Evolution* **69:** 1321–1328.

Middelboe, M. (2000) Bacterial growth rate and marine virus-host dynamics. *Microbial Ecol* **40:** 114–124.

Middelboe, M., Hagstrom, A., Blackburn, N., Sinn, B., Fischer, U., Borch, N.H., *et al.* (2001) Effects of bacteriophages on the population dynamics of four strains of pelagic marine bacteria. *Microbial Ecol* **42:** 395–406.

Middelboe, M., Holmfeldt, K., Riemann, L., Nybroe, O., and Haaber, J. (2009) Bacteriophages drive strain diversification in a marine Flavobacterium: implications for phage resistance and physiological properties. *Environ Microbiol* **11:** 1971–1982.

Moebus, K. (1996a) Marine bacteriophage reproduction under nutrient-limited growth of host bacteria.2. Investigations with phage-host system H3:H3/1. *Mar Ecol Prog Ser* **144:** 13–22.

Moebus, K. (1996b) Marine bacteriophage reproduction under nutrient-limited growth of host bacteria.1. Investigations with six phage-host systems. *Mar Ecol Prog Ser* **144:** 1–12.

Mojica, K.D.A., van de Poll, W.H., Kehoe, M., Huisman, J., Timmermans, K.R., Buma, A.G.J., *et al.* (2015) Phytoplankton community structure in relation to vertical stratification along a north-south gradient in the Northeast Atlantic Ocean. *Limnol Oceanogr* **60:** 1498–1521.

Motegi, C., and Nagata, T. (2007) Enhancement of viral production by addition of nitrogen or nitrogen plus carbon in subtropical surface waters of the South Pacific. *Aquat Microbial Ecol* **48:** 27–34.

Newman, M.E.J. (2006) Modularity and community structure in networks. *Proc Natl Acad Sci U S Am* **103:** 8577–8582.

Nikolin, V.M., Osterrieder, K., von Messling, V., Hofer, H., Anderson, D., Dubovi, E., *et al.* (2012) Antagonistic pleiotropy and fitness trade-offs reveal specialist and generalist traits in strains of canine distemper virus. *Plos One* **7:** 9.

Parada, V., Herndl, G.J., and Weinbauer, M.G. (2006) Viral burst size of heterotrophic prokaryotes in aquatic systems. *J Mar Biol Assoc U K* **86:** 613–621.

Riemann, L., and Grossart, H.-P. (2008) Elevated lytic phage production as a consequence of particle colonization by a marine Flavobacterium (Cellulophaga sp.). *Microbial Ecol* **56:** 505–512.

Short, S.M. (2012) The ecology of viruses that infect eukaryotic algae. *Environ Microbiol* **14:** 2253–2271.

Slapeta, J., Lopez-Garcia, P., and Moreira, D. (2006) Global dispersal and ancient cryptic species in the smallest marine eukaryotes. *Mol Biol Evol* **23:** 23–29.

Suttle, C.A. (1994) The significance of viruses to mortality in aquatic microbial communities. *Microbial Ecol* **28:** 237–243.

Suttle, C.A. (2005) Viruses in the sea. *Nature* **437:** 356–361.

Suttle, C.A. (2007) Marine viruses - major players in the global ecosystem. *Nat Rev Microbiol* **5:** 801–812.

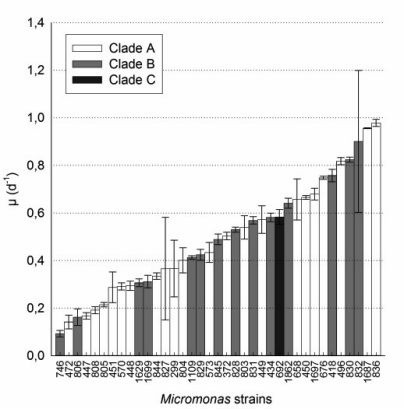
Suttle, C.A., and Chan, A.M. (1994) Dynamics and distribution of cyanophages and their effect on marine synechococcus spp. *Appl Environ Microbiol* **60:** 3167–3174.

Thingstad, T.F. (2000) Elements of a theory for the mechanisms controlling abundance, diversity, and biogeochemical role of lytic bacterial viruses in aquatic systems. *Limnol Oceanogr* **45:** 1320–1328.
Thingstad, T.F., and Lignell, R. (1997) Theoretical models for the control of bacterial growth rate, abundance, diversity and carbon demand. *Aquat Microbial Ecol* **13:** 19–27.
Thingstad, T.F., Vage, S., Storesund, J.E., Sandaa, R.A., and Giske, J. (2014) A theoretical analysis of how strain-specific viruses can control microbial species diversity. *Proc Natl Acad Sci U S A* **111:** 7813–7818.
Thomas, R., Grimsley, N., Escande, M.L., Subirana, L., Derelle, E., and Moreau, H. (2011) Acquisition and maintenance of resistance to viruses in eukaryotic phytoplankton populations. *Environ Microbiol* **13:** 1412–1420.
Thomsen, H.A., and Buck, K.R. (1998a) Nanoflagellates of the central California waters: taxonomy, biogeography and abundance of primitive, green flagellates (Pedinophyceae, Prasinophyceae). *Deep-Sea Res Part II-Top Stud Oceanogr* **45:** 1687–1707.
Thomsen, H.A., and Buck, K.R. (1998b) Nanoflagellates of East Pacific coastal waters: Morphology, taxonomy, and biogeography of weakly calcified coccolithophorids (Prymnesiophyceae). *Cryptogamie Algologie* **19:** 29–48.
Våge, S., Storesund, J.E., and Thingstad, T.F. (2013) Adding a cost of resistance description extends the ability of virus-host model to explain observed patterns in structure and function of pelagic microbial communities. *Environ Microbiol* **15:** 1842–1852.
Waterbury, J.B., and Valois, F.W. (1993) Resistance to cooccurring phages enables marine synechococcus communities to coexist with cyanophages abundant in seawater. *Appl Environ Microbiol* **59:** 3393–3399.
Weitz, J.S. (2008) Evolutionary ecology of bacterial viruses. *Microbe* **3:** 171–178.
Weitz, J.S. (2016) *Quantitative Viral Ecology: Dynamics of Viruses and Their Microbial Hosts*. Princeton, NJ: Princeton University Press.
Weitz, J.S., Poisot, T., Meyer, J.R., Flores, C.O., Valverde, S., Sullivan, M.B., and Hochberg, M.E. (2013) Phage-bacteria infection networks. *Trends Microbiol* **21:** 82–91.
Whitlock, M.C. (1996) The red queen beats the jack-of-all-trades: The limitations on the evolution of phenotypic plasticity and niche breadth. *Am Nat* **148:** S65–S77.
Worden, A.Z., Nolan, J.K., and Palenik, B. (2004) Assessing the dynamics and ecology of marine picophytoplankton: The importance of the eukaryotic component. *Limnol Oceanogr* **49:** 168–179.

Supporting information

Additional Supporting Information may be found in the online version of this article at the publisher's website.

Table S1. *Micromonas* strain information, including geographical origin and taxonomical clade based on previously determined 18S rRNA sequence data (Guillou *et al*., 2004; Slapeta *et al*., 2006; Lovejoy *et al*., 2007). NI= No information.
Table S2. MicV strains used in this study, their isolation information, along with the respective *Micromonas* strain in which each viral stock is prepared.
Fig. S1. Growth rate of the *Micromonas* strains used in the infection experiment, calculated according to Levasseur *et al*. (1993). Values correspond to the control samples and *Micromonas* clades names A, B and C correspond to those of Guillou *et al*. (2004).
Fig. S2. Growth rate (μ) and number of viral strains infecting each algal strain correlation. Growth rate values were normalized between 0 and 1. We can observe that a higher number of viruses infect the algae as the growth rate of these increases.
Fig. S3. Viral production (A) and maximum viral production per MicV strain (B).
Fig. S4. As with maximal viral production, average viral production also increased significantly with expanding host-range.



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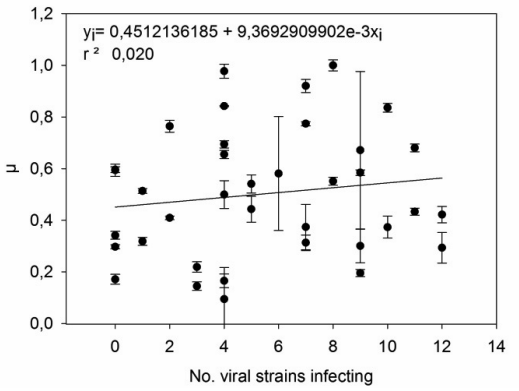

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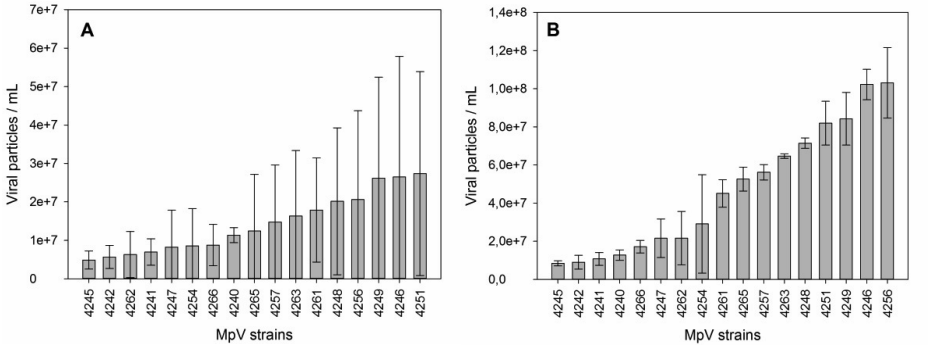

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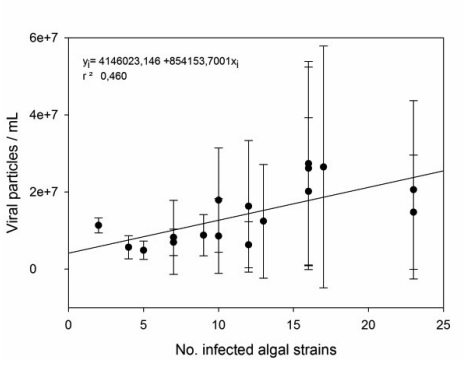

Figure S4. As with maximal viral production, average viral production also increased significantly with expanding host-range.

