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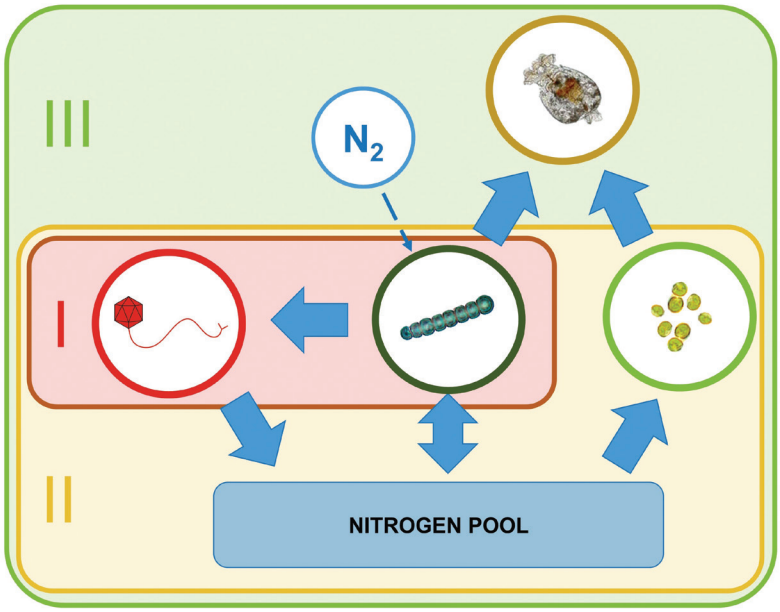
Ecological and Evolutionary Effects of Cyanophages on Experimental Plankton Dynamics



DEPARTMENT OF MICROBIOLOGY
FACULTY OF AGRICULTURE AND FORESTRY
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UNIVERSITY OF HELSINKI

Faculty of Agriculture and Forestry
Department of Microbiology
University of Helsinki, Finland

ECOLOGICAL AND EVOLUTIONARY EFFECTS OF CYANOPHAGES ON EXPERIMENTAL PLANKTON COMMUNITY DYNAMICS

Sebastián Coloma

ACADEMIC DISSERTATION

To be presented, with permission of the Faculty of Agriculture and Forestry of the University of Helsinki, for public examination in the auditorium 1041 at Biocenter 2, Viikinkaari 5, on March 27th 2018, at 12 o'clock noon.

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What is a scientist after all?

It is a curious man looking through a keyhole, the keyhole of nature, trying to know what's going on.

Jacques-Yves Cousteau

Contents

List of original publications

This thesis is based on the following original manuscript and publications:

I **Coloma S**, Dienstbier A, Bamford D, Sivonen K, Roine E and Hiltunen T. 2016. Newly isolated *Nodularia* phage influences cyanobacterial community dynamics. Environmental Microbiology 19:273–286.

II Cairns J, **Coloma S**, Sivonen K and Hiltunen T. 2016. Evolving interactions between diazotrophic cyanobacterium and phage mediate nitrogen release and host competitive ability. Royal Society Open Science 3:160839

III **Coloma S**, Gaedke U, Sivonen K and Hiltunen T. Frequency of phage-resistant cyanobacterial genotype determines experimental plankton community dynamics. Ecology. *Submitted manuscript*.

The author's contribution

I Sebastián Coloma contributed to the design of the study and performed all the microcosm experiments. He acquired, analysed and interpreted the data collected from the microcosm experiments. He performed the statistical analysis and wrote the first draft of the article. He carried critical revision of the article together with the co-authors.

II Sebastián Coloma carried out part of the experiments, participated in the analysis and interpretation of data, and collaborated with the other authors in the critical revision of the article. The work was based on material collected by S. Coloma in study I.

III Sebastián Coloma took part in the design of the experiments. He was responsible for performing the experiments, data collection, data analysis and interpretation, and drafting the article.

Abbreviations and definitions

DN	Dissolved nitrogen
DIN	Dissolved inorganic nitrogen
DOM	Dissolved organic matter
DON	Dissolved organic nitrogen
dsDNA	Double-stranded deoxyribonucleic acid
Evolution	Inherited change in a population over generations
Fitness	Ability to reproduce relative to other members of a population
Genotype	All or part of the genetic constitution of an individual or group
Lysogeny	Viral reproduction cycle where viral nucleic acid is integrated into the host genome
Lytic cycle	Viral reproduction cycle where host cells are lysed with the release of virus progeny at the end of the cycle
Mutation	Permanent alteration in the nucleotide sequence of a genome
OD	Optical density
Phenotype	Observable expression of a genotype
Prophage	Latent form of virus where viral genome has been integrated into the host bacterial genome
RMANOVA	Repeated measures analysis of variance
Trade-off	Gaining a trait with the cost of losing another

Abstract

Biotic and abiotic factors are known to influence the formation of blooms by diazotrophic cyanobacteria, which may dramatically modify the nutrient environment affecting the pelagic food web and the plankton community. Abiotic factors, e.g. nutrient availability and weather conditions, have been widely studied and discussed. However, biotic factors such as the impact of phages remain less studied. This study aims to describe the effects of a Baltic Sea cyanophage on a filamentous cyanobacterium (*Nodularia spumigena*) and other aspects in plankton communities utilizing an experimental approach. Specifically, the study addresses bacteria-phage interactions, plankton community dynamics, and nitrogen transfer between the plankton components (phytoplankton species and rotifers) in the food web.

To perform the experimental study, a Baltic Sea cyanophage infecting *Nodularia* was isolated, characterized and named 2AV2. The cyanophage 2AV2 belongs to the Siphovirus family with a lytic life cycle between 12–18 hours with a restricted host range of 12 out of 45 tested *Nodularia* strains. Lysis of the susceptible host caused an approximately 80% reduction in the cyanobacterial population resulting in selection for phage-resistant *Nodularia* cells.

The evolution of phage resistance significantly reduced the release of nitrogen resulting from lysis of susceptible host cells in the presence of phage. In addition, isolates from the phage-resistant population had two morphotypes, short filaments (40%) and long filaments (60%), while the susceptible population only displayed long filaments. Further, differences between these morphotypes were detected in traits such as growth rate and buoyancy. The divergence in phenotypic traits among phage-resistant cyanobacteria is suggested to represent an evolutionary trade-off between phage resistance and fitness in the absence of phage.

This is the first study to show a change in morphology (filament length) in *Nodularia spumigena* after the evolution of phage resistance. In an experimental plankton community, *Nodularia*, cyanophage 2AV2, *Chlorella* and rotifer biomasses developed differently between treatments with different initial frequencies of phage phage-resistant *Nodularia*, determining the ecological succession and nitrogen transfer in the food web. This study supports the hypothesis that cyanophages not only affect cyanobacterial populations but they also have a wider influence on plankton community dynamics and nitrogen transfer in food webs.

Tiivistelmä

Bioottisten ja abioottisten ympäristötekijöiden tiedetään vaikuttavan typpeä sitoviin syanobakteerikukintoihin, jotka muokkaavat ravinneympäristöä, mikä puolestaan vaikuttaa pelagiseen ravintoverkkoon ja planktiseen yhteisöön. Abioottiset tekijät, kuten ravinteiden saatavuus ja sääolosuhteet, ovat laajasti tutkittuja. Vähemmän tutkittua on bioottisten tekijöiden kuten faagien vaikutus kukintoja muodostaviin typpeä sitoviin syanobakteereihin. Tämän tutkimuksen tavoite on kuvata faagin vaikutusta syanobakteeriin (*Nodularia spumigena*) ja ravinneympäristöön laajemmassa planktisessa yhteisössä kokeellisen lähestymistavan kautta. Lisäksi tämä tutkimus tarkastelee isännän vastustuskyvyn eli faagiresistenssin evoluution vaikutusta kokeelliseen planktonyhteisödynamiikkaan (sis. filamenttinen syanobakteeri *Nodularia spumigena*, syanofaagi 2AV2, viherlevä *Chlorella vulgaris* ja rataseläinpopulaatioita) sekä typen kiertoon planktonkomponenttien välillä ravintoverkossa.

Kokeellista tutkimusta varten Itämerestä eristettiin ja karakterisoitiin syanofaagi, jolle annettiin nimi 2AV2. Syanofaagi 2AV2 kuuluu Siphovirus-heimoon, ja sillä on 12–18 tunnin pituinen lyyttinen sykli sekä kapea isäntäkirjo (12 altista isäntäkantaa 45 testatusta *Nodularia*-suvun kannasta). Faagi-infektio johti alttiiden isäntäsolujen lyysautumiseen ja vastustuskykyisten solujen valintaan. Resistenssievoluutio vähensi merkittävästi typen vapautumista, joka johtuu faagi-infektion aiheuttamasta solujen hajoamisesta. Lisäksi resistentin populaation isolaateista erottui kaksi eri morfotyyppiä, joista 40 % oli lyhyt- ja 60 % pitkärihmaisia, kun taas 100 % alttiista isännistä oli pitkärihmaisia. Lisäksi havaittiin eroavaisuuksia näiden morfotyyppien ominaisuuksissa, kuten kasvunopeudessa ja keijuvuudessa. Ominaisuuksien eroavaisuudet resistenttien syanobakteerien kesken kuvastavat vaihtokauppaa faagiresistenssin ja kelpoisuuden välillä faagin poissaollessa.

Tämä on ensimmäinen tutkimus, jossa havaitaan faagiresistenssievoluution jälkeisiä morfologisia muutoksia *Nodulariassa* (rihmojen pituuksissa). Kokeellisessa planktonyhteisössä *Nodularian*, syanofaagi 2AV2:n, *Chlorellan* ja rataseläimen biomassat vaihtelivat riippuen resistentin syanobakteerin esiintymistiheydestä kasvatuksen alussa. Resistenssin esiintymistiheys (frekvenssi) syanobakteeripopulaatiossa oli ratkaiseva tekijä ekologisessa sukkessiossa ja typen kierrossa ravintoverkossa. Tämä tutkimus tukee hypoteesia, että syanofaagit eivät ainoastaan vaikuta syanobakteeripopulaatioihin vaan myös laajemmin planktonyhteisön dynamiikkaan ja typen kiertoon ravintoverkossa.

