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PROSYNFEST2020

Celebrating the decades of research since the discovery of ***Prochlorococcus*** and ***Synechococcus***

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WELCOME

Discovered respectively about 30 and 40 years ago, *Prochlorococcus* and *Synechococcus* are becoming the most well-studied model marine microbes. What began as mere observations of small novel marine cyanobacteria now emerges as one the major advances of the last century in biological oceanography: these closely related microorganisms constitute a key genetically and physiologically diverse group of phytoplankton that represents a core engine of marine microbial communities and global biogeochemical cycles.

Building on the success of the first *Prochlorococcus*Fest (2008) that celebrated the 20th anniversary of the discovery of *Prochlorococcus*, this meeting will bring together both veterans and newbies alike to highlight past achievements, current research and future directions in the study of these important organisms. All areas and modes of inquiry are welcome. Participants working in diverse areas are welcome, such as people studying predators, biogeochemical influences, and other microbes that exchange metabolites with these significant primary producers.

ProSynFest 2020 will take place from 17 to 19 March 2022 at the Córdoba Conference and Convention Center (Córdoba, Spain). On March 16th, the day before the formal symposium, we will hold an informal, interactive, workshop geared toward sharing and discussing various approaches to working with these (sometimes recalcitrant!) model microorganisms.

We look forward to seeing old friends and making new ones in Córdoba in 2022.

VALUES

Inclusion and international cooperation

The organizers of ProSynFest2020 are working hard to create a stimulating meeting, which will bring together scientists from around the world to document the past, present and chart the future of marine cyanobacterial science. This meeting will coalesce a broad diversity of participants and we value and encourage their participation regardless of their race, religion, ethnicity, gender identity, sexual orientation, country of origin or nationality.

The site we have chosen for the meeting, Córdoba (Spain), has a rich and varied history and is recognized for its tolerance of diverse religions and convergence of cultures: Christians, Jews and Muslims coexisted peacefully in the Caliphate of Córdoba, in a time when Córdoba was the main center of science and culture in Europe.

This spirit will provide inspiration for the collaborative, respectful atmosphere we want to stimulate during ProSynFest2020. To help foster a safe, productive and welcoming environment we have crafted a statement on inclusion and international cooperation (see below) that we expect all participants to uphold to help achieve the colloquium's goals of advancing scientific discussion and promoting collaboration.

Statement on inclusion and international cooperation

The study of marine microbes crosses many disciplines and is an international enterprise that relies on scientists from countries around the world. ProSynFest2020 values the importance of inclusiveness and of the exchange of information and ideas beyond national boundaries in a safe, productive and welcoming environment for all participants.

Rights and privileges should not be based on gender identity, skin color, religion or country of origin. We know that diversity in thought and background is critical to good science. As values of equality and diversity are threatened worldwide, we commit ourselves to welcoming and including all attendees regardless of their race, religion, ethnicity, gender identity, sexual orientation, country of origin or nationality in an environment free of discrimination, harassment, intimidation and bullying.

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Contributors: *Debbie Lindell*

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11:30-13:00 **Vesicles and Lipids**
Contributors: *Steven Biller, María del Carmen Muñoz-Marín*

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14:00-15:30 **Genetics in Cyanobacteria**
Contributors: *David Kehoe, David Lea-Smith, Nikolai Radzinski, Giovanna Capovilla, Sean Kearney*

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Bonnie Hurwitz

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14:00-15:30 **Cyanorak v2.1, a scalable information system dedicated to the visualization and expert curation of genomes from the Cyanobacteria Cluster 5 radiation**

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MARCH 17, 2022

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12:25-12:40 **S1-short talk 5: How will ocean environmental change affect the resource limitation of marine microbes in the low latitude oceans?** *Alessandro Tagliabue*

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Laura Alonso Saez

17:35-17:50 **S1-short talk 10: *Prochlorococcus* strains employ different photosynthetic strategies under distinct conditions of oxygen concentration and light availability: mining the Ocean Protein Portal**
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David J. Scanlan

Session 2 (continued): Physiology, gene regulation and metabolism.
Chairs: P. Berube (morning) / A. Martiny (afternoon)

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Frédéric Partensky

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11:00-11:25 **S2-invited talk 12: Chromatic acclimation in *Synechococcus*: Mechanisms, regulation, and evolution**
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Claudia Steglich