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Article

Emerging Interaction Patterns in the *Emiliania huxleyi*-EhV System

Eliana Ruiz [1,*], Monique Oosterhof [1,2], Ruth-Anne Sandaa [1], Aud Larsen [1,3] and António Pagarete [1]

1 Department of Biology, University of Bergen, Bergen 5006, Norway; Ruth.Sandaa@uib.no (R.-A.S.); Antonio.Pagarete@uib.no (A.P.)
2 NRL for fish, Shellfish and Crustacean Diseases, Central Veterinary Institute of Wageningen UR, Lelystad 8221 RA, The Nederlands; Monique.oosterhof@wur.nl
3 Uni Research Environment, Nygårdsgaten 112, Bergen 5008, Norway; Aud.Larsen@uni.no
* Correspondence: Eliana.Martinez@uib.no; Tel.: +47-5558-8194

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Abstract: Viruses are thought to be fundamental in driving microbial diversity in the oceanic planktonic realm. That role and associated emerging infection patterns remain particularly elusive for eukaryotic phytoplankton and their viruses. Here we used a vast number of strains from the model system *Emiliania huxleyi*/Emiliania huxleyi Virus to quantify parameters such as growth rate (μ), resistance (R), and viral production (Vp) capacities. Algal and viral abundances were monitored by flow cytometry during 72-h incubation experiments. The results pointed out higher viral production capacity in generalist EhV strains, and the virus-host infection network showed a strong co-evolution pattern between *E. huxleyi* and EhV populations. The existence of a trade-off between resistance and growth capacities was not confirmed.

Keywords: *Phycodnaviridae*; Coccolithovirus; Coccolithophore; *Haptophyta*; Killing-the-winner; cost of resistance; infectivity trade-offs; algae virus; marine viral ecology; viral-host interactions

1. Introduction

Since the discovery of high viral concentrations in the marine environment, normally ranging between 10^7 and 10^{11} virions/L [1], hypotheses regarding the potential impact those viruses could have on their microbial host populations, have been put forward. Viral-induced microbial lysis in Earth's oceans could amount to an impressive 10^{23} new infections per second, releasing up to 10^9 tons of cellular carbon every day [2,3]. Consequently, viral lysis contributes greatly to marine biogeochemical cycling of nutrients as well as reducing the transport of organic matter to upper trophic levels in a process known as viral shunt [4–6]. Through horizontal gene transfer and the lysis of their hosts, marine viruses contribute to structuring the diversity and composition of microbial communities [7–11].

Viral activity has been suggested as a plausible mechanism contributing to explain Hutchinson's paradox, which questions the existence of highly diverse planktonic communities in nutrient limited environments [4,12,13]. Viral strain or species-specific lysis may potentially explain the coexistence of cells with different growth and resistance capacities [14,15]. This scenario is contemplated in the Killing-the-Winner (KtW) hypothesis, notably with the concept that resistance has an inherent cost. This trade-off, also known as cost of resistance (COR), ultimately regulate the co-existence of competition specialists (with higher growth rates) and defence specialists (with higher immune capacity against viral infection), respectively [16].

COR can be detected by analysing the virus-host infection network patterns (VHINs) that emerge after cross-infectivity experiments [17–19]. The most frequently tested VHIN patterns are nestedness

and modularity [17,20]. Nested patterns are characterized by specialist viruses tending to infect the most susceptible hosts, while the viruses with broader host-range infect hosts that are more resistant [21]. On the other hand, in modular patterns the interactions tend to occur within different groups of viruses and hosts, but not between groups [17,22].

The role of viruses as an important driver of microbial diversity has become clear in prokaryotic-virus systems [23–27] such as the *Pseudoalteromonas* [28] and the *Pseudomonas aeruginosa* host-virus systems, in which resistant cells emerging after infection were less competitive than the sensitive ones [24]. In other prokaryote-virus systems that role remains elusive [29–34]. The very few examples of trade-off between resistance and growth rate in eukaryotic hosts include studies on the prasinophyte *Ostreococcus tauri* [35] and the trebouxiophyte *Chlorella variabilis* [36].

Here we aim at getting insight on the main emerging patterns that result from eukaryotic host-virus interactions in the planktonic realm by focusing on *Emiliania huxleyi*, the most abundant and widely distributed calcifying haptophyte in our oceans [37], and its lytic viruses. Mostly known for its impressive blooms [38,39] this microalga is an important player in global geochemical cycles [40,41]. This photosynthetic unicellular eukaryote is infected by *Emiliania huxleyi* viruses (EhV), lytic giant viruses belonging to the genus *Coccolithovirus*, within the *Phycodnaviridae* family. These viruses are ubiquitous in the marine environment [42] and abundant, reaching 10^7/mL in natural seawater during bloom conditions and from 10^8 to 10^9/mL in laboratory cultures [43]. Genomic and metagenomic EhV characterizations show both a global consistency of this viral genome on a planetary scale as well as the maintenance of specific localized genetic traits. For example, despite the high levels of sequence similarity (>95%) between EhV isolates from a Norwegian fjord and the English Channel, these viral populations also contain distinctive genetic traits [44–50]. It is surprising that these genetic traits have been maintained through decades although no geographical isolation and speciation have occurred to date [45], allowing these viral communities to infect hosts from distant geographic places [44,51].

Taking advantage of the large number of *E. huxleyi* cell and EhV lines available for this host-virus system, from diverse geographical origins that include the major oceanic regions, an extensive array of cross-infectivity experiments was conducted in order to investigate parameters such as growth rate (μ), resistance (R), and viral production (Vp). We then confronted possible existence of correlations between those parameters with the theoretical hypotheses (Table 1) that delimit our conception of virus-microbe interactions in the oceans and the way we model those interactions.

Table 1. Hypotheses tested in the current study based on outcome of previous virus-host interaction studies. μ: growth rate; R: resistance; Vp: viral production.

Number	Hypothesis	Reference
1	Resistance is associated with reduced growth rates (COR).	Prokaryotes: [23–25,27–29,52–55] Eukaryotes: [35,36,56,57]
2	Host strains with higher μ produce more viruses.	[58–66]
3	Host strains with higher μ are infected by more viral strains.	[36]
4	Host strains with higher R produce fewer viruses.	[56,67]
5	Specialist viruses have higher Vp than generalists.	[14,68]

2. Materials and Methods

2.1. Emiliania Huxleyi and EhV Strains

Algal strains were obtained from the Roscoff Culture Collection, France; and from the University of Bergen, Norway. A total of 49 *E. huxleyi* strains (Table S1) were maintained in 30 mL polystyrene flasks with IMR $\frac{1}{2}$ medium [69] at 16 °C and a 14:10 h light:dark illumination cycle at 155 μmol photon m^{-2}/s irradiance.

A total number of 13 viral strains were obtained from the Plymouth Marine Laboratory, UK; and from the University of Bergen, Norway (Table S2). For all viral isolates, viral stocks were produced by infection of exponentially growing *E. huxleyi* RCC1257 strain. Viral lysates were centrifuged at

12,000 × g for 20 min and the supernatant was filtered through a 0.45 µm syringe filter (Whatman plc, GE Healthcare Life Sciences, Kent, UK) to remove cellular debris. Viral stocks were kept at 4 °C in the dark and were renewed so often as to never be more than 2 weeks old before inoculation in order to preserve the agent's viability. Plaque assays were not conducted as haptophytes in general do not grow on agar plates and have only been achieved for a few *E. huxleyi* strains [70,71].

2.2. Cross-Infectivity Experiments

Cross-infectivity experiments were performed between all the *E. huxleyi* and EhV strains (Table S3). Prior to each experiment, *E. huxleyi* cultures were maintained in exponential growth phase with cell concentrations ranging from 10^5 to 10^6 cells/mL. The experiments were performed in 24 well culture plates under the same temperature and light conditions as the general culturing conditions described above. Triplicates of 2 mL of each algal culture (1×10^5 cells/mL) were inoculated with each of the 13 viral strains at a concentration of 1×10^6 viral particles/mL, resulting in a virus to host ratio (VHR) of 10. Three replicates of uninfected culture were also used as a control for each *E. huxleyi* strain. An incubation time of 72 h was chosen because this is consistent with the time scales reported for *E. huxleyi*/EhV selection dynamics observed in the natural environment [72–74]. Moreover, preliminary growth tests [75] performed on several *E. huxleyi* strains, did not indicate that prolonged incubation period would contribute essential knowledge on the growth capacity of each strain.

2.3. Enumeration of Algae and Viruses

At times 0 h and 72 h, 500 µL was subsampled from each well to determine algae and virus concentrations using a FACSCalibur BC flow cytometer (Becton–Dickinson, Biosciences, Franklin Lakes, NJ, USA) [76–78] provided with an air-cooled laser procuring 15 mW at 488 nm. Viral samples were fixed with 20 µL of glutaraldehyde (25%) for 30 min at 4 °C, and frozen at −80 °C until further use. For flow cytometry analysis, samples were thawed, diluted 500-fold in TE buffer (10:1 mM Tris:EDTA, pH 8, filtered through 0.2 µm), and stained with SYBR Green I 100× diluted (Invitrogen, 1600 Faraday Avenue, PO Box 6482, Carlsbad CA, 92008 United States) for 10 min at 80 °C before analysis. Algal enumeration was conducted on fresh samples, and cell populations were discriminated using chlorophyll auto-fluorescence (670 LP) and SSC signals. Virus populations were determined and enumerated on basis of their green fluorescence (530/30) and SSC signals.

2.4. Growth Rate, Resistance, Viral Production

Growth rates (µ) were calculated for each *E. huxleyi* strain using the control non-inoculated incubations according to the following formula [79]:

$$\mu = \mathrm{Ln}\,(N2/N1)/t \quad (1)$$

where N1 and N2 were the cell concentrations at the beginning and end of the experiment, respectively, and t was the incubation time in days.

The level of resistance of each *E. huxleyi* strain to viral infection was measured in two manners. The first manner (R_1) was based on the difference of cells that were not lysed after incubation with viruses, compared to the non-inoculated controls. For each *E. huxleyi* strain a resistance value was hence calculated against each of the 13 EhV strains and the 13 resistance values were then averaged to obtain an overall resistance capacity for each alga strain (R_1). Resistance was also estimated as the number of EhV strains that successfully produced progeny on that host (R_2).

A value of viral production (Vp), corresponding to the capacity of each viral strain to produce new progeny on a certain host, was calculated for each virus — host pair as the difference between final and initial viral concentrations. These values were averaged to obtain a global infectivity capacity for each viral strain, per algal strain. The maximum amount of viruses that each EhV strain, per algal strain, produced was registered as "Maximum viral production".

Potential correlations between the different parameters (growth rate, resistance, and viral production) were investigated with regression slopes and statistical probability analyses, using either Anova (*F*) or Pearson analysis.

A potential impact of domestication on these parameters was also investigated. An analysis was performed on two groups of *E. huxleyi* strains, which were isolated in different periods of time. The periods before and after 2009, respectively, were chosen for an apparent increase in Vp was preliminary observed in strains as old or younger than 2009 (Figure S1).