Resumen

Los factores bióticos y abióticos son conocidos por influir en la formación de las floraciones masivas (bloom) de cianobacterias diazotróficas, las cuales pueden modificar de manera radical la cantidad de nutrientes en el entorno, el cual afecta a la trama trófica y la comunidad planctónica. Los factores abióticos, por ejemplo, la disponibilidad de nutrientes y las condiciones climáticas han sido ampliamente estudiadas y debatidas. Sin embargo, el impacto de los bacteriófagos en la dinámica poblacional de las cianobacterias filamentosas diazotróficas formadoras de bloom no ha sido investigada a fondo.

Este estudio tiene como objetivo describir el rol de un cianófago aislado del Mar Báltico en la dinámica comunitaria de una especie de cianobacterias (*Nodularia spumigena*) y el efecto sobre los nutrientes en el medio acuático a través de un enfoque evolutivo experimental. Además, esta tesis explora la influencia de la evolución de cianobacterias resistentes a bacteriófagos en la dinámica comunitaria de plancton experimental (que incluye poblaciones de *Nodularia spumigena*, cianófago 2AV2, *Chlorella vulgaris* y rotíferos) y la transferencia de nitrógeno entre los componentes del plancton. Para realizar el estudio experimental se aisló un cianófago infectante de *Nodularia* del Mar Báltico, caracterizado y nombrado como 2AV2. Este cianófago pertenece a la familia Siphoviridae con un ciclo de vida lítico entre 12-18 horas y con un rango de hospedero estrecho de 12 cepas susceptibles de 45 cepas testeadas.

La infección del bacteriófago redujo aproximadamente en un 80% la población de cianobacterias hospedadores, seleccionando solo las cianobacterias resistentes a estos. Los hospederos restantes evolucionaron y crecieron en una población estable resistente a los fagos. La evolución de la fago-resistencia redujo significativamente la liberación de nitrógeno a partir de la lisis celular mediada por fagos. Además, los filamentos aislados de la población resistente a fagos tenían 2 morfotipos, 40% de filamentos cortos y 60% de filamentos largos, mientras que la población susceptible tenía 100% de estos últimos. Se detectaron diferencias de rasgos fenotípicos entre estos morfotipos, como la tasa de crecimiento y la flotabilidad.

Se sugiere que la divergencia en los rasgos fenotípicos entre las cianobacterias resistentes a los fagos se debió a una compensación evolutiva para mejorar el fitness. Este es el primer estudio que registra un cambio en la morfología (longitud del filamento) después de la evolución de la resistencia a los bacteriófagos en *Nodularia spumigena.* En la comunidad

de plancton experimental, las biomasas de *Nodularia*, cianofago 2AV2, *Chlorella* y rotíferos desarrollaron diferencias entre los tratamientos con diferente frecuencia de *Nodularia* resistente al fago. La frecuencia de fago-resistencia en la población de cianobacterias determinó en la sucesión ecológica y la transferencia de nitrógeno en la cadena alimenticia.

Esta tesis apoya la hipótesis de que los cianófagos no solo afectan a las poblaciones de cianobacterias, sino que tienen una influencia más amplia en la dinámica de la comunidad planctónica y la transferencia de nitrógeno en la cadena alimenticia. Por otra parte, este estudio muestra las ventajas de utilizar un enfoque de evolución experimental en el estudio de la dinámica acuática bacteriófago-hospedador.

1. Introduction

Key role of cyanobacteria in Baltic Sea pelagic food webs

Cyanobacteria are oxygen producing photoautotrophs belonging to the bacteria domain of life. Together with eukaryotic algae they contribute significantly to oceanic primary production. Cyanobacteria are one of the oldest life forms on Earth with fossil evidence dated 3.5 billion years old (Schopf, 2012). The ancient evolution of oxygenic photosynthesis in cyanobacteria provided the first significant biotic source of oxygen on Earth, having a pivotal role in the oxygenation of the atmosphere and permitting the evolution of plants and animals (Buick, 1992; Hamilton *et al.*, 2016). Cyanobacteria are widely distributed in different types of environments, and are highly diverse containing a wide range of morphologies: these include unicellular and unbranched or branched filamentous cell organization (Rippka *et al.*, 1979). Certain species possess differentiated cell types such as heterocysts (nitrogen fixing cells) and akinetes (dormant cells) which are structurally modified cells with specific functions (Adams *et al.*, 1981). In addition, cyanobacteria can produce a variety of bioactive compounds including those toxic to mammals (Sivonen and Börner, 2008; Catherine *et al.*, 2017).

Carbon, nitrogen and phosphorus are among the essential nutrients for cyanobacterial growth and primary production in general, since they are needed for the synthesis of nucleic acids, phospholipids, amino acids and proteins. Primary producers fix inorganic carbon (carbon dioxide) and assimilate inorganic nitrogen (mostly nitrate and ammonium) and phosphorus dissolved in aquatic environments (Ogawa and Kaplan, 2003; Chaffin and Bridgeman, 2014). However, in vast areas of the Baltic Sea (Baltic proper and Kattegat), inorganic nitrogen is scarce in surface waters with N:P ratios above the 16:1 Redfield ratio, limiting the growth of non-nitrogen fixing phytoplankton during spring (Granéli *et al.*, 1990). Such nitrogen limited conditions are advantageous for heterocyst-forming filamentous cyanobacteria whose growth in the Baltic Sea is limited by phosphorus and iron (Stal *et al.*, 1999; Moisander *et al.*, 2003; Degerholm *et al.*, 2006).

It is known that diazotrophic filamentous cyanobacteria fix nitrogen through heterocysts in the absence of dissolved inorganic nitrogen. However, nitrogen fixation in cyanobacteria is thought to involve higher energy expenditure than the assimilation of dissolved inorganic nitrogen (DIN). This energy aspect regulates heterocyst formation and pathways for nitrogen uptake (Adams *et al.*, 1981; Cheng *et al.*, 1999). A schematic representation of the uptake and

release of nitrogen by a diazotrophic filamentous cyanobacterium is presented in Fig. 1.

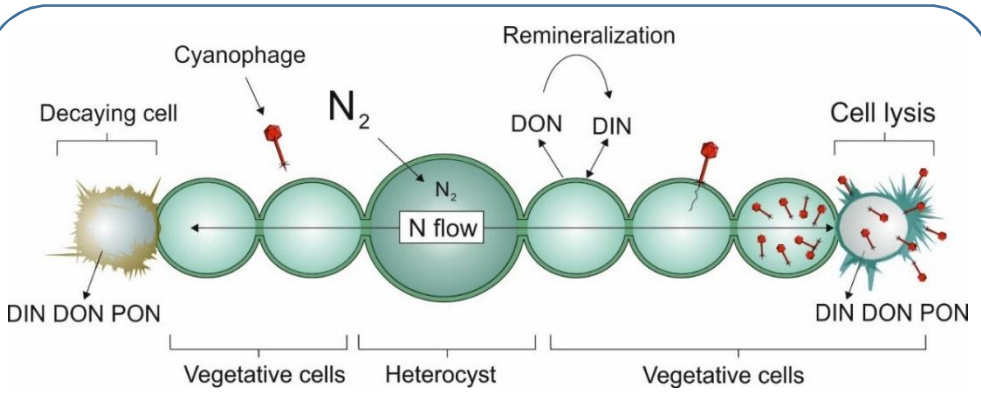

Figure 1. Schematic representation of nitrogen flow in a diazotrophic filamentous cyanobacterium. Once gaseous nitrogen (N_2) is fixed in heterocysts, the nitrogen is distributed to vegetative cells along the filament. Part of the fixed nitrogen is then utilized to build cellular structures while part of it will leak out from the cells as dissolved inorganic nitrogen (DIN) or dissolved organic nitrogen (DON). DON can then be transformed into DIN by bacteria (remineralization) becoming thus available for reuse (Haaber and Middelboe, 2009). In addition, DIN, DON and particulate organic nitrogen (PON) are released from decaying or lysed cells considerably increasing the nitrogen concentration in the surrounding aquatic environment (Glibert and Bronk, 1994; Larsson *et al.*, 2001; Ploug *et al.*, 2010; 2011; Buchan *et al.*, 2014; Svedén *et al.*, 2016). Consequently, the recently fixed nitrogen may be transferred not only to other members of the bacterioplankton and phytoplankton community but also to primary consumers such as zooplankton that graze on fresh, decaying or phage-infected cyanobacteria (Ohlendieck *et al.*, 2000; Haaber and Middelboe, 2009; Karlson *et al.*, 2015).

During summer, cyanobacterial nitrogen fixation can contribute importantly to the nitrogen fluxes in the Baltic Sea, stimulating the net primary production in pelagic food webs (Larsson *et al.*, 2001), increasing bacterio-, phyto-, and zooplankton biomasses and ultimately even pelagic fish production (Paerl and Pinckney, 1996; Ohlendieck *et al.*, 2000; Stevenson and Waterbury, 2006; Hansson *et al.*, 2007; Ploug *et al.*, 2011; Karlson *et al.*, 2015). In the presence of optimal weather and nutrient conditions during the summer period, cyanobacterial populations may grow into high densities developing

massive blooms, dominating the plankton community, altering local food webs and having an important impact on the regional ecosystem (Kanoshina *et al.*, 2003; Kozlowsky-Suzuki *et al.*, 2007). When cyanobacterial blooms are composed of toxin-producing strains, the concentration of toxins can have negative effects on fish growth (Persson *et al.*, 2011) and cause illness and death in other wild and domestic vertebrates (Sivonen *et al.*, 1990; Smayda, 1997; Carmichael, 2001). The increasing occurrence of diazotrophic cyanobacterial blooms during recent decades increases their importance in the ecology and biogeochemical cycles of pelagic food webs in the Baltic Sea (Fig. 2). The cyanobacterial host used in this study is *Nodularia spumigena*, one of the most common bloom-forming filamentous cyanobacteria in the Baltic Sea, a diazotrophic (nitrogen fixing) and toxin-producing species from the Nostocales order (Sivonen *et al.*, 1989a; Kahru *et al.*, 1994; Finni *et al.*, 2001).

Figure 2. Diazotrophic filamentous cyanobacterial bloom on the shore of Loviisa, Southern Coast of Finland, in 2015.

Factors regulating cyanobacterial community dynamics and bloom formation

Blooms of cyanobacteria occur every summer in the Baltic Sea, threatening the ecological integrity and sustainability of aquatic ecosystems (Sivonen *et al.*, 1989a; Kononen, 1992; Laamanen *et al.*, 2005; Karjalainen *et al.*, 2007). Several studies have described the factors that regulate the occurrence and intensity of cyanobacterial blooms, however, with the main focus being on abiotic factors (Laamanen, 1997; Wasmund, 1997; Paerl and Otten, 2013).