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Douglas Campbell

12:45-13:00 **S2-short talk 13 (on-line): The evolution of siderophore use in *Prochlorococcus***
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13:00-14:00 Lunch in conference center

14:00-14:25 **S2-invited talk 15: Impacts of plastic leachate on marine cyanobacteria**
Lisa R. Moore

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Solange Duhamel

14:50-15:05 **S2-short talk 14 (on-line): Arsenic biotransformation by *Prochlorococcus*: comparative physiology, protein expression and production of methylarsenicals**
Gabrielle Rocap

16:00-17:00 Afternoon: Free time or commented visit of the Mosque

Evening: Dinner in town. Attendees on their own

MARCH 19, 2022

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Chairs: D. Kehoe

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Osvaldo Ulloa

09:25-9:50 **S2-invited talk 18: Emerging complexity of N cycling in *Prochlorococcus* populations.**
Paul M. Berube

9:50-10:15 **S2-invited talk 19: Identifying the role of transport in open ocean cyanobacterial symbionts by expression in model heterologous systems.**
Rachel Foster

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10:30-10:45 **S2-short talk 16: Stabilization of extensive fine-scale diversity by dispersal and ecologically driven spatio-temporal chaos.** *Daniel Fisher, Michael Pearce, Atish Agarwala*

10:45-11:05 Coffee break

Session 3: Predation and trophic interactions.
Chairs: E. Zinser (morning) / D. Lindell (afternoon) / R. Foster (after RT)

11:05-11:30 **S3-invited talk 20: Cyanobacteria-cyanophage interactions.**
Debbie Lindell

11:30-11:45 **S3-short talk 17: Temporal transcriptional patterns of cyanophage genes suggest synchronized infection of cyanobacteria in the oceans.** *Qinglu Zeng*

11:45-12:00 **S3-short talk 18: Extracellular vesicles secreted by *Synechococcus*: effect of stress in their production and content.** *María del Carmen Muñoz-Marín, Steven J. Biller, Jesús Díez, José Manuel García-Fernández*

12:00-12:15 **S3-short talk 20: Integrative elements abundant in marine vesicles shape *Prochlorococcus* genomic plasticity** *Thomas Hackl*

12:15-12:30 **S3-short talk 20: Biochemical characterization of cyanophage encoded auxiliary metabolic proteins give insights into their role during infection.** *Nicole Frankenberg-Dinkel*

12:30-12:45 **S3-short talk 21: Nutrient cycling in picocyanobacteria-heterotroph interactions.** *Joseph A. Christie-Oleza, Despoina Sousoni, James Kerr, Maria del Mar Aguilo-Ferretjans, David J. Scanlan*

12:45-13:00 **S3-short talk 22: Macroplanktonic pelagic tunicates prey on picocyanobacterial.** *Anne Thompson*

13:00-14:00 Lunch in conference center

14:00-14:25 **S3-invited talk 21: *Prochlorococcus* and *Synechococcus*: Friends, enemies, frenemies?**
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14:25-14:50 **S3-invited talk 22 (on-line): Cyanobacterial extracellular vesicles: origins, contents, and community interactions.**
Steven J. Biller

14:50-15:05 **S3-short talk 23: *Prochlorococcus* rely on mixotrophy and microbial interactions rather than on chlorotic resting stages to survive long-term stress** *Daniel Sher*

15:05-15:20 **S3-short talk 24 (on-line): *Proteomics of nitrogen incorporation in Synechococcus, Prochlorococcus and their phage.*** *Jacob Waldbauer*

15:05-15:20 S3-short talk 25 (on-line): Cyanovirocell infochemicals driving microbial interactions. *Sheri Floge*

15:35-16:00 Coffee break.