2.5. Host-Virus Network Analysis

In order to test the structure of the infection network, we used the BiMat package for Matlab [21]. This network-based analysis was applied on a binary matrix where 0 referred to no lysis and 1 to lysis. The NODF algorithm was used to measure nestedness and is based on overlap and decreasing fill [80]. It returns a score between 0 and 1, where 1 corresponds to a perfectly nested structure. Modularity (Qb) was calculated using the Leading-Eigenvector algorithm [81]. The value Qb, introduced by Barber [82], is calculated using the standard bipartite modularity function. To quantify the statistical significance of the nestedness (NODF) and modularity (Qb), 100 null random matrices (for each) were created with the null model Equiprobable (a random matrix in which all the interactions are uniformly permuted).

3. Results

Forty-nine *E. huxleyi* strains were characterized according to their ability to grow under a standard set of nutrients, light and temperature conditions. Growth rate (μ) varied significantly among *E. huxleyi* strains, ranging from 0.12 (SD $\pm$ 0.01) to 1.11 (SD $\pm$ 0.02)/d (registered in strains RCC4533 and RCC1744, respectively) (Figure S2). The difference in growth rate among the algal strains was not related to the ocean they were isolated from (one-way ANOVA F (2, 37) = 0.275, p = 0. 76).

We confronted the observed differences in resistance capacity with the parameters growth rate and viral production, respectively. The level of resistance of *E. huxleyi* to EhV infection was accessed in two manners: (R_1) percentage of cells that were not lysed after incubation with viruses (Figure S3) and (R_2) the number of EhV strains that successfully produced progeny on that host, meaning that lower R_2 levels indicate higher resistance capacity. A trade-off between resistance and growth rate capacities (hypotheses 1 and 3 in Table 1**)** was not confirmed with our results. Neither types of resistance, R_1 and R_2, were significantly correlated to growth rate (Pearson's r = -0.131, p = 0.370, and Pearson's r = -0.0959, p = 0.512; respectively) (Figures 1 and 2). R_1 was indirectly correlated with viral production (Figure 3) (Pearson's r = -0.499, $p > 0.01$), in accordance with hypothesis 4. R_2 was significantly and positively correlated with maximum viral production (Pearson's r = 0.614, $p < 0.01$), which means that the *E. huxleyi* strains that were susceptible to more EhV types were also the ones that presented higher maximum viral production (Figure 4). Viral production and growth rate did not correlate significantly (Pearson's r = 0.1, p = 0.494) (Figure S4) and hence did not confirm hypothesis 2 (Table 1).

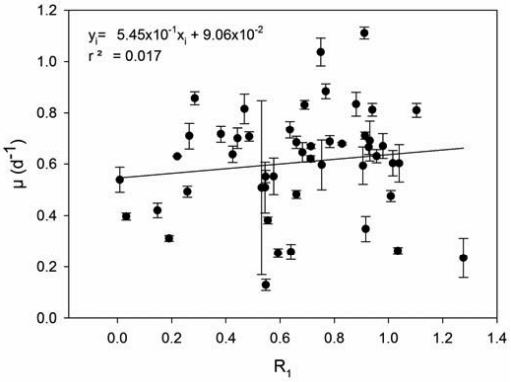

Figure 1. Resistance capacity R_1 (calculated as the ratio between the number of cells that did not lyse after incubation with viruses and the number of cells in the non-inoculated controls) plotted against growth rate (μ). Error bars show standard deviation (n = 3).

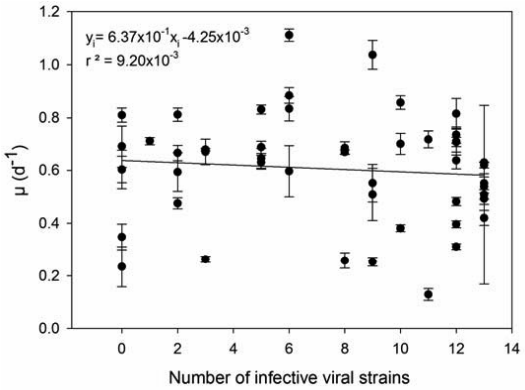

Figure 2. Resistance capacity R_2 (number of viral strains infecting each algal strain) plotted against growth rate (μ). Error bars show standard deviation (n = 3).

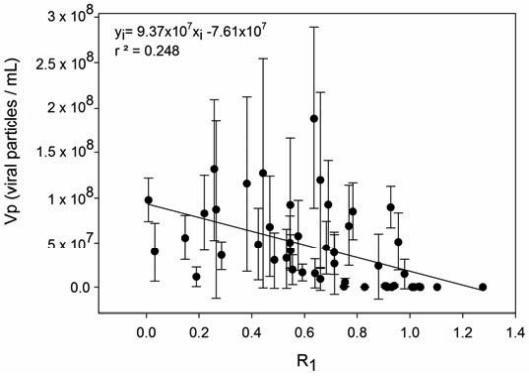

Figure 3. Viral production (Vp) plotted against resistance capacity R_1. Error bars show standard deviation (n = 13).

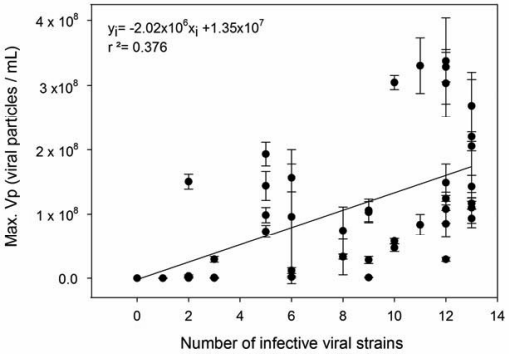

Figure 4. Number of viral strains infecting each algal strain and maximum viral production correlation. Error bars show standard deviation (n = 3).

Seven out of the 49 *E. huxleyi* strains (RCC1259, RCC1269, RCC3856, 371, P847, PERU15-40 and SO52) were susceptible to infection by all the EhV strains tested, while 6 *E. huxleyi* strains (RCC1211, RCC1218, RCC1235, RCC1256, RCC1276 and RCC3548) were resistant to infection by all the EhV strains tested. When analysing these two groups of *E. huxleyi* strains, no significant differences in growth rate were found (one-way ANOVA F (1, 11) = 0.01592, p = 0.90188), while their R_1 values were significantly different (one-way ANOVA F (1, 11) = 36.8593, $p = 8.1 \times 10^{-5}$).

A significant higher viral production was found in the most recently isolated algal strains (one-way ANOVA F (1, 47) = 30.36, $p = 1.5 \times 10^{-6}$). For the other parameters (growth rate, R_1 and R_2) there were no significant differences between younger and older strains (one-way ANOVA F (1, 47) = 1.094, p = 0.30; one-way ANOVA F (1, 47) = 0.106, p = 0.745; one-way ANOVA F (1, 47) = 0.909, p = 0.345; respectively).

We observed significant variation in "Maximum viral production" capacity among the different EhV strains (Figure 5). Those differences did not translate into significant differences in "Average Viral Production" (Figure S5), as the capacity of each EhV to produce progeny depended very much on which host strain it was infecting. Host-ranges among EhV strains also proved very variable, from generalists that infected up to 36 host strains (e.g., EhV-207) to specialists capable of infecting only 1 strain (e.g., EhV-99b1). Surprisingly, and against the prediction in hypothesis 5, generalist viral strains (EhV-164, EhV-202, EhV-208, EhV-201 and EhV-207) produced significantly more virus progeny viral production (one-way ANOVA F (1, 8) = 8.123, p = 0.021) than specialist strains (EhV-99b1, EhV-203, EhV-156, EhV-86, and EhV-145).

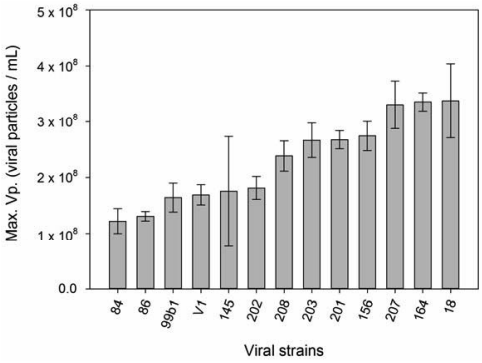

Figure 5. Differences between maximum viral production among EhV strains. Error bars show standard deviation (n = 49).

The bipartite network analysis applied to the whole host-range matrix displayed a nested structure (Figure 6) with a NODF value of 0.60. In that nested pattern there was a tendency for hosts with higher resistance to only be infected by more generalist viruses, while specialist viruses tend to infect the most sensitive hosts.

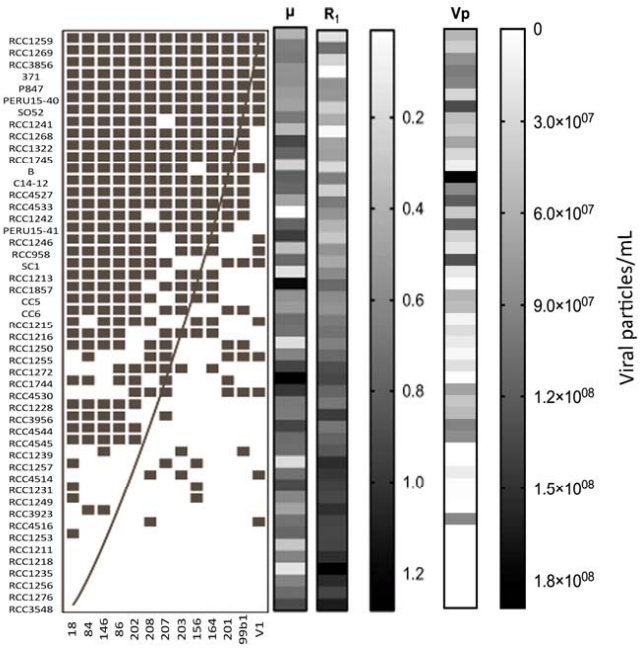

Figure 6. Viral-host infectivity network with a clear nested pattern (NODF value of 0.60) where specialist viruses tend to infect the most susceptible hosts, while viruses with broader host-range infect hosts with higher resistance. ■: infection; □: no infection. Sidebars represent μ, R_1 and Vp parameters, respectively.

4. Discussion

Since Hutchinson first stated the Paradox of the phytoplankton in the early sixties, many hypotheses explaining the high diversity in the oceans have been postulated [13]. Among these, viral activity has proven to be a potential disrupter on equilibrium in planktonic communities [4,12]. Due to the lack of quantitative data for viral-host interactions, especially in marine micro-eukaryotic organisms, we therefore decided to perform a vast survey on strains of the ubiquitous and environmentally relevant coccolithophorid *Emiliania huxleyi* sp. (*E. huxleyi*) (Lohman) and its virus, *Emiliania huxleyi* virus (EhV), and investigate for emerging patterns resulting from this arms race.