Planktonic blooms are typically allocated in patches, determined by the physical variability of the water body (Galat and Verdin, 1989; Kononen and Leppänen, 1997). In addition to naturally occurring optimal bloom conditions, the recent intensification of the blooms has been linked to eutrophication and climate change (Kahru *et al.*, 1994; Lundberg *et al.*, 2005; Raateoja *et al.*, 2005; Paerl *et al.*, 2011; Suikkanen *et al.*, 2013). For instance, anthropogenic nutrient loading, rising temperatures, changes in wind intensity and the inflow of low-oxygen saline waters enhance vertical stratification in the Baltic Sea that appears to be a critical factor determining bloom intensity (Wasmund, 1997). Vertical stratification accompanied by anoxic conditions in the lower water bodies contributes to the release of phosphorus from sediments and, consequently, to a decreased N:P ratio in the water column. Low N:P ratios cause proliferation of phosphorus limited cyanobacteria, while high N:P ratios allow other species to dominate the phytoplankton community over cyanobacteria (Kahru *et al.*, 2000).

Winds have an important role in the distribution of cyanobacteria in the water column. For instance, calm weather may cause filamentous cyanobacteria containing gas vesicles to float towards higher irradiance, accumulating near the water surface, while strong winds may cause mixing of water that disperses cyanobacteria (Walsby *et al.*, 1997; Stal *et al.*, 2003).

Besides the aforementioned abiotic factors, biotic factors such as the grazing of zooplankton on cyanobacteria and phage-mediated lysis of cyanobacterial cells can also affect cyanobacterial community dynamics and bloom formation (Suttle, 2007; Brussaard *et al.*, 2008; Clokie *et al.*, 2011; Engström-Öst *et al.*, 2013; Storesund *et al.*, 2015; Fu *et al.*, 2017). It is well established that zooplankton and protists may exert top-down control on the abundance and diversity of picocyanobacteria in the Baltic Sea (Motwani and Gorokhova, 2013). However, due to the specific traits of filamentous cyanobacteria (e.g. production of bioactive compounds, poor edibility of filamentous, colonial formations and low nutrient value), they have long been considered to be

inadequate food for zooplankton and to obstruct the transfer of energy to higher trophic levels (Gulati and DeMott, 1997; Ger *et al.*, 2016).

Zooplankton have been shown to feed preferentially on other phytoplankton species and only opportunistically on cyanobacteria (Meyer-Harms *et al.*, 1999). Low grazing rates on cyanobacteria indicate that grazers discriminate against toxic cyanobacteria and graze selectively (DeMott and Moxter, 1991). The reduced grazing pressure on cyanobacteria and lower competition for resources (due to reduction of competitors by grazers) may enhance cyanobacterial growth resulting in bloom formation (Gorokhova and Engström-Öst, 2009; Lehtinen *et al.*, 2010; Rose *et al.*, 2017). However, generalist grazers such as small crustaceans (i.e. *Daphnia* sp.) may control blooms when cyanobacteria are within the edible size (Sarnelle, 2007; Urrutia-Cordero *et al.*, 2016). Therefore, in pelagic ecosystems with a high abundance of toxic filamentous cyanobacteria, zooplankton and phytoplankton can experience a significant uncoupling.

Alongside phyto- and zooplankton species, viruses are described as a major biotic factor influencing microbial community structure and dynamics, making them an important component of Baltic Sea food webs (Šulčius and Holmfeldt, 2016). Cyanophages, viruses that infect cyanobacteria, are known both to impose constraints and to increase the diversity of cyanobacterial communities and, subsequently, to interfere with microbial-driven global biogeochemical processes in marine environments (Fuhrman, 1999; Brussaard *et al.*, 2008; Middelboe *et al.*, 2009; Rohwer and Thurber, 2009; Jover *et al.*, 2014; Brum and Sullivan, 2015). Although Suttle (2007) describes phages as ‘by far the most abundant “life forms” in the ocean’ to emphasize their high abundance and diversity, cyanophages and their ecological and evolutionary roles have thus far been poorly characterized (Brum *et al.*, 2015).

Ecological and evolutionary role of cyanophages

Cyanophages affect the composition and dynamics of cyanobacterial populations and are thus important entities affecting primary productivity (Suttle *et al.*, 1990). Cyanophages possess double-stranded DNA (dsDNA) genomes and belong to three major virus families based on tail morphology: Podoviridae, Myoviridae, and Siphoviridae (Suttle, 2000). Despite the long history of cyanophage research (Safferman and Morris, 1963; Granhall and Hofsten, 1969), to my knowledge, only 17 cyanophages infecting filamentous cyanobacteria have been characterized from the Baltic Sea (Jenkins and Hayes, 2006; Šulčius *et al.*, 2015; Šulčius and Holmfeldt, 2016). These cyanophages belong to the Myoviridae and Siphoviridae virus families with lytic cycles (Table 1). In line with previous findings, the characterized cyanophage in this thesis belong to the Siphoviridae virus family with a dsDNA genome and lytic cycle (Fig. 3). The phage was named vB_NpeS-2AV2 but will be referred to in the text as phage 2AV2. The host for this phage is the filamentous cyanobacterium *Nodularia spumigena.*

Phages infect host cells by attaching to suitable receptors on the host cell surface and introducing their viral genome into the host cell. In a lytic cycle, the viral genome takes over the cell machinery and produces viral structures which are assembled forming new virus particles. Finally, the new virus particles are released by breaking the cell surface, causing lysis of the host cell (Fig. 1).

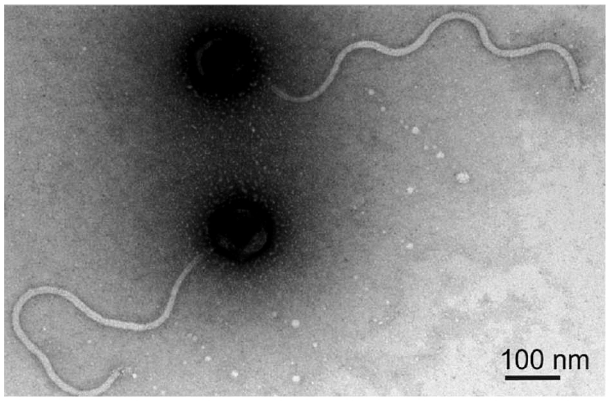

Figure 3. Transmission electron micrograph of 2AV2 virions. The virions have an icosahedral head approx. 95 nm in diameter and a noncontractile tail approx. 795 nm long.

Table 1. Diazotrophic filamentous cyanobacteria infecting cyanophages isolated from the Baltic Sea. All the cyanophages here have a doubled stranded DNA genome and lytic cycle.

Host species	Virus strain	Virus family	Capsid diameter (nm)	Tail length (nm)	Cross infectivity	Reference
N. spumigena	vB_NpeS-2AV2	Siphoviridae	95 × 95	795	12/45	Article I
N. spumigena	N-BM1	Myoviridae	80 × 80	133	4/9	Jenkins and Hayes, 2006
N. spumigena	N-BM2	Myoviridae	79 × 118	126	2/3	
N. spumigena	N-BM3	Myoviridae	57 × 72	45	1/1	
N. spumigena	N-BM4	Myoviridae	ND	ND	2/5	
N. spumigena	N-BS1	Siphoviridae	137 × 120	834	4/7	
N. spumigena	N-BS2	Siphoviridae	53 × 53	245	2/3	
N. spumigena	N-BS3	Siphoviridae	54 × 60	188	2/9	
N. spumigena	N-BS4	Siphoviridae	127 × 122	888	4/4	
N. spumigena	N-BS5	Siphoviridae	ND	ND	1/3	
N. spumigena	11	Unclassified	ND	ND	2/5	
N. spumigena	12	Unclassified	ND	ND	5/7	
N. spumigena	13	Unclassified	50 × 52	119	1/3	
N. spumigena	14	Unclassified	ND	ND	1/3	
N. spumigena	15	Unclassified	ND	ND	1/3	
N. spumigena	16	Unclassified	ND	ND	1/5	
N. spumigena	17	Unclassified	ND	ND	1/4	
A. flos aquae	Vb-AphaS-CL131	Siphoviridae	97	361	2/7	Šulciuš *et al.*, 2015

*Characterized in this thesis (I). The table has been modified from Šulciuš *et al.*, 2016.

However, in a chronic infection, the host releases phage progeny without cell lysis. Another well-known viral cycle is the lysogenic cycle or lysogeny where the phage genome is integrated into the host genome. Such a viral genome is called a prophage and can be transmitted from parent cell to progeny or through horizontal gene transfer (Sullivan *et al.*, 2003). A latent state where no replication of the prophage genome and no progeny production occurs is called pseudolysogeny (Clokie *et al.*, 2011). This state may explain the long-term survival of viruses in unfavourable conditions. However, events such as variation in UV radiation, temperature, pressure or common pollutants can cause stress in the host cell resulting in the activation of the lytic cycle (Jiang and Paul, 1996; Fuhrman, 1999).

Lytic phages have been found to supress cyanobacterial and algal populations significantly (Proctor and Fuhrman, 1990; Bratbak *et al.*, 1993; Brussaard *et al.*, 2005; Šulčius *et al.*, 2015). When progeny phages are released and the host cell is lysed, intracellular materials are released as well, such as particulate or dissolved organic material (POM or DOM, respectively), causing a redirection of nutrients in a process called the viral shunt (Wilhelm and Suttle, 1999; Weitz and Wilhelm, 2012). The nutrients are then redirected back to the microbial loop, becoming available to other microorganisms instead of being transferred to higher trophic levels (primary and higher-level consumers) (Poorvin *et al.*, 2004). This recycling process permits also the retention of nutrients in the euphotic zone of the water column avoiding the transfer of nutrients to larger organisms which will eventually sink, transporting the nutrients to deeper waters (Fuhrman, 1999) Overall, lytic phages have large ecological effect on nutrient (carbon, nitrogen and phosphorus) bioavailability, marine biogeochemical cycles and food webs (Brussaard *et al.*, 2008; Jover *et al.*, 2014).

Cyanophages play an important role also in the evolution and diversification of cyanobacterial communities (Biller *et al.*, 2015; Shestakov and Karbysheva, 2015). In general, lytic phages have been shown to have a strong influence on planktonic bacterial population dynamics and to sustain the genetic diversity of prokaryotes (Middelboe *et al.*, 2001).