16:00-17:35 2nd Poster session (starting with 2-min flash talks by poster presenters Set #2) **+ 3 parallel roundtables:**

RT1: Phylogenomics and species/genus concept
Co-chairs: F. Partensky, W. Hess, P. Cabello-Yeves & V. Salazar

RT2: New flow technologies for analyzing picocyanos
Co-chairs: A. Thompson, F. Ribalet & K. Hunter-Cevera

RT3: Exploiting the wealth of metagenomics data
Co-chairs: D. Scanlan, L. Garczarek & A. Martiny

17:35-18:00 **S3-invited talk 23 (on-line): Experimental Evolution of a Simple *Prochlorococcus* Community**.
Jeffrey Morris

18:00-18:50 Keynote speech 3: Cyanobacteria in symbiosis: learning from new models.
Jonathan P. Zehr

18:50-19:20 Wrap up, general discussion and concluding remarks.
Z. Johnson, J.M. García-Fernández, F. Partensky & S.W.Chisholm

19:20 End of conference

Dinner in town. Attendees on their own

WORKSHOP

One-day Workshop

The workshop is designed to provide a solid understanding of the nuances of experimentation and data analysis on picocyanobacteria. Open to both novices and experts alike, it will be a forum for sharing established protocols and omics tools as well as those under development. All the sessions will be method-oriented and include videos of hands-on experiments and time for discussion with participants on technical questions.

Track 1 - Laboratory track: "Tools of the Trade: Picocyanobacteria Culture and Manipulation"

Laboratory topics will include: Strategies for culturing in large and small volumes, continuous and semi-continuous cultures, co-culture with heterotrophs, cryopreservation, isolating cells from natural seawater, agar plating; cyanophage methods such as plaque assays, or how to knock out genes in the cyanophage genome; progress in the development of genetic tools for both *Prochlorococcus* and *Synechococcus*, the lessons to be learned and discussion of paths forward, with recent examples of applications from their 'model' sister, *Synechocystis*; the challenges of working with picocyanobacteria lipids and vesicles. **The aim is to disseminate expertise in working with these fascinating (but finicky) microbes**! As a bonus, participants will also have an opportunity to select from a large diversity of characterized cultured strains across the diversity of the genus which will be mailed to them after the conference.

Track 2 - Computational Track: "Databases and Omics Tools for studying Picocyanobacteria Biology and Ecology"

In parallel, a separate track will feature the most relevant bioinformatics databases and tools available to handle the ever-growing amount of 'omics' data and metadata available on these microbes – presented by their developers: the IMG database, the new home for Proportal; "Planet Microbe", a marine-specific portal for iMicrobe/muSCOPE; The BioCyc database collection gathering 14,735 pathway/genome databases, plus software tools for exploring them; "Cyanorak", a manually curated database of clusters of orthologs built from 97 picocyanobacteria genomes; and CMAP, a visualization platform for oceanic datasets.

Track 1: Laboratory Track

09:00-10:00 **Culturing picocyanobacteria**
Contributors: Allison Coe, Lisa Moore

Methods for culturing picocyanobacteria and their associated marine heterotrophs have been in continuous development over the past 4 decades. This workshop will highlight the most popular methods and detail the intricacies of culturing *Prochlorococcus*, *Synechococcus*, and associated marine heterotrophs. Topics will include isolation strategies, media recipe comparisons, plating techniques, rendering cultures axenic, growing in large and small volumes, cryopreserving cells, and flow cytometry techniques

10:00-11:00 **Marine Cyanophages**
Contributors: Debbie Lindell

Marine cyanophages have been the subject of research for almost three decades. In this session we will discuss a variety of traditional and newly developed methods for their

isolation, enumeration and characterization. These will include the plaque assay and polonies, determining taxonomy and host-range, measuring cyanophage growth and infection properties, including the latent period and burst size. We will also touch on experimental considerations for assessing impacts of cyanophages on their cyanobacterial hosts and present a new method for the genetic engineering of cyanophages.

For the genetic system, growth curves and infection properties see poster **S2P52;** For enumeration by polonies see posters **S3P65**, **S1P28** and **S1P32**.

11:30-13:00 **Vesicles and Lipids**
Contributors: Steven Biller, María del Carmen Muñoz-Marín

Extracellular vesicles – ~50-200nm membrane-bound particles released by *Prochlorococcus*, *Synechococcus*, and most other marine microbes – are emerging as a fascinating aspect of cyanobacterial biology. In this session, we will discuss current methodologies for studying these tiny structures in both cultures and in the field. Subjects to be discussed include cyanobacterial lipid purification, vesicle isolation and enumeration, distinguishing between different classes of small particles in aquatic systems, vesicle labeling and tracking, and considerations for -omics analysis. Advances in this area will require overcoming a number of methodological challenges, and we hope that participants will help us brainstorm new ideas and approaches.