Among the different hypotheses tested (Table 1) was the existence, or not, of a clear trade-off between resistance and growth rate (COR). COR has been previously confirmed in some bacteria-virus systems [26–30], and is fundamental in the formulation of the Killing the Winner model [19]. In our study we did not observe a clear COR trade-off in the *E. huxleyi*-EhV system. Instead, we found that highly resistant algal strains were capable of growing at high rates. This indicates that, at least in this system, viruses may not be the main selective force acting upon their hosts or, that if they are, their impact is camouflaged by antagonic impacts from other selective factors (e.g., different adaptation to the standard culture conditions used). However, it could be that viral-imposed selection was so strong that it would result in an emerging global cost of resistance observable on *E. huxleyi* strains independently of their inherent local adaptations. An approximation to such global "cost of resistance"

is precisely the parameter value used when trying to model the interactions between viruses and their hosts [83]. Its prominence in current models justified the present attempt to evaluate its real extension.

When Avrani and colleagues [29] observed a similar response in viral resistant *Prochlorococcus* strains, they also found that the reduced growth rates increased after 7 months and that these strains reduced their resistance against the viruses [84]. The changes in growth rate and resistance occurred as independent events, indicating that the selection pressure on these phenotypes was decoupled. Decoupled selective pressure for growth rate and resistance may be the reason for the lack of correlation between these parameters in our study as well.

COR not being observed for the *E. huxleyi*-EhV system using our approach is not necessarily proving it does not exist or that it is irrelevant. As also tried in the current study, COR is often measured as reduction of growth rates in the resistant host [23–27], but other CORs, like altered susceptibility to other viruses and possibly also to some bacteria [85], have also been argued [29,84,86–88]. Trade-off might also emerge when strains with different resistance capacities are put under competition for a limited level of nutrients [30,33,89,90], and this is the logical follow up to our study. Another aspect to take into account is the potential impact that domestication has on the isolated strains [91]. In vitro growing conditions (nutrients, light, temperature) are inevitably different from what the cells would be experiencing in the natural environment. Particularly, in vitro cells are released from viral pressure, a situation that, with time, could potentially erase the selective traits that viruses might impose on cells in the natural environment. A sign of domestication-related effects in our case was the lower viral production capacity observed for "older" strains (isolated before 2009).

Patterns other than COR that shed light on the global interaction between *E. huxleyi* and EhV did, however, emerge in this study. Contrary to our expectations [14,68], we observed a tendency for generalist viruses (e.g., EhV-207) to produce more progeny than the specialists (e.g., EhV-86). It was recently reported that a generalist EhV strain could outcompete a specialist 8 h post infection [92]. One explanation for this apparent difference in infective success between generalist and specialist viruses may thus be a trade-off where high host-range/replication rates are associated with hindered progeny (new virions) fitness [64,93–96]. An alternative possibility could be the presence of an "un-costly" strong adaptive potential to new hosts, as shown for the Tobacco etch potyvirus (TEV) [97]. It also has to be taken into consideration that viral infective performance; such as viral adsorption coefficient and burst size also depends strongly on host traits. In the current study, a set of *E. huxleyi* strains were the ones that presented the higher viral production, independently of the EhV strain that was infecting them. Such added levels of complexity create niches for different strains of viruses and hosts with different infection and resistance capacities, respectively, to coexist. The patterns emerging from the interaction between *E. huxleyi* and EhV indicate that there's a plethora of niches that create the possibility for co-existence of viruses and hosts with unexpected trait capacities. Notably, viral strains with narrower host-ranges and smaller virion production competing with generalist strains. Future studies should try to evaluate the possibility of take-over in the case of two specialist or generalist strains.

The emerging virus-host interaction network (VHIN) pattern showed a significant nestedness match between viral strains and their hosts. A nested structure like this is considered to result from sequential gene-for-gene (GFG) adaptations [98,99]. In the GFG model one genotype replaces another leading to continued fitness improvements of both, host and virus populations, resulting in an everlasting arms race dynamics. Different mesocosm studies on natural *E. huxleyi*/EhV communities [73,74] have shown that host and viral strain diversity can co-change in very short periods of just a few days during *E. huxleyi* blooms. This supports the Arms Race dynamics indicated by our VHIN. Future studies should evaluate the potential for strains with similar host-range capacity to take-over one another. The currently observed cross-infection network did not however have a perfect nested structure. An alternative co-evolution mechanism, termed diffuse co-evolution, appears to be more adequate for multi-species and/or multi-strain communities where selection pressures due to one species, can change in the presence of other species [17,100]. In order to predict diffuse

co-evolution, however, experiments in which the different species/strains could interact, allowing real fitness costs associate to both, viruses and hosts, to arise [100] are necessary.

As also previously shown, the same *E. huxleyi* viruses (isolated in the English Channel and the Norwegian fjords) proved able to infect *E. huxleyi* hosts isolated in a large spatio-temporal scale [44,51], indicating a strong genomic consistency between geographically distant EhV strains. Nonetheless, and despite high abundance of conserved genomic sequences among these strains, significant genomic variety is also documented [44,73,101]. As EhVs are enveloped viruses [102], their entry mechanism should be endocytosis or fusion of the envelope with the host's membrane and the progeny release through a budding mechanism [103]. Such an infection mechanism potentially generates a highly lipid-specific contact between host and virus. The host, *E. huxleyi*, has high phenotypic plasticity [104–108] and adaptation capacity [104,109–112] that could result in ecotypes that respond differently to viral infection [37,108,112]. Even if genes associated with virus susceptibility have been found within non-core regions of the *E. huxleyi* genome [108], our results did not show significant differences in growth rate, resistance, or viral production in hosts from very distant geographical locations. Hence, despite the recognized genetic variability in both host and virus, our results suggest a globally, non-segregated evolution process between *E. huxleyi* and EhV [113].

In conclusion, and despite a lack of supporting evidence of a trade-off between resistance and growth capacities, our results did indeed, through the nested host-virus interaction pattern, demonstrate a strong co-evolution pattern between *E. huxleyi* and EhV populations. The absence of trade-off between growth rate and resistance, invites us to think that EhVs may not be the main force driving the *E. huxleyi* selection, and that other fitness costs, which passed unnoticeably in the present study, exist. Further work should aim at unravelling these.

Supplementary Materials: The following are available online at www.mdpi.com/1999-4915/9/3/61/s1, Table S1: *E. huxleyi* strain information, in blank = No information; Table S2: EhV strain information; Table S3: Measured parameters from the cross-infectivity experiments between each *E. huxleyi*-EhV pair. fC = final concentration of *E. huxleyi* cells in cells/mL, μ = *E. huxleyi* growth rate, R_1 = percentage of cells that were not lysed after incubation with viruses, compared to the non-inoculated controls, Vp = viral production in viral particles/mL, R2 = number of EhV strains that successfully produced progeny on that host, R1 AV = averaged R1 for each algal strain, Vp AV = averaged Vp for each algal strain in viral particles/mL, Max. Vp AV = averaged maximum Vp for each algal strain in viral particles/mL, SD = standard deviation; Figure S1: Correlation between viral production per host cell (Vp) and isolation year of the algal strains. Error bars show standard deviation (n = 13); Figure S2: Growth rates (μ/d) measured for control samples of all of the *E. huxleyi* strains (see Table S1 for strain information) used in the infection experiment measured over a period of x days and, calculated according to Levasseur et al. (1993). Values correspond to the control samples. Error bars show standard deviation (n = 3); Figure S3: Resistance strategy (R) for each *E. huxleyi* strain. Error bars show standard deviation (n = 13); Figure S4: Correlation between growth rate (μ) and viral production per host cell (Vp), in viral particles/mL. Error bars show standard deviation (n = 13); Figure S5: Average viral production per EhV strain, for all the algal strains. Error bars show standard deviation (n = 49).

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Conflicts of Interest: The authors declare no conflict of interest. The founding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

References

1. Wilhelm, S.W.; Suttle, C.A. Viruses and nutrient cycles in the sea—Viruses play critical roles in the structure and function of aquatic food webs. *Bioscience* **1999**, *49*, 781–788. [CrossRef]
2. Suttle, C.A. Marine viruses—Major players in the global ecosystem. *Nat. Rev. Microbiol.* **2007**, *5*, 801–812. [CrossRef] [PubMed]
3. Breitbart, M. Marine viruses: Truth or dare. *Annu. Rev. Mar. Sci.* **2012**, *4*, 425–448. [CrossRef] [PubMed]