Lytic phages can reduce the number of susceptible cells in a cyanobacterial community by selecting for rare phage-resistant cells (Šulčius *et al.*, 2015). Phages may sustain bacterial diversity through suppressing dominant competitors (i.e. “killing the winner”), allowing the coexistence of less competitive populations (Thingstad and Lignell, 1997; Jiang and Paul, 1998). The magnitude of the reduction of cyanobacterial abundance can be affected, among others, by the frequency of susceptible cells and the virulence of the cyanophages, since they spread randomly and face the problem of finding the

next host while remaining infectious (Fuhrman, 1999; Mann, 2003). Nevertheless, in a phage-resistant cell dominated population, phages may be present at low frequencies due to the presence of low numbers of susceptible cells in the cyanobacterial community (Waterbury and Valois, 1993). The ecological and evolutionary dynamics are therefore driven by the altering selection pressures imposed by phages and the genetic variability generated in the process (Weinbauer and Rassoulzadegan, 2004).

Sustained interactions between cyanophages and cyanobacteria may generate not only one-way but also reciprocal evolutionary changes, a process known as (antagonistic) coevolution. Coevolution can result in the evolution of diverse viral attack and host cell defence mechanisms (Buckling and Rainey, 2002; Martiny *et al.*, 2014). A variety of defence strategies have evolved against phage predation, such as mechanisms associated with preventing phage adsorption, restriction-modification systems, and clustered regularly interspaced short palindromic repeats and CRISPR-associated (*cas*) genes (CRISPRs-Cas) systems (Labrie *et al.*, 2010; Stern and Sorek, 2011; Chénard *et al.*, 2016).

In marine unicellular cyanobacteria, phage resistance is most likely achieved through mutations in genes involved in the attachment of the phage to the cell surface (Stoddard *et al.*, 2007; Avrani *et al.*, 2011). The mutations conferring resistance may affect the function of other traits in the host. In cyanobacteria, the evolution of phage resistance typically involves a cost in host fitness, which can be observed as a reduction in host growth rate in the absence of phage (Bohannan and Lenski, 2000; Lennon *et al.*, 2007). Interestingly, phages can also incorporate new traits into the host by introducing beneficial genes, through lysogenic conversion (transduction), and thereby increase host fitness.(Sandaa, 2008). Disentangling the consequences of the evolution of phage resistance on cyanobacterial communities is important to better understand cyanobacterial bloom formation and plankton community dynamics.

In this thesis, I seek to understand the role of cyanophages in the ecology and evolution of cyanobacterial populations and the implications of these interactions on planktonic community dynamics. My main hypothesis is that cyanophages have an important role in the ecology and evolutionary dynamics of the host population, reducing temporarily host abundance, releasing intracellular nitrogen and driving host phage-resistance. If this is the case, I expect that the impact of phages influences the nutrient environment and planktonic community dynamics, affecting the interactions between food web components.

2. Study aims

In this thesis, I used an experimental evolution / ecology approach to explore interactions between the cyanophage 2AV2 and a *Nodularia* host population. In addition, I examined the implications of the evolution of phage resistance and nitrogen release for experimental planktonic community dynamics. The main focus of the studies in this thesis is outlined below and illustrated in Fig. 4.

This thesis is based on three studies which focus on the role of cyanophages on:

I	cyanobacterial community dynamics
II	host nitrogen release and evolved phenotypic traits
III	experimental planktonic community dynamics

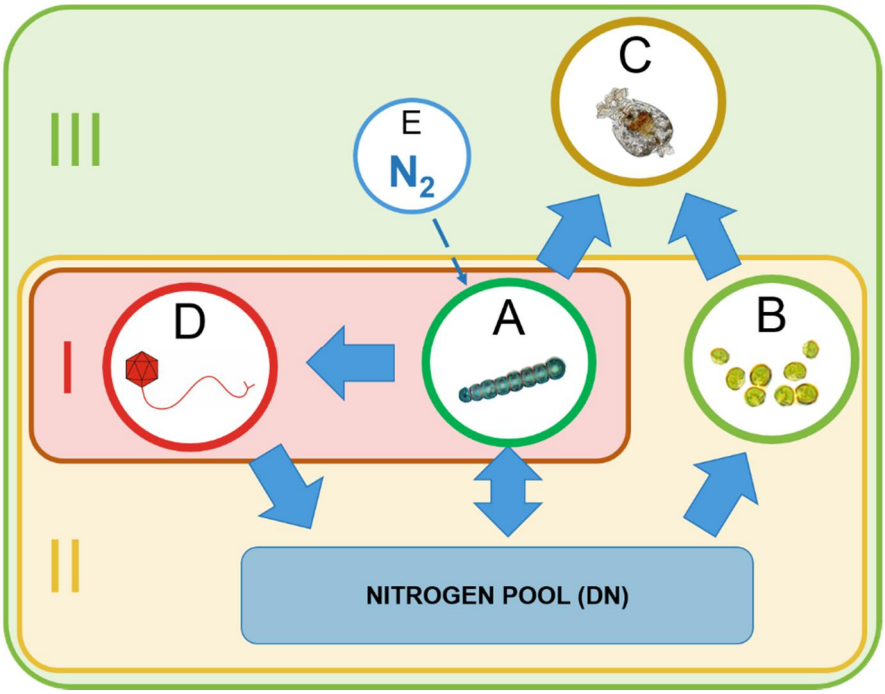

Figure 4. Schematic representation of the organisms and their interactions studied in this thesis. (A) Diazotrophic filamentous cyanobacterium *Nodularia spumigena*, (B) phytoplankton, (C) rotifer and (D) phage. Arrows (blue) indicate hypothetical nitrogen fluxes between community components in the food web. The nitrogen sources considered include dissolved nitrogen (DN) and gaseous nitrogen (E). Coloured areas highlight the focus of subprojects: study I (red), study II (yellow) and study III (green).

The specific aims and *hypotheses* were:

i. To determine the effect of cyanophage 2AV2 on a previously unexposed (naïve) *Nodularia* population under controlled environmental conditions (**I**)

H_i: *Cyanophage infection reduces host Nodularia population (susceptible cell type) size and promotes the evolution of phage resistance*

ii. To quantify nitrogen release from *Nodularia* populations (naïve and evolved) caused by phage-induced cell lysis (**I** and **II**)

H_{ii}: *Phage-induced cell lysis considerably increases the nitrogen concentration in the surrounding environment when the Nodularia population is dominated by susceptible host (naïve) cells*

iii. To determine the capacity of non-diazotrophic cyanobacteria and other phytoplankton species to reuse the nitrogen released from the phage-induced *Nodularia* cell lysis (**I** and **II**)

H_{iii}: *Phytoplankton species are able to recycle the nutrients released through phage-induced host cell lysis*

iv. To identify potential fitness costs of phage resistance in *Nodularia* (**II**)

H_{iv}: *Evolution of phage resistance involves costs of resistance which affect host fitness*

v. To investigate the ecological impact of the evolution of phage-resistant *Nodularia* on experimental plankton community dynamics (**III**)

H_v: *In a cyanobacteria-dominated phytoplankton community the evolution of phage resistance in cyanobacteria affects the interaction between planktonic food web components, in turn, influencing overall planktonic community dynamics*

3. Summary of materials and methods

The methods used in the articles comprising this thesis (**I**, **II** and **III**) are listed in Table 2 and described in detail in each article. The organisms used in the studies are listed in Table 3. The strains belonging to the genus *Nodularia* used to test for phage host range are listed in Supplementary Table S1 and isolated *Nodularia spumigena* clones in Supplementary Table S2.

Table 2. Methods used in this thesis.

Method		Article
Cultivation of study organisms		
	Algal species	II, III
	Cyanophage and cyanobacteria	I, II, III
	Rotifers	III
Molecular biology analyses		
	Viral DNA extraction and purification	I
	Bacterial DNA extraction	I
	PCR amplification	I
	Sequencing	I
Quantification		
	Cell counting	I, II, III
	Optical density (spectrophotometry)	II
	Plaque assay	I, II, III
Chemical analyses		
	HPLC	I
	Analysis of total nitrogen	I, II, III
Microscopy		
	Light and fluorescence microscopy	I, II, III
	Transmission electron microscopy (TEM)	I
Bioinformatics		
	Viral genome assembly and annotation	I
	Bacterial community analysis (16S rRNA gene)	I
Statistical analysis using SPSS		I, II, III

Table 3. Organisms used in this thesis.

Phylum/group	Strain	Study
Bacillariophyta	*Thalassiosira pseudonana* TV5	II
Bacillaryophyta	*Phaedoactylum tricornutum* TV 335	II
Chlorophyta	*Chlamydomonas reindardtii* UTEX 89	II
Chlorophyta	*Chlorella pyrenoidosa* TV 216	II
Chlorophyta	*Chlorella vulgaris* UTEX 26	II, III
Chlorophyta	*Scenedesmus obliquus*	II
Cryptophyta	*Rhodomonas* sp. Crypto07-B1	II
Cyanobacteria (filamentous)	*Nodularia spumigena* UHCC 0040*	I, II, II
Cyanobacteria (unicellular)	*Synechococcus* sp. TV65	I, II
Cyanobacteria (unicellular)	*Synechococcus* sp. CCY 0417	II
Cyanobacteria (unicellular)	*Synechococcus* sp. CCY 0435	II
Cyanobacteria (unicellular)	*Synechocystis* sp. UHCC 0318	II
Cyanophage (dsDNA virus)	Siphovirus vB_NpeS-2AV2	I, II, III
Rotifera	*Brachionus plicatilis*	III

*Previously named AV2.

4. Results and discussion

Host specificity and relevance of phage infecting *Nodularia* to cyanobacterial community dynamics (I, II)

Cyanobacterial community dynamics are influenced by abiotic factors such as weather and nutrient conditions and biotic factors such as competitors, parasites and grazers. Although several factors among these can cause strong selection pressure and drive cyanobacterial diversification, here I will focus on the role of cyanophages. To comprehend the influence of the studied cyanophage 2AV2 on filamentous cyanobacteria, I first investigated its host specificity by conducting infection assays with several *Nodularia* strains from the UHCC/HAMBI collection.

The cyanophage 2AV2 was shown to have relatively high specificity as it was only able to lyse 12 *Nodularia* strains among the 45 tested strains (Table S1). This host range is in line with those described by Jenkins and Hayes (2006) for other Baltic Sea cyanophages, where susceptible and resistant *Nodularia* strains were found within the same genotypic group. The tested strains (susceptible and resistant) had a wide spatial and temporal distribution in the Baltic Sea (Fig. 5). Interestingly, susceptible and resistant *Nodularia* AV strains were found to coexist in the same area when sampled within 8 days, indicating high host specificity.