14:00-15:30 **Genetics in Cyanobacteria**
Contributors: David Kehoe, David Lea-Smith, Nikolai Radzinski, Giovanna Capovilla, Sean Kearney

Recent decades of study of picocyanobacteria has greatly expanded our understanding of these organisms and their role in global ecosystems. However, such work has been hampered by limitations on our capacity to perform genetics on *Prochlorococcus* and other picocyanobacteria. This includes a general inability to modify the genome of *Prochlorococcus*, as well as difficulties particular to known techniques developed for other picocyanobacteria. Here we will discuss several recent advancements in the development of techniques for genetic modification of picocyanobacteria such as *Prochlorococcus* and *Synechococcus*, covering such topics as electroporation, transformation using suicide plasmids and autonomously replicating plasmids, CRISPR-Cas12a and CRISPR-Cpf1, transposon mutagenesis, the use of reporter genes, efficient purification of transformants, and natural competence. Additionally, this workshop will provide detailed analysis into the latest techniques involved in engineering model cyanobacterial species using Golden Gate-based CyanoGate plasmids and how these techniques are being used to develop a *Synechocystis* sp. PCC 6803 mutant library.

Track 2: Computational Track

09:00-11:00 **Comparative Genomics for Gene Discovery using Integrated Microbial Genomes (IMG).** Contributor: Rekha Seshadri

The Integrated Microbial Genomes & Microbiomes (IMG/M) system helps bridge that gap by serving as a resource for gene discovery and comparative analysis of genomic and metagenomic datasets (for more details: see abstract of poster **S0P05**).

11:30-13:00 **Planet Microbe: a cyberinfrastructure for integrating oceanographic omics, environmental and physiochemical data layers** Contributor: Bonnie Hurwitz

Planet Microbe, a federated resource to enable data discovery and open data sharing for historical and on-going oceanographic sequencing efforts. In this project, several historical oceanographic 'omics datasets (Hawaii Ocean Time-series (HOT), Bermuda Atlantic Time-series (BATS), Global Ocean Sampling Expedition (GOS), C-DEBI) are integrated into Planet Microbe and reconnected to their physiochemical measurements. New oceanic large-scale datasets such as the Tara Ocean Expedition and Ocean Sampling Day (OSD) are also integrated into the platform (for more details: see abstract of poster **S0P03**).

14:00-15:30 **Cyanorak v2.1: a scalable information system dedicated to the visualization and expert curation of genomes from the Cyanobacteria Cluster 5 radiation** Contributor: Frédéric Partensky

Cyanorak v2.1 (http://abims.sb-roscoff.fr/cyanorak) is a scalable information system dedicated to the expert curation of clusters of likely orthologs (CLOGs) built from 97 genomes of *Prochlorococcus*, marine *Synechococcus* and *Cyanobium*. This genome set covers most of the wide genetic and pigment diversity known so far in Cyanobacteria Cluster 5 (*sensu* Herdman et al. 2001; Bergey's manual). All ca. 26,000 CLOGs of the database have been automatically assigned rich functional annotations, which were then manually refined for more than 1,700 of them. Cyanorak also includes several useful plugins including BLAST search tools, comparative genome context, visualisation of individual genomes as well as various export options. This demo will present both the public and private parts of the Cyanorak v2.1 information system and a glimpse of the next release (Cyanorak v3) that encompasses a selection of >150 new non-redundant whole genome sequences (WGS), MAGs and SAGs and includes new features such as the orthologs in model heterotrophic bacteria and cyanobacteria outside Cluster 5.

16:00-17:30 **A Curated *Synechococcus* elongatus Pathway/Genome Database at BioCyc.org** Contributor: Peter Karp

BioCyc.org is an extensive web portal for microbial genomes and metabolic pathways. BioCyc contains 14,700 microbial genomes including 66 *Synechococcus* genomes, 8 *Synechocystis* genomes, and 18 *Prochlorococcus* genomes (for more details: see abstract of poster **S0P04**).