4. Fuhrman, J.A. Marine viruses and their biogeochemical and ecological effects. *Nature* **1999**, *399*, 541–548. [CrossRef] [PubMed]
5. Wommack, K.E.; Colwell, R.R. Virioplankton: Viruses in aquatic ecosystems. *Microbiol. Mol. Biol. Rev.* **2000**, *64*, 69–114. [CrossRef] [PubMed]
6. Weinbauer, M.G.; Rassoulzadegan, F. Are viruses driving microbial diversification and diversity? *Environ. Microbiol.* **2004**, *6*, 1–11. [CrossRef] [PubMed]
7. Brussaard, C.P.D. Viral control of phytoplankton populations—A review. *J. Eukaryot. Microbiol.* **2004**, *51*, 125–138. [CrossRef] [PubMed]
8. Suttle, C.A.; Chan, A.M. Dynamics and distribution of cyanophages and their effect on marine *Synechococcus* spp. *Appl. Environ. Microbiol.* **1994**, *60*, 3167–3174. [PubMed]
9. Waterbury, J.B.; Valois, F.W. Resistance to cooccurring phages enables marine synechococcus communities to coexist with cyanophages abundant in seawater. *Appl. Environ. Microbiol.* **1993**, *59*, 3393–3399. [PubMed]
10. Weinbauer, M.G. Ecology of prokaryotic viruses. *FEMS Microbiol. Rev.* **2004**, *28*, 127–181. [CrossRef] [PubMed]
11. Jiang, S.C.; Paul, J.H. Gene transfer by transduction in the marine environment. *Appl. Environ. Microbiol.* **1998**, *64*, 2780–2787. [PubMed]
12. Fuhrman, J.A.; Suttle, C.A. Viruses in marine planktonic systems. *Oceanography* **1993**, *6*, 51–63. [CrossRef]
13. Hutchinson, G.E. The paradox of the plankton. *Am. Nat.* **1961**, *95*, 137–145. [CrossRef]
14. Winter, C.; Bouvier, T.; Weinbauer, M.G.; Thingstad, T.F. Trade-offs between competition and defense specialists among unicellular planktonic organisms: The "killing the winner" hypothesis revisited. *Microbiol. Mol. Biol. Rev.* **2010**, *74*, 42–57. [CrossRef] [PubMed]
15. Thingstad, T.F.; Vage, S.; Storesund, J.E.; Sandaa, R.A.; Giske, J. A theoretical analysis of how strain-specific viruses can control microbial species diversity. *Proc. Natl. Acad. Sci. USA* **2014**, *111*, 7813–7818. [CrossRef] [PubMed]
16. Sheldon, B.C.; Verhulst, S. Ecological immunology: Costly parasite defences and trade-offs in evolutionary ecology. *Trends Ecol. Evol.* **1996**, *11*, 317–321. [CrossRef]
17. Weitz, J.S.; Poisot, T.; Meyer, J.R.; Flores, C.O.; Valverde, S.; Sullivan, M.B.; Hochberg, M.E. Phage-bacteria infection networks. *Trends Microbiol.* **2013**, *21*, 82–91. [CrossRef] [PubMed]
18. Poullain, V.; Gandon, S.; Brockhurst, M.A.; Buckling, A.; Hochberg, M.E. The evolution of specificity in evolving and coevolving antagonistic interactions between a bacteria and its phage. *Evolution* **2008**, *62*, 1–11. [CrossRef] [PubMed]
19. Meyer, J.R.; Dobias, D.T.; Weitz, J.S.; Barrick, J.E.; Quick, R.T.; Lenski, R.E. Repeatability and contingency in the evolution of a key innovation in phage lambda. *Science* **2012**, *335*, 428–432. [CrossRef] [PubMed]
20. Fortuna, M.A.; Stouffer, D.B.; Olesen, J.M.; Jordano, P.; Mouillot, D.; Krasnov, B.R.; Poulin, R.; Bascompte, J. Nestedness versus modularity in ecological networks: Two sides of the same coin? *J. Anim. Ecol.* **2010**, *79*, 811–817. [CrossRef] [PubMed]
21. Flores, C.O.; Poisot, T.; Valverde, S.; Weitz, J.S. Bimat: A matlab package to facilitate the analysis of bipartite networks. *Methods Ecol. Evol.* **2016**, *7*, 127–132. [CrossRef]
22. Gomez, P.; Buckling, A. Bacteria-phage antagonistic coevolution in soil. *Science* **2011**, *332*, 106–109. [CrossRef] [PubMed]
23. Benmayor, R.; Buckling, A.; Bonsall, M.B.; Brockhurst, M.A.; Hodgson, D.J. The interactive effects of parasitesf disturbance, and productivity on experimental adaptive radiations. *Evolution* **2008**, *62*, 467–477. [CrossRef] [PubMed]
24. Brockhurst, M.A.; Buckling, A.; Rainey, P.B. The effect of a bacteriophage on diversification of the opportunistic bacterial pathogen, pseudomonas aeruginosa. *Proc. R. Soc. B-Biol. Sci.* **2005**, *272*, 1385–1391. [CrossRef] [PubMed]
25. Brockhurst, M.A.; Rainey, P.B.; Buckling, A. The effect of spatial heterogeneity and parasites on the evolution of host diversity. *Proc. R. Soc. B-Biol. Sci.* **2004**, *271*, 107–111. [CrossRef] [PubMed]
26. Middelboe, M.; Holmfeldt, K.; Riemann, L.; Nybroe, O.; Haaber, J. Bacteriophages drive strain diversification in a marine flavobacterium: Implications for phage resistance and physiological properties. *Environ. Microbiol.* **2009**, *11*, 1971–1982. [CrossRef] [PubMed]
27. Riemann, L.; Grossart, H.-P. Elevated lytic phage production as a consequence of particle colonization by a marine flavobacterium (*Cellulophaga* sp.). *Microb. Ecol.* **2008**, *56*, 505–512. [CrossRef] [PubMed]

28. Middelboe, M. Bacterial growth rate and marine virus-host dynamics. *Microb. Ecol.* **2000**, *40*, 114–124. [PubMed]
29. Avrani, S.; Wurtzel, O.; Sharon, I.; Sorek, R.; Lindell, D. Genomic island variability facilitates prochlorococcus-virus coexistence. *Nature* **2011**, *474*, 604–608. [CrossRef] [PubMed]
30. Meaden, S.; Paszkiewicz, K.; Koskella, B. The cost of phage resistance in a plant pathogenic bacterium is context-dependent. *Evolution* **2015**, *69*, 1321–1328. [CrossRef] [PubMed]
31. Lennon, J.T.; Khatana, S.A.M.; Marston, M.F.; Martiny, J.B.H. Is there a cost of virus resistance in marine cyanobacteria? *ISME J.* **2007**, *1*, 300–312. [CrossRef] [PubMed]
32. Lythgoe, K.A.; Chao, L. Mechanisms of coexistence of a bacteria and a bacteriophage in a spatially homogeneous environment. *Ecol. Lett.* **2003**, *6*, 326–334. [CrossRef]
33. Bohannan, B.J.M.; Lenski, R.E. The relative importance of competition and predation varies with productivity in a model community. *Am. Nat.* **2000**, *156*, 329–340. [CrossRef]
34. Mizoguchi, K.; Morita, M.; Fischer, C.R.; Yoichi, M.; Tanji, Y.; Unno, H. Coevolution of bacteriophage pp01 and escherichia coli o157: H7 in continuous culture. *Appl. Environ. Microbiol.* **2003**, *69*, 170–176. [CrossRef] [PubMed]
35. Thomas, R.; Grimsley, N.; Escande, M.L.; Subirana, L.; Derelle, E.; Moreau, H. Acquisition and maintenance of resistance to viruses in eukaryotic phytoplankton populations. *Environ. Microbiol.* **2011**, *13*, 1412–1420. [CrossRef] [PubMed]
36. Frickel, J.; Sieber, M.; Becks, L. Eco-evolutionary dynamics in a coevolving host-virus system. *Ecol. Lett.* **2016**, *19*, 450–459. [CrossRef] [PubMed]
37. Brown, C.W.; Yoder, J.A. Coccolithophorid blooms in the global ocean. *J. Geophys. Res.-Oceans* **1994**, *99*, 7467–7482. [CrossRef]
38. Tyrrell, T.; Taylor, A.H. A modelling study of *Emiliania huxleyi* in the ne atlantic. *J. Mar. Syst.* **1996**, *9*, 83–112. [CrossRef]
39. Egge, J.K.; Heimdal, B.R. Blooms of phytoplankton including *Emiliania-huxleyi* (haptophyta)—Effects of nutrient supply in different n-p ratios. *Sarsia* **1994**, *79*, 333–348. [CrossRef]
40. Broerse, A.T.C.; Ziveri, P.; van Hinte, J.E.; Honjo, S. Coccolithophore export production, species composition, and coccolith-caco3 fluxes in the ne atlantic (34 degrees n 21 degrees w and 48 degrees n 21 degrees w). *Deep-Sea Res. Part II Top. Stud. Oceanogr.* **2000**, *47*, 1877–1905. [CrossRef]
41. Burkill, P.H.; Archer, S.D.; Robinson, C.; Nightingale, P.D.; Groom, S.B.; Tarran, G.A.; Zubkov, M.V. Dimethyl sulphide biogeochemistry within a coccolithophore bloom (disco): An overview. *Deep-Sea Res. Part II Top. Stud. Oceanogr.* **2002**, *49*, 2863–2885. [CrossRef]
42. Cottrell, M.T.; Suttle, C.A. Wide-spread occurrence and clonal variation in viruses which cause lysis of a cosmopolitan, eukaryotic marine phytoplankter, micromonas-pusilla. *Mar. Ecol. Prog. Ser.* **1991**, *78*, 1–9. [CrossRef]
43. Nissimov, J.I.; Napier, J.A.; Kimmance, S.A.; Allen, M.J. Permanent draft genomes of four new coccolithoviruses: Ehv-18, ehv-145, ehv-156 and ehv-164. *Mar. Genom.* **2014**, *15*, 7–8. [CrossRef] [PubMed]
44. Allen, M.J.; Martinez-Martinez, J.; Schroeder, D.C.; Somerfield, P.J.; Wilson, W.H. Use of microarrays to assess viral diversity: From genotype to phenotype. *Environ. Microbiol.* **2007**, *9*, 971–982. [CrossRef] [PubMed]
45. Pagarete, A.; Kusonmano, K.; Petersen, K.; Kimmance, S.A.; Martinez, J.M.; Wilson, W.H.; Hehemann, J.H.; Allen, M.J.; Sandaa, R.A. Dip in the gene pool: Metagenomic survey of natural coccolithovirus communities. *Virology* **2014**, *466*, 129–137. [CrossRef] [PubMed]
46. Pagarete, A.; Lanzen, A.; Puntervoll, P.; Sandaa, R.A.; Larsen, A.; Larsen, J.B.; Allen, M.J.; Bratbak, G. Genomic sequence and analysis of ehv-99b1, a new coccolithovirus from the norwegian fjords. *Intervirology* **2013**, *56*, 60–66. [CrossRef] [PubMed]
47. Nissimov, J.I.; Worthy, C.A.; Rooks, P.; Napier, J.A.; Kimmance, S.A.; Henn, M.R.; Ogata, H.; Allen, M.J. Draft genome sequence of the coccolithovirus ehv-84. *Stand. Genom. Sci.* **2011**, *5*, 1–11. [CrossRef] [PubMed]
48. Nissimov, J.I.; Worthy, C.A.; Rooks, P.; Napier, J.A.; Kimmance, S.A.; Henn, M.R.; Ogata, H.; Allen, M.J. Draft genome sequence of four coccolithoviruses: *Emiliania huxleyi* virus ehv-88, ehv-201, ehv-207, and ehv-208. *J. Virol.* **2012**, *86*, 2896–2897. [CrossRef] [PubMed]
49. Nissimov, J.I.; Worthy, C.A.; Rooks, P.; Napier, J.A.; Kimmance, S.A.; Henn, M.R.; Ogata, H.; Allen, M.J. Draft genome sequence of the coccolithovirus *Emiliania huxleyi* virus 202. *J. Virol.* **2012**, *86*, 2380–2381. [CrossRef] [PubMed]