Numerous studies on phage-host interactions show that phages are not able to infect all bacteria but are rather divided into generalist and specialist phages (Flores *et al.*, 2011). Furthermore, previous studies suggest that the host specificity of phages has important implications for host ecology and evolution (Bohannan and Lenski, 2000; Gorter *et al.*, 2015). Cyanophages are mainly species- or strain-specific and have a lytic cycle i.e. reproduce and release progeny through host cell lysis (Sullivan *et al.*, 2003; Yoshida *et al.*, 2006; Suttle, 2007). High specificity might delay phage-host encounters and limit phage dispersal (Sullivan *et al.*, 2003). Nevertheless, locally adapted phages with high specificity can reproduce successfully when targeting local host species or strains (Koskella *et al.*, 2011).

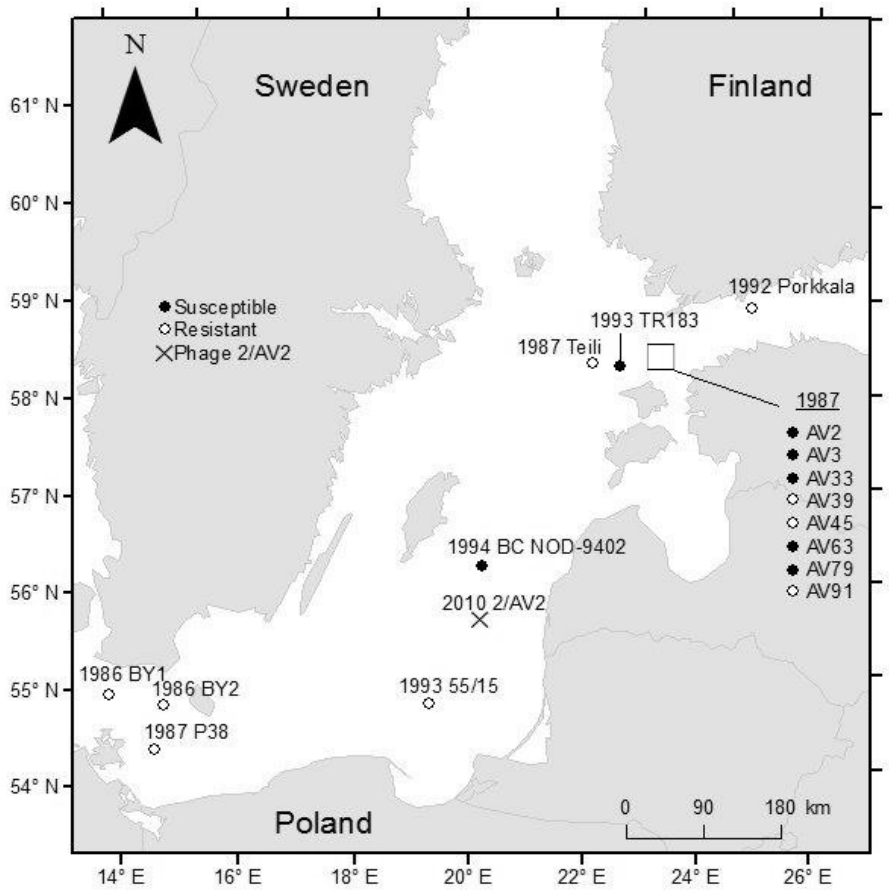

Figure 5. Spatial and temporal occurrence of 16 *Nodularia* strains among the 45 strains tested for phage host range. The isolation site of the phage (×), and susceptible (●) and resistant *Nodularia* strains (○) are indicated in the map. (Strain details are provided in Table S1.)

The phage susceptible *Nodularia spumigena* strain UHCC 0040 (previously named AV2) was chosen to further examine phage-host interactions with the isolated phage 2AV2. An experimental evolution approach was used, involving the construction of microcosms to examine the ecological and evolutionary relevance of the phage in cyanobacterial community dynamics (Fig. 6). This method allows efficient investigation of how biotic and abiotic factors affect bacterial communities. In addition, it has been shown to be a powerful tool in studying the role of the phages in host community dynamics and the evolution of phage-host interactions (Koskella and Meaden, 2013).

Figure 6. Microcosm setup consisting of semi-continuous batch cultures. The cultures are kept at 25°C ± 2°C at a constant light intensity of 5–8 µmol $m^{-2}s^{-1}$.

In the microcosm experiment (**I**), phage infection caused an approximately 80% reduction of the initial *Nodularia* population size (Fig. 7). The phage lysed susceptible *Nodularia* cells, selecting for phage-resistant *Nodularia* cells. The growth of phage-resistant *Nodularia* subsequently led to restoration of a high population size and stable dynamics.

Antagonistic coevolution with alternating mutations in the host bacterium and counter-mutations in the phage have been frequently shown in laboratory cultures (Koskella and Brockhurst, 2014). However, here phage coevolution resulting in re-infection of the *Nodularia* population dominated by cells resistant to the initial phage was not detected. A similar scenario where phage coevolution was not detected and the resistant host reached more stable dynamics was recently observed in a virus-host system with the green algae *Chlorella* (Frickel *et al.*, 2016).

Despite the lysis of the susceptible host and the evolution of phage resistance in the *Nodularia* population, the phage persisted at low densities until the end of the experiment. Explanations for low phage densities here include the susceptible host remaining at low density in populations and the persistence of non-degraded phages.

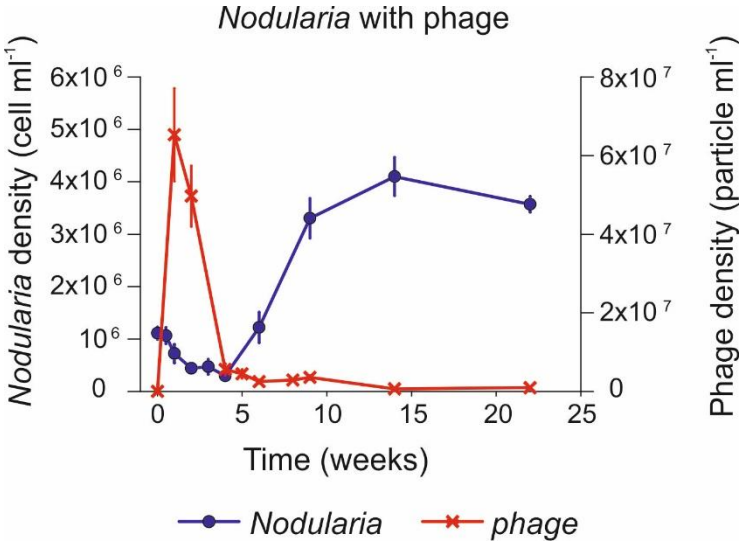

Figure 7. Phage-host dynamics during 22-week long experiment (n=3; mean ± s.e.). *Nodularia* (blue line) densities decreased for the first 4 weeks due to phage-induced cell lysis, which was followed by growth of a phage-resistant population. Phage (red line) density peaked during the first week after which it decreased rapidly, remaining at a low level after week 4.

Influence of phage-mediated nitrogen release on phytoplankton growth (I, II)

Exudation of nitrogenous compounds by diazotrophic cyanobacteria has been shown to promote the co-occurrence of phyto- and bacterioplankton species (Ohlendieck *et al.*, 2000; Ploug *et al.*, 2011; Woodland *et al.*, 2013; Woodhouse *et al.*, 2016). This was also demonstrated here by the growth of the single-celled non-nitrogen-fixing picocyanobacterium *Synechococcus* in mixed culture with *Nodularia* in nitrogen limited medium (**I**). In contrast, in monocultures in the same nitrogen limited medium, the non-nitrogen fixing *Synechococcus* went extinct (Fig. 8). Interestingly, both organisms reached higher densities when cultured together, suggesting the presence of mutualistic interactions.

Nitrogen fixing and non-nitrogen-fixing cyanobacterial species have been shown to be associated with distinctive bacterial communities (Woodhouse *et al.*, 2016; Zhu *et al.*, 2016). Similarly, the bacterial communities associated with *Nodularia* and *Synechococcus* monocultures were found to differ from each other (**I**) (Fig. S1).

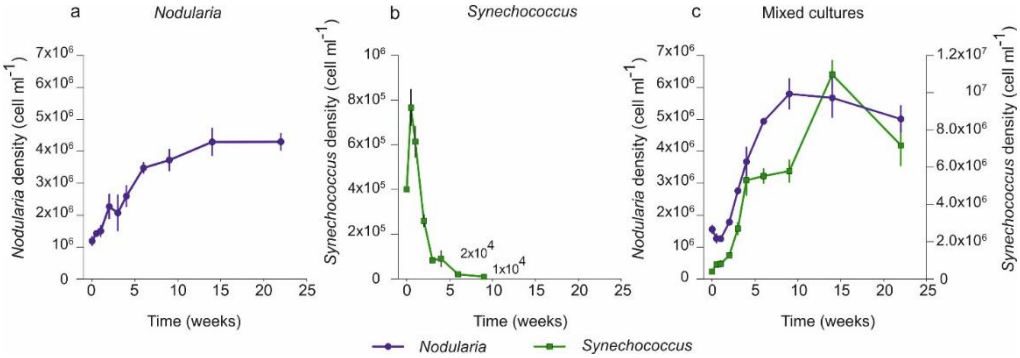

Figure 8. Cyanobacterial community dynamics during 22-week long microcosm experiment (mean ± s.e.). (a) *Nodularia* monoculture (blue line), (b) *Synechococcus* monoculture (green line), (c) mixed culture with *Nodularia* and *Synechococcus*.

Exudation of nitrogen by *Nodularia* and phage-induced cell lysis may modify the nutrient environment when cyanobacteria occur at high abundances (Fuhrman, 1999; Wilhelm and Suttle, 1999). Although the first study revealed only an increase in the total nitrogen in the medium over time, the second study was able to show that nitrogen release is caused by phage-induced host cell lysis. The sampling time of once per week (**I**) was insufficient for identification of the effect of phages, requiring shorter sampling times (**II**). In cultures of phage susceptible *Nodularia* (hereafter, referred to as "naïve" to indicate assumed lack of previous phage encounter), phage-induced cell lysis resulted in a significant increase in the nitrogen concentrations in cell-free filtrates (Fig. 9; RMANOVA, $F_{1,4} = 90.1$, $P < 0.001$). In contrast, in cultures of phage-resistant *Nodularia* (hereafter, referred to as "evolved" to indicate assumed presence of evolutionary changes caused by phage exposure), nitrogen concentrations in the cell-free filtrate remained stable in the absence of host cell lysis, without significant difference between the times points (**II**).