17:30-18:30 **Simons CMAP: an interconnected oceanic data portal** Contributor: Ginger Armbrust & Mohammad Dehghani Ashkezari

Simons Collaborative Marine Atlas Project (Simons CMAP) is an open-source data portal interconnecting data sets across Oceanography disciplines. It enables scientists and the public to dive into the vast and often underutilized ocean datasets to retrieve custom subsets of data, create data visualizations and run analyses. CMAP database is hosting global multi-decade remote sensing (e.g. satellite temperature, chlorophyll), several decades of global biogeochemical models (e.g. MIT Darwin, Mercator-Pisces), and decades of field measurements (e.g. ~100 cruise expeditions, Argo floats, Hawaii Ocean Time series; for more details, see poster **S0P05**).

KEYNOTE ADDRESS

Keynote speech 1

Prochlorococcus: Then and now

Sallie W. (Penny) Chisholm
Massachusetts Institute of Technology, Cambridge MA USA

The discovery of marine *Synechococcus* in 1979 changed our view of the drivers of primary production in the sea. In addition to the very dynamic and patchily distributed eukaryotic phytoplankton, there was now a ubiquitous lawn of tiny and extremely abundant cyanobacteria underpinning the marine food web. That seminal breakthrough led to the discovery of *Prochlorococcus* which was even smaller and more numerous, further establishing picocyanobacteria as major components of ocean ecosystems.

The story of the discovery of *Prochlorococcus* illuminates the role of serendipity in science and the power of teamwork. Over the years since then, this tiny cell has taught us a lot about the oceans and its microbial inhabitants. We have learned, for example, that not all *Prochlorococcus* can utilize nitrate challenging textbook renderings of the drivers of ocean biogeochemistry. Further, *Prochlorococcus* led us to the discovery that extracellular vesicles are shed by a plethora of marine bacteria, revealing a new feature of marine ecosystems. And we have learned that the *Prochlorococcus* collective embodies a vast open pangenome, which expands with every new cell sequenced. The processes governing the distribution of the pangenome among different lineages, and different habitats, are the subject of intensive research. Studies of *Prochlorococcus*' survival under extended darkness, interactions with heterotrophs, and their production of novel secondary metabolites, have presented us with a rich array of research paths to follow. Our approach has always been to let the cells tell us what is important to them and where to look next. They have never let us down.

Keynote speech 2

Four decades of research on marine *Synechococcus*: a globally important phototroph

David J. Scanlan
School of Life Sciences University of Warwick

Since their discovery in the late 1970s marine cyanobacterial picoplankton of the genus *Synechococcus* have become known as key primary producers of global importance. Their characteristic orange fluorescence, which underlies possession of a complex phycobilisome structure that acts as the major light-harvesting antennae of all marine *Synechococcus* strains, facilitated rapid assessment of their numerical abundance in oceanic waters via flow cytometry. As a result, we now know these organisms to be one of the most abundant phototrophs on Earth. Moreover, with a ubiquitous distribution across waters ranging in temperature from 2-30°C and relatively high CO_2 fixation rates means they are responsible for around 20% of global net production and hence a key player in the marine carbon cycle. Over the last 40 years tremendous progress has been made understanding the physiological, genetic and genomic diversity of these organisms, particularly in the context of how these organisms populate the world's oceans. More challenging has been obtaining a good understanding of the factors controlling the productivity and abundance of these organisms, particularly the role of biotic (grazing, viral lysis) versus abiotic variables, and how these organisms interact with the plethora of microbes with which they co-exist. This talk seeks to give a broad overview on all these points, highlighting both the impressive advances that have been made but also attempting to emphasize those aspects of *Synechococcus* biology that remain to be elucidated or at least need further work.

Keynote speech 3

Cyanobacteria in symbiosis: learning from new models

Jonathan P. Zehr
University of California-Santa Cruz, USA

The diversification of cyanobacteria was important in shaping Earth's biology and biogeochemistry. *Prochlorococcus* and *Synechococcus*, among the most important photosynthetic microbes on Earth, were some of the first marine microbes to be cultivated. *Prochlorococcus* and *Synechococcus* strains exhibit a spectrum of evolutionary and adaptational strategies, from dramatically streamlined genomes (*Prochlorococcus*) to great metabolic flexibility (*Synechococcus*). But much is yet to be learned about the diverse cyanobacteria that are so important in Earths diverse habitats.