50. Nissimov, J.I.; Worthy, C.A.; Rooks, P.; Napier, J.A.; Kimmance, S.A.; Henn, M.R.; Ogata, H.; Allen, M.J. Draft genome sequence of the coccolithovirus *Emiliania huxleyi* virus 203. *J. Virol.* **2011**, *85*, 13468–13469. [CrossRef] [PubMed]
51. Pagarete, A.J. Functional Genomics of Coccolithophore Viruses. Ph.D. Thesis, Pierre and Marie Curie University, Paris, France, 2010; p. 222.
52. Middelboe, M.; Jacquet, S.; Weinbauer, M. Viruses in freshwater ecosystems: An introduction to the exploration of viruses in new aquatic habitats. *Freshw. Biol.* **2008**, *53*, 1069–1075. [CrossRef]
53. Bouvier, T.; del Giorgio, P.A. Key role of selective viral-induced mortality in determining marine bacterial community composition. *Environ. Microbiol.* **2007**, *9*, 287–297. [CrossRef] [PubMed]
54. Chao, L.; Levin, B.R.; Stewart, F.M. A complex community in a simple habitat: An experimental study with bacteria and phage. *Ecology* **1977**, *58*, 369–378. [CrossRef]
55. Lenski, R.E.; Levin, B.R. Constraints on the coevolution of bacteria and virulent phage: A model, some experiments, and predictions for natural communities. *Am. Nat.* **1985**, *125*, 585–602. [CrossRef]
56. Thyrhaug, R.; Larsen, A.; Thingstad, T.F.; Bratbak, G. Stable coexistence in marine algal host-virus systems. *Mar. Ecol. Prog. Ser.* **2003**, *254*, 27–35. [CrossRef]
57. Haaber, J.; Middelboe, M. Viral lysis of phaeocystis pouchetii: Implications for algal population dynamics and heterotrophic c, n and p cycling. *ISME J.* **2009**, *3*, 430–441. [CrossRef] [PubMed]
58. Moebus, K. Marine bacteriophage reproduction under nutrient-limited growth of host bacteria.2. Investigations with phage-host system h3:H3/1. *Mar. Ecol. Prog. Ser.* **1996**, *144*, 13–22. [CrossRef]
59. Moebus, K. Marine bacteriophage reproduction under nutrient-limited growth of host bacteria.1. Investigations with six phage-host systems. *Mar. Ecol. Prog. Ser.* **1996**, *144*, 1–12. [CrossRef]
60. Parada, V.; Herndl, G.J.; Weinbauer, M.G. Viral burst size of heterotrophic prokaryotes in aquatic systems. *J. Mar. Biol. Assoc. UK* **2006**, *86*, 613–621. [CrossRef]
61. Motegi, C.; Nagata, T. Enhancement of viral production by addition of nitrogen or nitrogen plus carbon in subtropical surface waters of the South Pacific. *Aquat. Microb. Ecol.* **2007**, *48*, 27–34. [CrossRef]
62. Bratbak, G.; Jacobsen, A.; Heldal, M.; Nagasaki, K.; Thingstad, F. Virus production in phaeocystis pouchetii and its relation to host cell growth and nutrition. *Aquat. Microb. Ecol.* **1998**, *16*, 1–9. [CrossRef]
63. Baudoux, A.-C.; Brussaard, C.P.D. Influence of irradiance on virus-algal host interactions. *J. Phycol.* **2008**, *44*, 902–908. [CrossRef] [PubMed]
64. Demory, D.; Arsenieff, L.; Simon, N.; Six, C.; Rigaut-Jalabert, F.; Marie, D.; Ge, P.; Bigeard, E.; Jacquet, S.; Sciandra, A.; et al. Temperature is a key factor in micromonas–virus interactions. *ISME J.* **2017**, *13*, 601–612. [CrossRef] [PubMed]
65. Maat, D.S.; Crawfurd, K.J.; Timmermans, K.R.; Brussaard, C.P.D. Elevated CO_2 and phosphate limitation favor micromonas pusilla through stimulated growth and reduced viral impact. *Appl. Environ. Microbiol.* **2014**, *80*, 3119–3127. [CrossRef] [PubMed]
66. Maat, D.S.; de Blok, R.; Brussaard, C.P.D. Combined phosphorus limitation and light stress prevent viral proliferation in the phytoplankton species phaeocystis globosa, but not in micromonas pusilla. *Front. Mar. Sci.* **2016**, *3*. [CrossRef]
67. Kendrick, B.J.; DiTullio, G.R.; Cyronak, T.J.; Fulton, J.M.; van Mooy, B.A.S.; Bidle, K.D. Temperature-induced viral resistance in *Emiliania huxleyi* (prymnesiophyceae). *PLoS ONE* **2014**, *9*, e112134. [CrossRef] [PubMed]
68. MacArthur, R.H.; Wilson, E.O. *The Theory of Island Biogeography*; Princeton University Press: Princeton, NJ, USA, 1967; p. 203.
69. Klochkova, T.A.; Kang, S.H.; Cho, G.Y.; Pueschel, C.M.; West, J.A.; Kim, G.H. Biology of a terrestrial green alga, *Chlorococcum* sp. (chlorococcales, chlorophyta), collected from the miruksazi stupa in Korea. *Phycologia* **2006**, *45*, 349–358. [CrossRef]
70. Wilson, W.H.; Tarran, G.A.; Schroeder, D.; Cox, M.; Oke, J.; Malin, G. Isolation of viruses responsible for the demise of an *Emiliania huxleyi* bloom in the english channel. *J. Mar. Biol. Assoc. UK* **2002**, *82*, 369–377. [CrossRef]
71. Vaughn, J.M.; Balch, W.M.; Novotny, J.F.; Vining, C.L.; Palmer, C.D.; Drapeau, D.T.; Booth, E.; Vaughn, J.M.; Kneifel, D.M.; Bell, A.L. Isolation of *Emiliania huxleyi* viruses from the gulf of maine. *Aquat. Microb. Ecol.* **2010**, *58*, 109–116. [CrossRef]
72. Highfield, A.; Evans, C.; Walne, A.; Miller, P.I.; Schroeder, D.C. How many coccolithovirus genotypes does it take to terminate an *Emiliania huxleyi* bloom? *Virology* **2014**, *466*, 138–145. [CrossRef] [PubMed]

73. Martinez, J.M.; Schroeder, D.C.; Larsen, A.; Bratbak, G.; Wilson, W.H. Molecular dynamics of *Emiliania huxleyi* and cooccurring viruses during two separate mesocosm studies. *Appl. Environ. Microbiol.* **2007**, *73*, 554–562. [CrossRef] [PubMed]
74. Sorensen, G.; Baker, A.C.; Hall, M.J.; Munn, C.B.; Schroeder, D.C. Novel virus dynamics in an *Emiliania huxleyi* bloom. *J. Plankton Res.* **2009**, *31*, 787–791. [CrossRef] [PubMed]
75. Ruiz, E. *Growth Rate Experiments in Emiliania Huxleyi*; Universitetet I Bergen: Bergen, Norway, 2014.
76. Brussaard, C.P.D.; Thyrhaug, R.; Marie, D.; Bratbak, G. Flow cytometric analyses of viral infection in two marine phytoplankton species, micromonas pusilla (prasinophyceae) and phaeocystis pouchetii (prymnesiophyceae). *J. Phycol.* **1999**, *35*, 941–948. [CrossRef]
77. Brussaard, C.P.D.; Marie, D.; Bratbak, G. Flow cytometric detection of viruses. *J. Virol. Methods* **2000**, *85*, 175–182. [CrossRef]
78. Brussaard, C.P.D.; Marie, D.; Thyrhaug, R.; Bratbak, G. Flow cytometric analysis of phytoplankton viability following viral infection. *Aquat. Microb. Ecol.* **2001**, *26*, 157–166. [CrossRef]
79. Levasseur, M.; Thompson, P.A.; Harrison, P.J. Physiological acclimation of marine-phytoplankton to different nitrogen-sources. *J. Phycol.* **1993**, *29*, 587–595. [CrossRef]
80. Almeida-Neto, M.; Guimaraes, P.; Guimaraes, P.R.; Loyola, R.D.; Ulrich, W. A consistent metric for nestedness analysis in ecological systems: Reconciling concept and measurement. *Oikos* **2008**, *117*, 1227–1239. [CrossRef]
81. Newman, M.E.J. Modularity and community structure in networks. *Proc. Natl. Acad. Sci. USA* **2006**, *103*, 8577–8582. [CrossRef] [PubMed]
82. Barber, M.J. Modularity and community detection in bipartite networks. *Phys. Rev. E* **2007**, *76*. [CrossRef] [PubMed]
83. Våge, S.; Storesund, J.E.; Thingstad, T.F. Adding a cost of resistance description extends the ability of virus-host model to explain observed patterns in structure and function of pelagic microbial communities. *Environ. Microbiol.* **2013**, *15*, 1842–1852. [CrossRef] [PubMed]
84. Avrani, S.; Lindell, D. Convergent evolution toward an improved growth rate and a reduced resistance range in prochlorococcus strains resistant to phage. *Proc. Natl. Acad. Sci. USA* **2015**, *112*, E2191–E2200. [CrossRef] [PubMed]
85. Segev, E.; Wyche, T.P.; Kim, K.H.; Petersen, J.; Ellebrandt, C.; Vlamakis, H.; Barteneva, N.; Paulson, J.N.; Chai, L.; Clardy, J.; et al. Dynamic metabolic exchange governs a marine algal-bacterial interaction. *Elife* **2016**, *5*, e17473. [CrossRef] [PubMed]
86. Marston, M.F.; Pierciey, F.J.; Shepard, A.; Gearin, G.; Qi, J.; Yandava, C.; Schuster, S.C.; Henn, M.R.; Martiny, J.B.H. Rapid diversification of coevolving marine synechococcus and a virus. *Proc. Natl. Acad. Sci. USA* **2012**, *109*, 4544–4549. [CrossRef] [PubMed]
87. Avrani, S.; Schwartz, D.A.; Lindell, D. Virus-host swinging party in the oceans: Incorporating biological complexity into paradigms of antagonistic coexistence. *Mob. Genet. Elements* **2012**, *2*, 88–95. [CrossRef] [PubMed]
88. Castillo, D.; Christiansen, R.H.; Espejo, R.; Middelboe, M. Diversity and geographical distribution of flavobacterium psychrophilum isolates and their phages: Patterns of susceptibility to phage infection and phage host range. *Microb. Ecol.* **2014**, *67*, 748–757. [CrossRef] [PubMed]
89. Holt, R.D.; Grover, J.; Tilman, D. Simple rules for interspecific dominance in systems with exploitative and apparent competition. *Am. Nat.* **1994**, *144*, 741–771. [CrossRef]
90. Leibold, M.A. A graphical model of keystone predators in food webs: Trophic regulation of abundance, incidence, and diversity patterns in communities. *Am. Nat.* **1996**, *147*, 784–812. [CrossRef]
91. Lakeman, M.B.; von Dassow, P.; Cattolico, R.A. The strain concept in phytoplankton ecology. *Harmful Algae* **2009**, *8*, 746–758. [CrossRef]
92. Nissimov, J.I.; Napier, J.A.; Allen, M.J.; Kimmance, S.A. Intragenus competition between coccolithoviruses: An insight on how a select few can come to dominate many. *Environ. Microbiol.* **2016**, *18*, 133–145. [CrossRef] [PubMed]
93. Duffy, S.; Turner, P.E.; Burch, C.L. Pleiotropic costs of niche expansion in the RNA bacteriophage phi 6. *Genetics* **2006**, *172*, 751–757. [CrossRef] [PubMed]
94. Elena, S.F.; Agudelo-Romero, P.; Lalic, J. The evolution of viruses in multi-host fitness landscapes. *Open Virol. J.* **2009**, *3*, 1–6. [CrossRef] [PubMed]