Later, the cell-free filtrates were used as growth medium to test for the ability of phytoplankton species to reuse nitrogen released via phage-induced cell lysis. Diatoms, single-celled picocyanobacteria and algae reached significantly higher maximum biovolumes in cultures where a cell-free filtrate of naïve (susceptible) *Nodularia* was used as medium compared to the biovolumes reached with cell-free filtrates obtained from evolved (phage-resistant) *Nodularia* cultures (Fig. 10). In nitrogen limited conditions, the phage 2AV2 had a positive overall effect on the growth of non-nitrogen-fixing cyanobacteria and other phytoplankton species able to uptake the newly available nitrogen. As a consequence, the evolution of phage resistance in *Nodularia* reduced the impact of phages on the local nutrient environment and planktonic community dynamics.

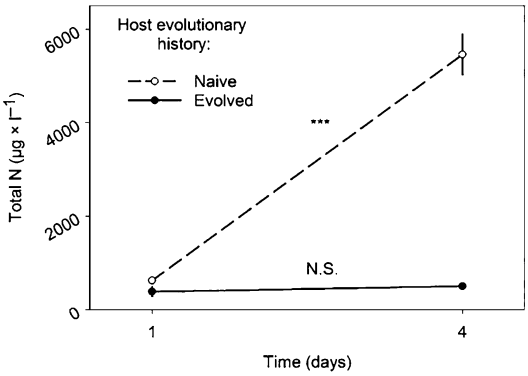

Figure 9. Total nitrogen concentrations in cell-free filtrates after phage inoculation (mean ± s.e.). Cell-free filtrates from naïve (dashed line) and evolved (solid line) *Nodularia* cultures were exposed to phage in a 4 day long experiment. After 4 days of culture, the total nitrogen concentration increased significantly in cell-free filtrates of naïve (susceptible) *Nodularia* cultures. In contrast, no significant changes in nitrogen concentrations were detected in filtrates of the evolved (phage-resistant) *Nodularia* cultures.

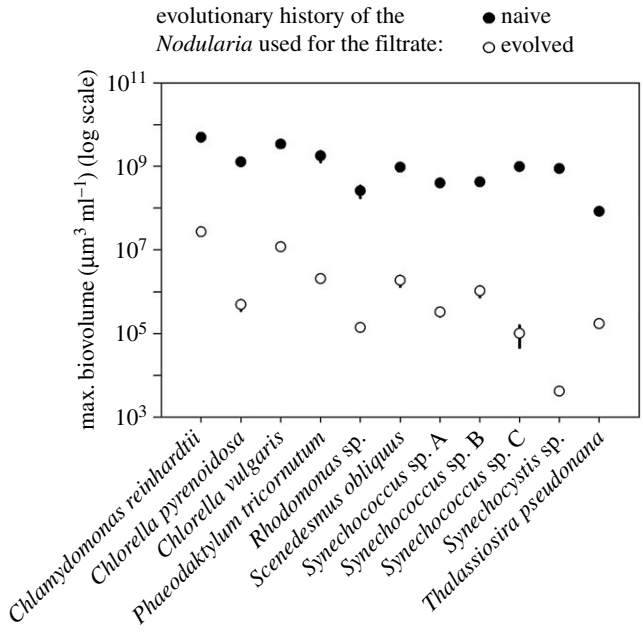

Figure 10. The maximum phytoplankton biovolume sustained by cell-free filtrates (mean ± s.e.). The biovolumes of phytoplankton strains cultured in filtrates from naïve and evolved *Nodularia* populations are indicated by black and empty circles, respectively.

Evolution and phenotypic traits of phage-resistant cyanobacteria (I, II)

Bacterial hosts have developed a wide range of mechanisms of resistance against phage infection (Labrie *et al.*, 2010). Intracellular mechanisms include CRISPR-Cas systems, which are adaptive immunity systems providing immunity to cells by recognising and degrading foreign genetic material (Barrangou *et al.*, 2007). Conversely, extracellular mechanisms include spontaneous mutations in cell surface related genes, which modify cell surface receptors preventing phage adsorption (Avrani *et al.*, 2011; Avrani and Lindell, 2015). The evolution of phage resistance promotes genotypic and phenotypic diversity in the host, increasing organismal complexity and evolvability (Zaman *et al.*, 2014; Wielgoss *et al.*, 2016). In this study, the mechanism responsible for phage resistance in *Nodularia* was not studied. Mechanisms that have been previously found in cyanobacteria include both mutations affecting cell surface receptors and CRISPR-cas systems (Avrani *et al.*, 2011; Wang *et al.*, 2012).

Phage resistance mutations may have a multitude of phenotypic effects involving a variety of physiological costs (Bohannan and Lenski., 2000). In this thesis, differences in phenotypic traits were detected between *Nodularia* isolates from the naïve and evolved populations (**II**). Regarding resistance phenotypes, isolates from the evolved populations (n = 58) were all resistant while isolates from the naïve population (n = 60) were all susceptible to phage 2AV2 infection.

In addition to resistance phenotypes, different filament length morphotypes were also detected among the isolates, with evolved (phage-resistant) *Nodularia* isolates exhibiting either a short- or long-filamentous morphotype (Table S2). All the phage susceptible isolates in this study possess a long-filamentous morphotype with filament length similar to that of the long-filamentous resistant morphotype (Tukey's HSD: $P = 0.339$). The short-filamentous resistant morphotype differs significantly in length from the susceptible isolates (Tukey's HSD: $P < 0.001$) (Fig. 11). In another later study, cyanophages were also shown to cause short filaments in the species *Aphanizomenon flos-aquae* (Šulčius *et al.*, 2017). In this study, long and short filaments further exhibited differences in growth rate and buoyancy. The novel short-filamentous phage-resistant *Nodularia* morphotype grew better in iron and phosphorus limited media compared to long-filamentous phage-resistant and susceptible *Nodularia* morphotypes.

Figure 11. Photomicrographs of Nodularia morphotypes; (a) long-filamentous phage susceptible morphotype, (b) long-filamentous phage-resistant morphotype and (c) short-filamentous phage-resistant morphotype.

Buoyancy is provided by gas vesicles, allowing planktonic cyanobacteria to float and sink within the photic zone of the water column to find optimal light intensity for photosynthesis, which is critical for survival (Walsby *et al.*, 1995; 1997). The impermeable gas vesicles are composed by proteins and mostly limited to plankton microorganisms. The hollow gas vesicles lower cell density, increasing floatability. Different mechanisms are responsible for the regulation of cyanobacterial buoyancy, e.g. regulation of gas vesicle gene expression, destruction of gas vesicles by pressure, and changes in carbohydrate content and other dense substances. Large amounts of carbohydrates are produced and stored during high irradiance by photosynthesis making the cell denser and thereby prone to sink. However, stored carbohydrates are depleted by respiration during night making cells less dense and permitting them float back to the surface (Walsby, 1994). In this study, the short-filamentous morphotype among phage-resistant *Nodularia* isolates was the only morphotype that sank to the bottom of culture bottles. The short-filamentous morphotype failed to recover buoyancy during cultivation for several generations.

Investment into defence mechanisms against phages typically includes a trade-off with fitness in the absence of phage (i.e. has a fitness cost). This has also been observed for cyanobacteria, and is usually detected as decreased growth rate (Avrani *et al.*, 2011). Here, decreased buoyancy and filament length, detected in 40% of evolved *Nodularia* isolates, could be considered to represent a new type of trade-off whereby morphological changes caused by phage resistance result in a fitness cost in the absence of phage. Furthermore, such changes in buoyancy or filament length as a result

of phage exposure may contribute to geno- and phenotypic diversity within *Nodularia* populations.

Speculatively, these findings also raise questions concerning the evolutionary history of non-buoyant benthic *Nodularia* species, considering that the diversification of *Nodularia* observed in this study (new morphotypes and loss of buoyancy) may permit colonization of new ecological niches, including the benthic environment. Together these findings provide an addition to the body of evidence demonstrating that phages are an important factor driving evolutionary processes in bacterial communities (Koskella and Brockhurst, 2014).

Role of phage-resistant cyanobacteria in planktonic community dynamics (III)

In this thesis, phages were observed to cause nitrogen release through cell lysis, and to influence various aspects of the ecology and evolution of *Nodularia* populations (**I** and **II**). To investigate the influence of phages in more complex plankton communities, an experimental plankton community was constructed (**III**). Together with phages, zooplankton are known to exert top-down control of phytoplankton (producers) (Jürgens and Matz, 2002; Brussaard *et al.*, 2008; Storesund *et al.*, 2015). Here rotifers were used as the zooplankton (consumers) component of the experimental plankton community. Because phage resistance was previously (**I** and **II**) found to reduce the effect of phages on phytoplankton community dynamics, *Nodularia* populations with different frequencies of the phage-resistant genotype were included. The experimental plankton community therefore included cyanobacteria (*Nodularia*), green algae (*Chlorella*), rotifers (*Brachionus*) and phages (2AV2). The organisms were cultured in nitrogen limited (N-lim) and rich (N-rich) media.

The results revealed that the experimental community dynamics were determined by the initial frequency of the phage-resistant genotype in *Nodularia* populations. The communities with a high frequency of the phage-resistant genotype (50% of initial *Nodularia* population) were dominated by *Nodularia* throughout the experiment. In contrast, when *Nodularia* populations had zero or a low initial frequency of the phage-resistant genotype (0% or 5% of population), *Nodularia* population densities decreased under the detection limit (extinction) and *Chlorella* dominated the community at the end of the experiment. Compared to *Chlorella*, *Nodularia* is a superior competitor allowing populations with high phage-resistant genotype frequencies to dominate the plankton community. However, under phage pressure,

Nodularia populations with low or zero frequency of the phage-resistant genotype were outcompeted by *Chlorella*. This result contrasts with the previous results (**I**) where mutualistic interactions were found between *Nodularia* and the non-nitrogen fixing cyanobacterium *Synechococcus*. However, it is known that competition efficiency, growth rates and nutrient affinity vary among species. Furthermore, the frequency of phage-resistant *Nodularia* in the naïve populations (**I**) is uncertain and might be higher compared to the experimental plankton communities (0% and 5% of the *Nodularia* population; **III**). The low frequency of phage-resistant *Nodularia* may explain the irrecoverable collapse of the population in the experimental communities.