95. Nikolin, V.M.; Osterrieder, K.; von Messling, V.; Hofer, H.; Anderson, D.; Dubovi, E.; Brunner, E.; East, M.L. Antagonistic pleiotropy and fitness trade-offs reveal specialist and generalist traits in strains of canine distemper virus. *PLoS ONE* **2012**, *7*, e50955. [CrossRef]
96. Keen, E.C. Tradeoffs in bacteriophage life histories. *Bacteriophage* **2014**, *4*, e28365. [CrossRef] [PubMed]
97. Bedhomme, S.; Lafforgue, G.; Elena, S.F. Multihost experimental evolution of a plant rna virus reveals local adaptation and host-specific mutations. *Mol. Biol. Evol.* **2012**, *29*, 1481–1492. [CrossRef] [PubMed]
98. Agrawal, A.F.; Lively, C.M. Modelling infection as a two-step process combining gene-for-gene and matching-allele genetics. *Proc. R. Soc. B-Biol. Sci.* **2003**, *270*, 323–334. [CrossRef] [PubMed]
99. Flor, H.H. Host-parasite interaction in flax rust—Its genetics and other implications. *Phytopathology* **1955**, *45*, 680–685.
100. Inouye, B.; Stinchcombe, J.R. Relationships between ecological interaction modifications and diffuse coevolution: Similarities, differences, and causal links. *Oikos* **2001**, *95*, 353–360. [CrossRef]
101. Rowe, J.M.; Fabre, M.F.; Gobena, D.; Wilson, W.H.; Wilhelm, S.W. Application of the major capsid protein as a marker of the phylogenetic diversity of *Emiliania huxleyi* viruses. *FEMS Microbiol. Ecol.* **2011**, *76*, 373–380. [CrossRef] [PubMed]
102. Mackinder, L.C.M.; Worthy, C.A.; Biggi, G.; Hall, M.; Ryan, K.P.; Varsani, A.; Harper, G.M.; Wilson, W.H.; Brownlee, C.; Schroeder, D.C. A unicellular algal virus, *Emiliania huxleyi* virus 86, exploits an animal-like infection strategy. *J. Gen. Virol.* **2009**, *90*, 2306–2316. [CrossRef] [PubMed]
103. Allen, M.J.; Howard, J.A.; Lilley, K.S.; Wilson, W.H. Proteomic analysis of the ehv-86 virion. *Proteome Sci.* **2008**, *6*. [CrossRef] [PubMed]
104. Medlin, L.K.; Barker, G.L.A.; Campbell, L.; Green, J.C.; Hayes, P.K.; Marie, D.; Wrieden, S.; Vaulot, D. Genetic characterisation of *Emiliania huxleyi* (haptophyta). *J. Mar. Syst.* **1996**, *9*, 13–31. [CrossRef]
105. Iglesias-Rodriguez, M.D.; Schofield, O.M.; Batley, J.; Medlin, L.K.; Hayes, P.K. Intraspecific genetic diversity in the marine coccolithophore *Emiliania huxleyi* (prymnesiophyceae): The use of microsatellite analysis in marine phytoplankton population studies. *J. Phycol.* **2006**, *42*, 526–536. [CrossRef]
106. Blanco-Ameijeiras, S.; Lebrato, M.; Stoll, H.M.; Iglesias-Rodriguez, D.; Muller, M.N.; Mendez-Vicente, A.; Oschlies, A. Phenotypic variability in the coccolithophore *Emiliania huxleyi*. *PLoS ONE* **2016**, *11*, e0157697. [CrossRef] [PubMed]
107. Paasche, E. A review of the coccolithophorid *Emiliania huxleyi* (prymnesiophyceae), with particular reference to growth, coccolith formation, and calcification-photosynthesis interactions. *Phycologia* **2001**, *40*, 503–529. [CrossRef]
108. Kegel, J.U.; John, U.; Valentin, K.; Frickenhaus, S. Genome variations associated with viral susceptibility and calcification in *Emiliania huxleyi*. *PLoS ONE* **2013**, *8*, e80684. [CrossRef] [PubMed]
109. Young, J.R.; Westbroek, P. Genotypic variation in the coccolithophorid species *Emiliania-huxleyi*. *Mar. Micropaleontol.* **1991**, *18*, 5–23. [CrossRef]
110. Young, J.R. *A Guide to Extant Coccolithophore Taxonomy*; International Nannoplankton Association: Bremerhaven, Germany, 2003; p. 121.
111. Cook, S.S.; Whitlock, L.; Wright, S.W.; Hallegraeff, G.M. Photosynthetic pigment and genetic differences between two southern ocean morphotypes of *Emiliania huxleyi* (haptophyta). *J. Phycol.* **2011**, *47*, 615–626. [CrossRef] [PubMed]
112. Hagino, K.; Bendif, E.; Young, J.R.; Kogame, K.; Probert, I.; Takano, Y.; Horiguchi, T.; de Vargas, C.; Okada, H. New evidence for morphological and genetic variation in the cosmopolitan coccolithophore *Emiliania huxleyi* (prymnesiophyceae) from the cox1b-atp4 genes. *J. Phycol.* **2011**, *47*, 1164–1176. [CrossRef] [PubMed]
113. Coolen, M.J.L. 7000 years of *Emiliania huxleyi* viruses in the black sea. *Science* **2011**, *333*, 451–452. [CrossRef] [PubMed]

viruses

MDPI

Correction

Correction: Ruiz, E. et al. Emerging Interaction Patterns in the Emiliania Huxleyi-EhV System. *Viruses* 2016, *9*, 61

Eliana Ruiz [1,*], Monique Oosterhof [1,2], Ruth-Anne Sandaa [1], Aud Larsen [1,3] and António Pagarete [1]

1 Department of Biology, University of Bergen, 5006 Bergen, Norway; Monique.oosterhof@wur.nl (M.O.); Ruth.Sandaa@uib.no (R.-A.S.); Aud.Larsen@uni.no (A.L.); Antonio.Pagarete@uib.no (A.P.)

2 NRL for Fish, Shellfish and Crustacean Diseases, Wageningen Bioveterinary Research of Wageningen UR, 8200 AB Lelystad, The Nederlands

3 Uni Research Environment, Nygårdsgaten 112, 5008 Bergen, Norway

* Correspondence: Eliana.Martinez@uib.no; Tel.: +47-5558-8194

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The authors wish to make the following change to their paper [1].

The viral strains in the x axis were not ordered correctly in the original Figure 6. The figure should be replaced with:

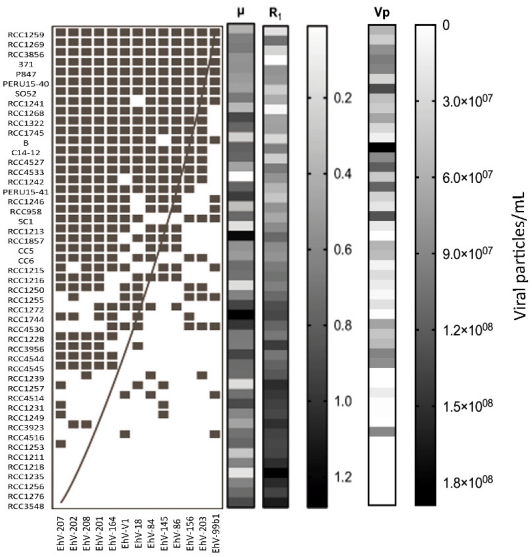

Figure 6. Viral-host infectivity network with a clear nested pattern (NODF value of 0.60) where specialist viruses tend to infect the most susceptible hosts, while viruses with broader host-range infect hosts with higher resistance. ■: infection; □: no infection. Sidebars represent μ, R_1 and Vp parameters, respectively.

The authors apologize for any inconvenience this may cause.

The change does not affect the scientific results. The manuscript will be updated and the original will remain online on the article webpage.

Reference

1. Ruiz, E.; Oosterhof, M.; Sandaa, R.-A.; Larsen, A.; Pagarete, A. Emerging interaction patterns in the emiliania huxleyi-EhV system. *Viruses* **2017**, *9*, 61. [CrossRef] [PubMed]

Supplementary Materials: Emerging Interaction Patterns in the *Emiliania huxleyi*-EhV System

Eliana Ruiz, Monique Oosterhof, Ruth-Anne Sandaa, Aud Larsen and António Pagarete

Table S1. *E. huxleyi* strain information, in blank= No information.

Strain	Isolation Site	Coordinates	Isolation Date
RCC1211	Atlantic Ocean	+35° 41', -7° 33'	1998
RCC1213	Mediterranean Sea	40.68, 14.14	2000
RCC1215	Mediterranean Sea	+41° 40', +2° 48	2001
RCC1216	Pacific Ocean	-42° 18', +169° 50'	1998
RCC1218	Pacific Ocean	-42° 18', +169° 50'	1998
RCC1228	Atlantic Ocean	+49° 24', -1° 8'	2003
RCC1231	Pacific Ocean	-42° 18', +169° 50'	1998
RCC1235	Mediterranean Sea	+43° 41', +7° 19'	2006
RCC1239	Pacific Ocean	+43° 13', +141° 1'	2002
RCC1241	Pacific Ocean	+41° 30', +141° 15'	2002
RCC1242	Pacific Ocean	-2° 67', -82° 72'	1991
RCC1246	Mediterranean Sea	+41° 36', +2° 39'	1999
RCC1249	Mediterranean Sea	+41° 28', +2° 19'	1998
RCC1250	Mediterranean Sea	+37° 10', -1° 13'	1999
RCC1253	Pacific Ocean	+43° 13', +141° 1'	2002
RCC1255	Atlantic Ocean	59.88, 10.67	1905
RCC1256	Atlantic Ocean	+63° 27', -20° 14'	1999
RCC1257	Atlantic Ocean	+63° 27', -20° 14'	1999
RCC1259	Atlantic Ocean	+42° 50', -69° 0'	1990
RCC1268	Atlantic Ocean	+49° 30', -10° 30'	2007
RCC1269	Atlantic Ocean	+49° 30', -10° 30'	2007
RCC1272	Atlantic Ocean	+49° 30', -10° 30'	2007
RCC1276	Atlantic Ocean	+50° 30', -10° 30'	2007
RCC1322	Mediterranean Sea	+36° 15', -1° 35'	1998
RCC1744	Atlantic Ocean	+48° 45', -3° 57'	2007
RCC1745	Atlantic Ocean	+48° 45', -3° 57'	2007
RCC1857	Mediterranean Sea	+34° 8', +18° 27'	2008
RCC3548			
RCC3856	Pacific Ocean	-30° 15', -71° 42'	2011
RCC3923	Pacific Ocean	-36° 39', -73° 20'	2011
RCC3956	Pacific Ocean	-30° 15', -71° 42	2011
371	Atlantic Ocean	32° N 62° W	1988
B			1991
C14-12			
CC5			
CC6			
P847			

Table S1. *Cont.*

Strain	Isolation Site	Coordinates	Isolation Date
PERU15-40	Pacific Ocean		2015
PERU15-41	Pacific Ocean		2015
RCC4514	Atlantic Ocean	+60° 16', +5° 12'	2009
RCC4516	Atlantic Ocean	+60° 16', +5° 12'	2009
RCC4527	Atlantic Ocean	38.080884, -26.254634	2010
RCC4530	Atlantic Ocean	38.080884, -26.254634	2010
RCC4533	Atlantic Ocean	38.035215, -25.462284,	2010
RCC4544	Atlantic Ocean	+27° 59', -15° 22'	2014
RCC4545	Atlantic Ocean	+27° 59', -15° 22'	2014
RCC958	Pacific Ocean	-8° 20', -141° 15'	2004
SC1			
SO52			

Table S2. EhV strain information.

Viral isolate	Isolation site	Isolation Date	Geographical Coordinates	Host Strain Used for Viral Propagation
EhV-18	English channel	2008	50°15′N/04°13′W	RCC1259
EhV-84	English channel	1999	50°15′N/04°13′W	RCC1259
EhV-86	English channel	1999	50°13,79′N/04°9,59′W	RCC1259
EhV-145	Lossiemouth, Scottland	2008	57°72′N/03°29′W	RCC1259
EhV-156	English channel	2009	50°15′N/04°13′W	RCC1259
EhV-164	Scottish shore of Fife	2008	56°26′N/02°63′W	RCC1259
EhV-201	English channel	2001	50°15′N/04°13′W	RCC1259
EhV-202	English channel	2001	50°15′N/04°13′W	RCC1259
EhV-203	English channel	2001	50°15′N/04°13′W	RCC1259
EhV-207	English channel	2001	50°15′N/04°13′W	RCC1259
EhV-208	English channel	2001	50°15′N/04°13′W	RCC1259
EhV-99b1	Raunfjorden, Norway	1999	60.2° N/5.2° E	RCC1259
EhV-V1	Raunfjorden, Norway	2003	60.2° N/5.2° E	RCC1259

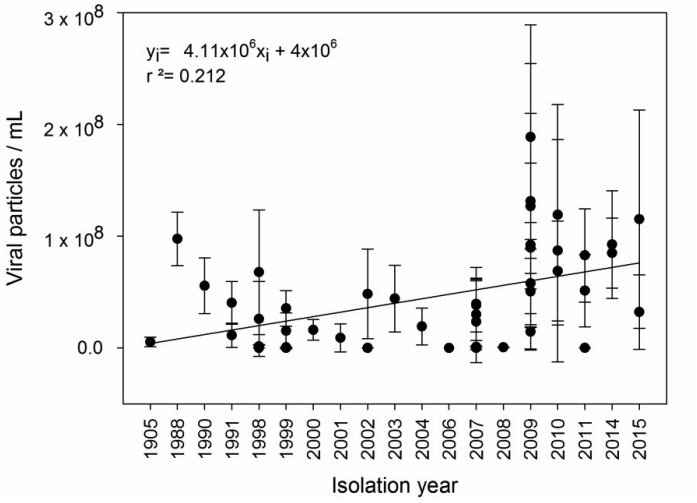

Figure S1. Correlation between viral production per host cell (Vp) and isolation year of the algal strains. Error bars show standard deviation (n = 13).

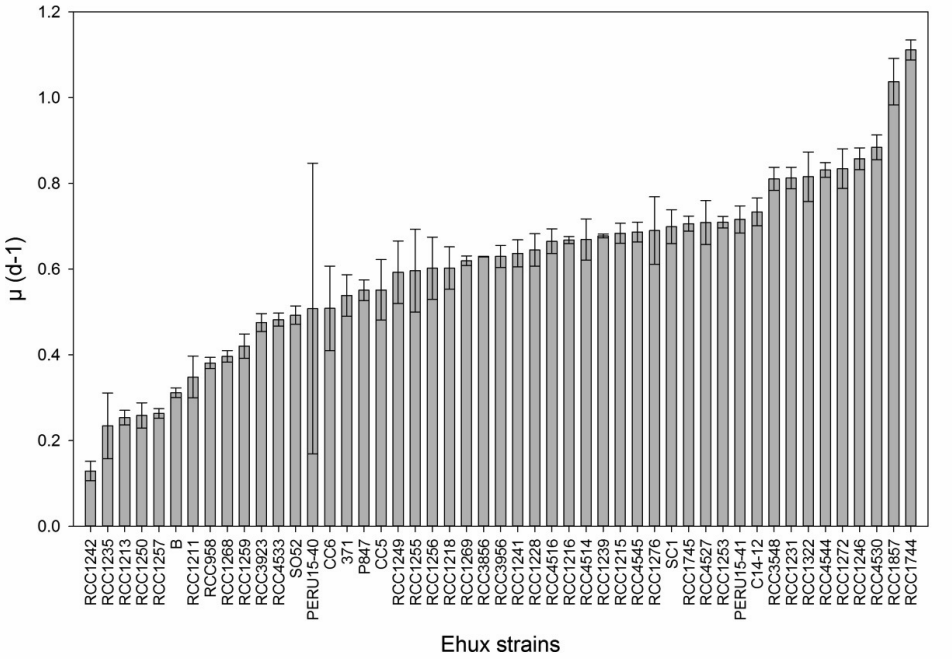

Figure S2. Growth rates (μ/d) measured for control samples of all of the *E. huxleyi* strains (see Table S1 for strain information) used in the infection experiment measured over a period of x days and, calculated according to Levasseur et al. (1993) [1]. Values correspond to the control samples. Error bars show standard deviation (n = 3).

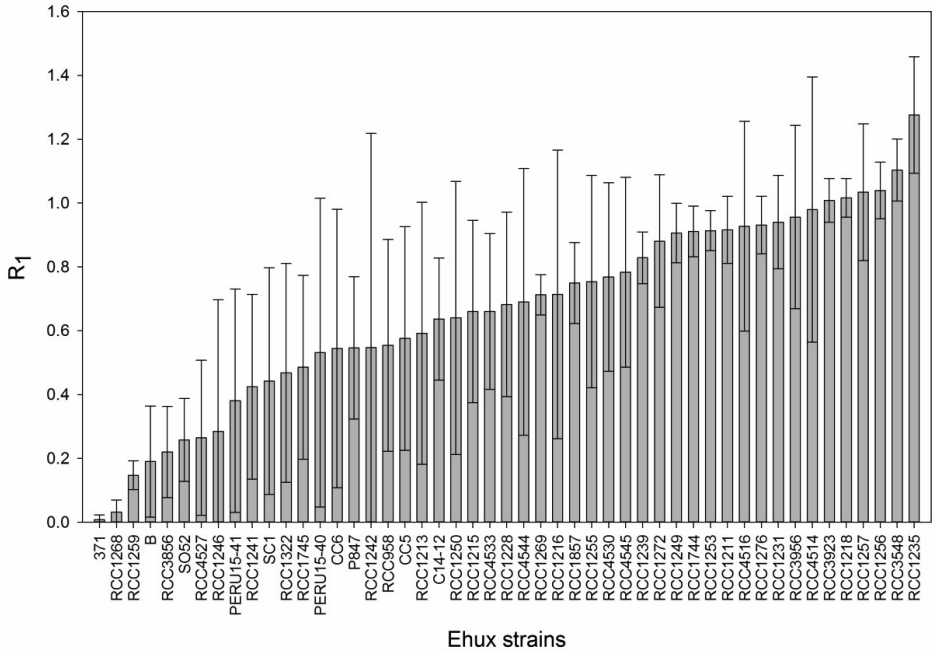

Figure S3. Resistance strategy (R) for each *E. huxleyi* strain. Error bars show standard deviation (n = 13).

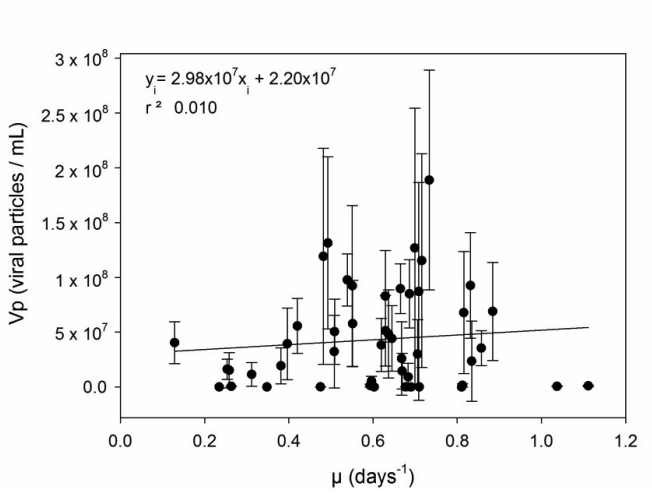

Figure S4. Correlation between growth rate (μ) and viral production per host cell (Vp), in viral particles/mL. Error bars show standard deviation (n = 13).

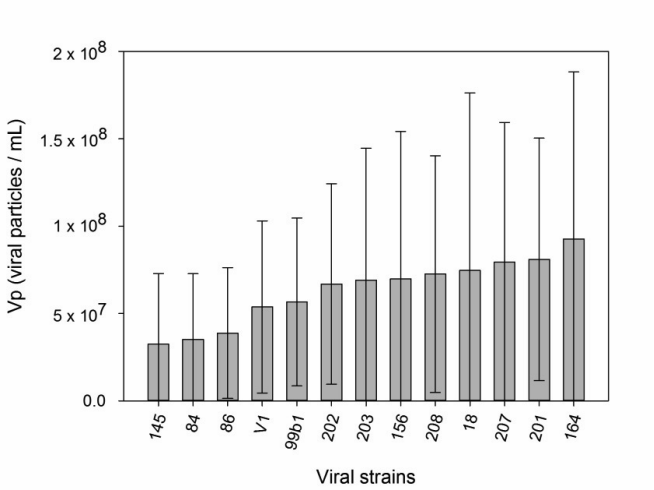

Figure S5. Average viral production per EhV strain, for all the algal strains. Error bars show standard deviation (n = 49).

Reference

1. Levasseur, M., P. A. Thompson, and P. J. Harrison. "Physiological Acclimation of Marine-Phytoplankton to Different Nitrogen-Sources." *Journal of Phycology* 29, no. 5 (1993): 587-95.