Regarding nitrogen flow in the plankton community, in the beginning of the experiment, most of the nitrogen in the plankton community (approx. 69–79%) was contained by the *Nodularia* population (Fig. 12). After phage infection, nitrogen was released from the lysed cells of phage susceptible *Nodularia.* In populations with zero or a low frequency of the phage-resistant *Nodularia* genotype, the released nitrogen was transferred to the *Chlorella* and rotifer populations. Consequently, at the end of the experiment, most of the nitrogen (approx. 93–99%) was contained by *Chlorella*. In cultures with a high frequency of the phage-resistant *Nodularia* genotype, most of the nitrogen was contained by the cyanobacterial population throughout the experiment. Therefore, the succession in the plankton community was determined by the frequency of the phage-resistant *Nodularia* genotype in the *Nodularia* population. Overall, the ecological succession in the community was not dramatically affected by the nitrogen content of the medium.

However, the nitrogen content in the rotifer population notably increased, accompanied by a decrease in *Chlorella*, on week 4 in N-rich compared to N-lim medium. Interestingly the free swimming rotifers, which are efficient grazers of suspended planktonic microorganisms and influence species composition in planktonic communities, were unable to recover after the peak on week 4 and maintained slightly lower but stable densities towards the end of the experiment (**III**). However, their contribution to the total nitrogen content in the food web decreased towards the end of the experiment. This reduction of the rotifer population could be due to the evolution of prey defence in *Chlorella* observed as multicellular colonies in the cultures (Fig. 13), since *Chlorella* is known to evolve to grow as large stable colonies that prevent predation and alter predator-prey cycles (Boraas *et al.*, 1998; Yoshida *et al.*, 2004). In cultures with a high frequency of phage-resistant *Nodularia* rotifers also faced difficulties in reaching *Chlorella* due to *Chlorella* densities decreasing under the threshold food concentration and the rigidity of *Nodularia* filaments which interfered with rotifer movement needed to reach

the food. Furthermore, *Chlorella* and rotifer population densities were higher in N-rich compared to N-lim medium, which was also seen in the total nitrogen content of the communities (Fig. 12).

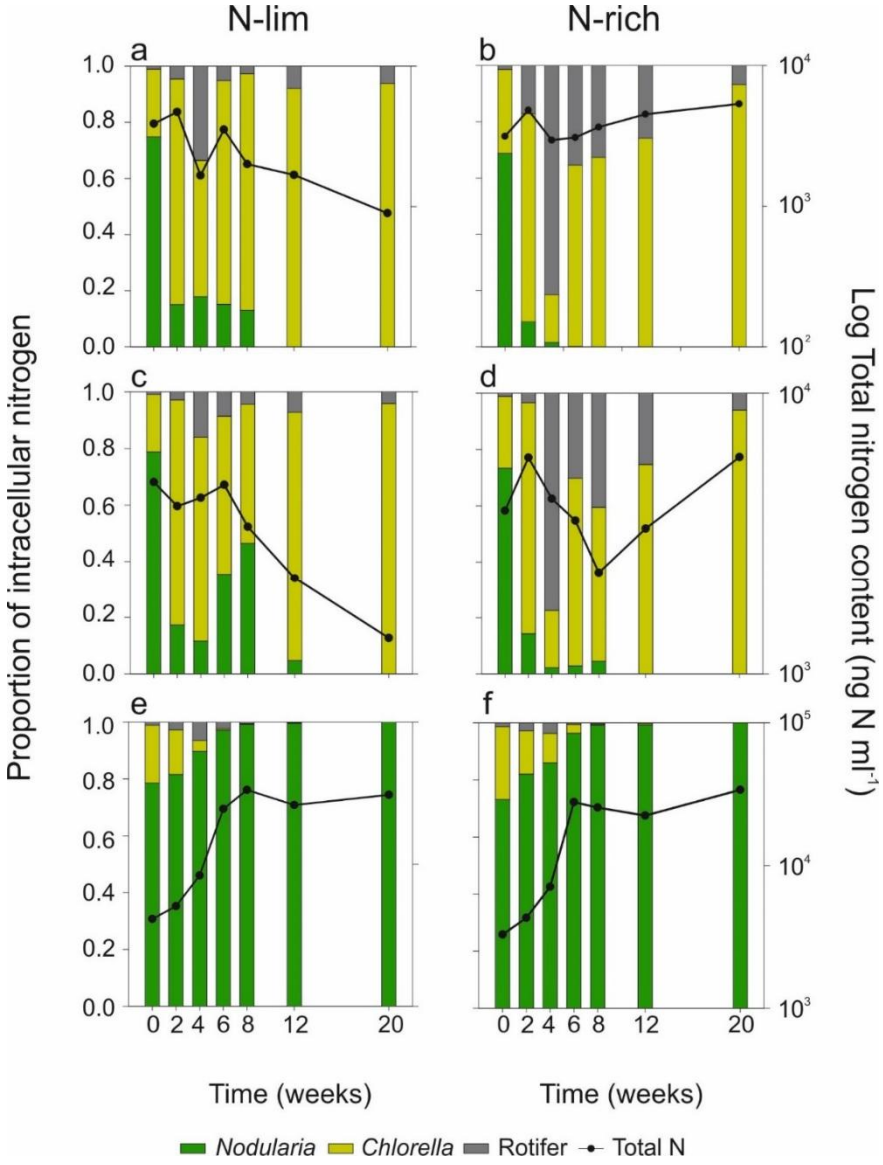

Figure 12. Temporal distribution of estimated intracellular nitrogen content in experimental planktonic community. The relative abundance of intracellular nitrogen in *Nodularia* (green), *Chlorella* (yellow), and rotifer (grey) is shown by bars and the estimated total nitrogen content by black lines (values shown on secondary axis). (a–b) *Nodularia* populations initially containing 0%, (c–d) 5%, and (e–f) 50% of the phage-resistant cyanobacterial genotype in N-lim and N-rich media.

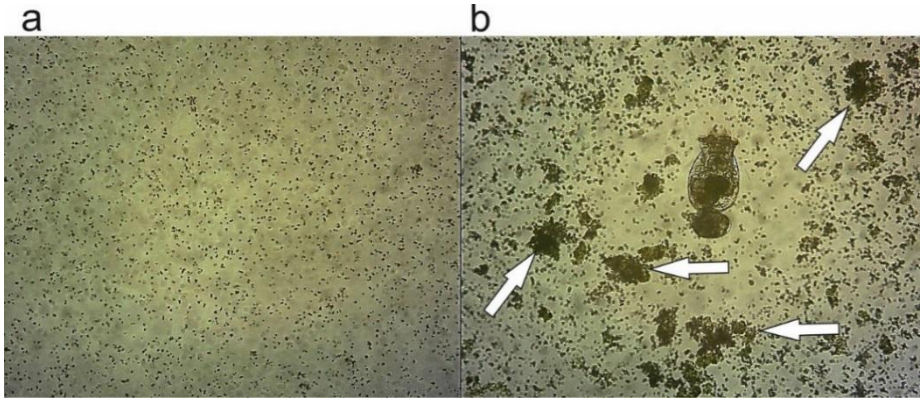

Figure 13. Photomicrographs of *Chlorella* defence formation against rotifer predation. (a) The *Chlorella* inoculum used in the beginning of the experiment shows single cells distributed evenly, and (b) a *Chlorella* culture after a few weeks of growing with rotifers forms multicellular colonies indicated by white arrows.

5. Concluding remarks and future directions

In this study, the effect of cyanophages on the population dynamics of diazotrophic filamentous cyanobacteria was described. The experimental evolution approach utilizing a semi-continuous microcosm setup allowed investigation of the ecological and evolutionary impact of the phage.

This thesis includes characterization of a novel phage isolated from the Baltic Sea which caused a temporary reduction in host population size selecting for the evolution of phage resistance (**I**). The host range test (specificity) performed for the phage was the largest known for filamentous cyanobacteria and included *Nodularia* strains isolated from a wide spatial and temporal distribution in the Baltic Sea (**II**). Phage-induced host lysis not only reduced host population size and released nutrients to the environment but also influenced the transfer of nitrogen to other phytoplankton species and acted as a driver of geno- and phenotypic divergence (**II**).

Despite the fact that large *in situ* studies continue to collect valuable data from naturally occurring plankton communities (Brum *et al.*, 2013; Sunagawa *et al.*, 2015), phage-host interactions are difficult to disentangle from such data. Through laboratory experiments, a more detailed understanding can be obtained about such interactions and the organisms involved. As seen in this thesis, in experimental conditions, phage resistance in a bloom forming diazotrophic cyanobacterium can determine the fate of plankton community dynamics and nitrogen flow in the planktonic food web (**III**). This study produces novel information regarding phage-host interactions between cyanophages and filamentous cyanobacteria.

This thesis represents an important contribution to knowledge regarding the role of cyanophages in plankton community dynamics and nitrogen cycle in the Baltic Sea. The use of more control treatments and more frequent sampling intervals would have provided a more detailed understanding about the effect of cyanophage. For further studies, it would be beneficial to measure not only total nitrogen concentration but also nitrogen ions (nitrate, nitrite and ammonium) to obtain more precise knowledge about nitrogen dynamics. Further molecular work is also needed to fill remaining gaps in knowledge concerning nitrogen transfer and phage resistance mechanisms. The next step would be to study the molecular evolution of *Nodularia* to characterise the dynamics of genomic factors producing the naïve and phage-resistant *Nodularia* phenotypes.

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8. Supplementary Information

Tables

Table S1. *Nodularia spumigena* strains, isolated from the Baltic Sea, used in this study to test for phage host range (**II**). Both recently designated culture collection codes for strains and earlier codes (previous name) are indicated.

Strain	(Previous name)	Year of isolation	Host[a]	References
UHCC 0176	(0208Porkkala)	1992	○	UHCC
UHCC 0117	(54/13)	1993	○	1
UHCC 0118	(55/15)	1987	○	1,9
UHCC 0067	(AN13a)	1994	●	2
UHCC 0069	(AN13c)	1994	●	2
UHCC 0040	(AV2)	1987	●	1,3,4
UHCC 0041	(AV3)	1987	●	1,9
UHCC 0042	(AV33)	1987	●	1,9
UHCC 0098	(AV39)	1989	○	1
UHCC 0093	(AV45)	1987	○	1
UHCC 0063	(AV63)	1987	●	1
UHCC 0079	(AV79)	1987	●	1
UHCC 0115	(AV91)	1987	○	1
BC Nod-9402		1994	●	5
UHCC 0188	(BY1)	1986	○	6,7,9
UHCC 0754	(BY2)	1986	○	6
CH301	-	–[b]	○	CH
CH307	-	1987	○	8
CH311	-	1987	○	8,13
UHCC 0764	(FL2)	1994	○	UHCC
UHCC 0177	(GR6)	1992	○	UHCC
UHCC 0155	(GR7b)	1992	○	UHCC
UHCC 0153	(GR8a)	1992	○	9
UHCC 0169	(GR8b)	1992	○	8-10
UHCC 0157	(GR9c)	1992	○	2
UHCC 0161	(GR9d)	1992	○	UHCC
UHCC 0145	(HV-36)	1991	○	2
UHCC 0120	(LL12)	1993	○	11
UHCC 0096	(P38)	1987	○	1,2,9
PCC 9350	(= axenic AV2)	1987	●	4,12

UHCC 0141	(Sr5a)	1991	●	2
UHCC 0143	(SR5i)	1991	●	2
UHCC 0116	(Teili)	1987	○	1,9
UHCC 0184	(TR183)	1993	●	2,9
UHCC 0104	(TR193)	1993	○	UHCC
UHCC 0080	(TR291a)	1994	○	UHCC
UHCC 0092	(TRO12b)	1994	○	UHCC
UHCC 0100	(TRO12d)	1994	○	UHCC
UHCC 0101	(TRO31a)	1994	○	2
UHCC 0102	(TRO31b)	1994	○	UHCC
UHCC 0070	(WP2a)	1994	○	UHCC
UHCC 0072	(WP2b)	1994	○	UHCC
UHCC 0073	(WP2c)	1994	○	2
UHCC 0074	(WP2d)	1994	○	2
UHCC 0076	(WP2f)	1994	○	2

Culture collections: PCC, Pasteur Culture Collection of Cyanobacteria; CH, Hübel, M. Hübel of E.-M.-Arndt University Greifswald, Biological Station Hiddensee, Kloster Germany; UHCC, University of Helsinki Cyanobacterial Collection HAMBI/UHCC. The number refer to following references: 1, Sivonen *et al.* (1989a); 2, Allahverdiyeva *et al.* (2010); 3, Koskenniemi *et al.* (2007); 4, Martin *et al.* (1990); 5, Hayes *et al.* (1997); 6, Sivonen *et al.* (1989b); 7, Moffit *et al.* (2001); 8, Fewer *et al.* (2013); 9, Lehtimäki *et al.* (2000); 10, Laamanen *et al.* (2001); 11, Kononen *et al.* (1993); 12, Iteman *et al.* (2002); 13, Lyra *et al.* (2005).

[a]The sensitive host strains were lysed (●) and the resistant strains where not affected (○).

[b] –, Unknown.

Table S2. Phenotypes of isolated clones from *Nodularia* populations after 22-week microcosm experiments. The naïve population refers to cyanobacteria previously unexposed and the evolved population to cyanobacteria previously exposed to the phage 2AV2 (evolved population). The statistical significance *p*-value is from a *t*-test for susceptibility test results.

Clone no.	Populations	Morphotype	Phage resistance	*p*-value
1	Naive	Long	Susceptible	0.006
2	Naive	Long	Susceptible	0.014
3	Naive	Long	Susceptible	0.006
4	Naive	Long	Susceptible	0.004
5	Naive	Long	Susceptible	0.014
6	Naive	Long	Susceptible	0.014
7	Naive	Long	Susceptible	0.022
8	Naive	Long	Susceptible	0.038
9	Naive	Long	Susceptible	0.032
10	Naive	Long	Susceptible	0.040
11	Naive	Long	Susceptible	0.000
12	Naive	Long	Susceptible	0.008
13	Naive	Long	Susceptible	0.014
14	Naive	Long	Susceptible	0.036
15	Naive	Long	Susceptible	0.007
16	Naive	Long	Susceptible	0.050
17	Naive	Long	Susceptible	0.045
18	Naive	Long	Susceptible	0.048
19	Naive	Long	Susceptible	0.002
20	Naive	Long	Susceptible	0.012
21	Naive	Long	Susceptible	0.002
22	Naive	Long	Susceptible	0.012
23	Naive	Long	Susceptible	0.008
24	Naive	Long	Susceptible	0.006
25	Naive	Long	Susceptible	0.026
26	Naive	Long	Susceptible	0.033
27	Naive	Long	Susceptible	0.000
28	Naive	Long	Susceptible	0.018
29	Naive	Long	Susceptible	0.036
30	Naive	Long	Susceptible	0.013
31	Naive	Long	Susceptible	0.011
32	Naive	Long	Susceptible	0.008

33	Naive	Long	Susceptible	0.000
34	Naive	Long	Susceptible	0.033
35	Naive	Long	Susceptible	0.013
36	Naive	Long	Susceptible	0.019
37	Naive	Long	Susceptible	0.000
38	Naive	Long	Susceptible	0.028
39	Naive	Long	Susceptible	0.021
40	Naive	Long	Susceptible	0.047
41	Naive	Long	Susceptible	0.035
42	Naive	Long	Susceptible	0.005
43	Naive	Long	Susceptible	0.010
44	Naive	Long	Susceptible	0.010
45	Naive	Long	Susceptible	0.017
46	Naive	Long	Susceptible	0.043
47	Naive	Long	Susceptible	0.033
48	Naive	Long	Susceptible	0.016
49	Naive	Long	Susceptible	0.000
50	Naive	Long	Susceptible	0.004
51	Naive	Long	Susceptible	0.016
52	Naive	Long	Susceptible	0.025
53	Naive	Long	Susceptible	0.022
54	Naive	Long	Susceptible	0.005
55	Naive	Long	Susceptible	0.029
56	Naive	Long	Susceptible	0.004
57	Naive	Long	Susceptible	0.026
58	Naive	Long	Susceptible	0.010
59	Naive	Long	Susceptible	0.000
60	Naive	Long	Susceptible	0.016
61	Evolved	Long	Resistant	0.096
62	Evolved	Long	Resistant	0.333
63	Evolved	Short	Resistant	0.292
64	Evolved	Long	Resistant	0.092
65	Evolved	Short	Resistant	0.145
66	Evolved	Long	Resistant	0.234
67	Evolved	Long	Resistant	0.262
68	Evolved	Long	Resistant	0.183
69	Evolved	Short	Resistant	0.775

70	Evolved	Short	Resistant	0.596
71	Evolved	Short	Resistant	0.948
72	Evolved	Long	Resistant	0.236
73	Evolved	N/A	N/A	N/D
74	Evolved	Short	Resistant	0.453
75	Evolved	Long	Resistant	0.661
76	Evolved	Long	Resistant	0.917
77	Evolved	Long	Resistant	0.283
78	Evolved	Long	Resistant	0.222
79	Evolved	Long	Resistant	0.090
80	Evolved	Long	Resistant	0.118
81	Evolved	Long	Resistant	0.126
82	Evolved	Long	Resistant	0.069
83	Evolved	Short	Resistant	0.916
84	Evolved	Long	Resistant	0.182
85	Evolved	Short	Resistant	0.246
86	Evolved	Short	Resistant	0.070
87	Evolved	Long	Resistant	0.198
88	Evolved	Short	Resistant	0.945
89	Evolved	Short	Resistant	0.356
90	Evolved	Long	Resistant	0.078
91	Evolved	Short	Resistant	0.690
92	Evolved	Long	Resistant	0.907
93	Evolved	N/D	N/D	N/D
94	Evolved	Long	Resistant	0.893
95	Evolved	Short	Resistant	0.728
96	Evolved	Long	Resistant	0.260
97	Evolved	Short	Resistant	0.595
98	Evolved	Short	Resistant	0.422
99	Evolved	Short	Resistant	0.844
100	Evolved	Long	Resistant	0.152
101	Evolved	Long	Resistant	0.064
102	Evolved	Long	Resistant	0.652
103	Evolved	Long	Resistant	0.960
104	Evolved	Long	Resistant	0.117
105	Evolved	Long	Resistant	0.340
106	Evolved	Long	Resistant	0.286

107	Evolved	Long	Resistant	0.139
108	Evolved	Long	Resistant	0.182
109	Evolved	Long	Resistant	0.233
110	Evolved	Long	Resistant	0.066
111	Evolved	Long	Resistant	0.747
112	Evolved	Long	Resistant	0.338
113	Evolved	Long	Resistant	0.457
114	Evolved	Long	Resistant	0.085
115	Evolved	Long	Resistant	0.173
116	Evolved	Short	Resistant	0.628
117	Evolved	Short	Resistant	0.322
118	Evolved	Long	Resistant	0.012
119	Evolved	Long	Resistant	0.237
120	Evolved	Short	Resistant	0.262

N/D = No data

The significance threshold (α) used for the *t*-test for $OD_{750\ nm}$ in wells with active vs. inactive phage was $p = 0.05$.

Figures

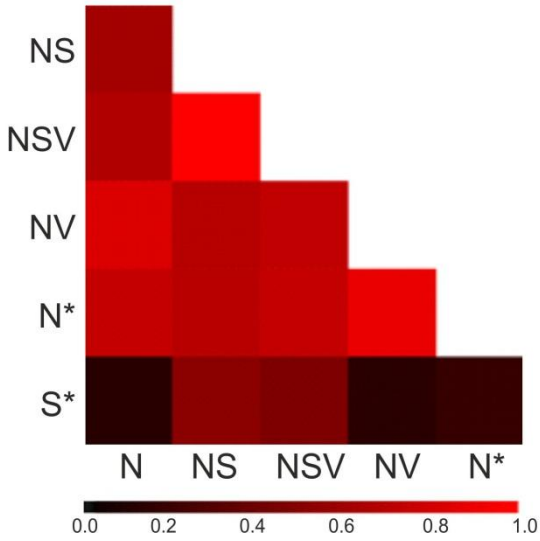

Figure S1. Similarity of associated bacterial communities in experimental culture conditions (N, *Nodularia* monoculture; NS, mixed culture; NSV, mixed culture with phage; NV, *Nodularia* monoculture with phage), initial *Nodularia* strain inoculum (N*) and initial *Synechococcus* strain inoculum (S*). Heatmap squares represent pairwise comparisons between bacterial communities using the Jaccard similarity index with a range from 0 (black) to 1 (red). The darker color in the comparison between S* and N, S* and NV, and S* and N*, suggest that the associated bacterial community differs significantly.

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