The nitrogen-fixing unicellular cyanobacterial symbiont UCYN-A (Candidatus Atelocyanobacterium thalassa), initially discovered in oligotrophic waters, has since been discovered to be widely distributed and active in nitrogen fixation in unexpected environments, such as coastal and high latitude waters. While challenging to study, new technologies have made it possible to research microorganisms such as UCYN-A as model systems, even prior to successful cultivation. Although appearing to be metabolically crippled by genome reduction resulting in the loss of key metabolic processes such as oxygenic photosynthesis, the TCA cycle, and components of the circadian clock, UCYN-A has elaborately co-evolved with its haptophyte partner, a single-celled alga, for ~100 million years. UCYN-A and its haptophyte host form a number of genetically distinct partnerships that range in size over an order of magnitude with a striking linear relationship of relative sizes of symbiont and host, presumably driven by metabolic dependencies and interactions. The haptophyte partner, related to Braarudosphaera bigelowii, has at least two different life cycle stages, one a calcified form and the other a typical flagellate. The symbiotic partnership is thus presented with challenges not only for cell division, but also for maintaining symbiosis through changes in cell morphology associated with these life cycles. Initially believed to be based on the simple exchange of fixed nitrogen for fixed carbon, genetic and genomic characterization of UCYN-A and its host show that even such simple systems may be based on much more complex interactions.

UCYN-A appears to be at the juncture of endosymbiosis and organellar evolution and has experienced different evolutionary selection pressures requiring different adaptations from the free-living unicellular cyanobacteria and thus presents a different model of cyanobacterial evolution and metabolism. UCYN-A is only one representative of the great diversity of important cyanobacteria in the environment and is at one end of the spectrum of interactions-much has yet to be learned from new cyanobacterial models, both uncultivated and cultivated.

INVITED TALKS

Session 1: Ecology, distribution and dynamics: past, present and future

S1-invited talk 1
A short story of the diversity and activity of cyanobacteria in the global ocean
Zackary Johnson *Duke University, USA*

Microbial communities are structured along marine environmental gradients with niche partitioning at the finest scales of diversity. However, relatively little is known about the relative activity of these microdiverse populations in situ. Here, we examine *Prochlorococcus*, a genetically diverse and biogeochemically important marine primary producer that partitions the global ocean. Using the 23S rRNA:rDNA ratio as a proxy for specific activity of in situ populations as well as other approaches, we assess *Prochlorococcus* communities across environmental gradients in the surface North Pacific Ocean to (1) determine the coupling between activity and abundance across taxonomic ranks and (2) examine differences in the specific activity among closely related taxa. We show that activity (rRNA) and abundance (rDNA) are highly correlated for 97% similar *Prochlorococcus* OTUs across all sites, suggesting a low proportion of inactive or dormant *Prochlorococcus* in the surface ocean. However, decomposition of these OTUs into finer resolution oligotypes (>99% similarity) reveals that closely-related sequence variants within an OTU exhibit uncoupling between activity and abundance likely due to density-dependent processes. Thus, highly correlated activity and abundance at the 97% OTU level may mask the distinct processes, such as differential predation, shaping microbial abundances at finer phylogenetic scales for this globally important organism.

S1-invited talk 2
Diversity and adaptation of *Synechococcus* in estuarine waters
Hongbin Liu[1], Xiaomin Xia[2]
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Synechococcus is one of the most widely distributed and abundant picocyanobacterial primary producers in the global oceans. Unlike its close relative *Prochlorococcus*, which only occur in warm oceanic waters, *Synechococcus* can be found in every corners of the ocean, from equator to polar waters and from estuaries to oceanic gyres. Marine Synechococcus are classified into three major types (type 1, 2, and 3) according to the composition of phycobilisomes, and more than 20 phylogenetic clades based on various gene markers. Moreover, due to different growth requirements, *Synechococcus* lineages can serve as indicators of different marine ecological niches. One area that has been long overlooked is the subtropical estuarine waters. In the past decade, we have investigated the distribution, diversity and special adaptation of the *Synechococcus* in the Pearl River Estuary and Hong Kong surrounding waters, and discovered unexpected high diversity and strong spatial and seasonal variabilities. In particular, we isolated a number of euryhaline, PC rich *Synechococcus* strains, as well as clade VIII and IX PE containing *Synechococcus* from estuarine and coastal waters. Genomic analysis of these strains revealed various adaptation strategies to tolerate a broad range of salinity and live in highly polluted environments. For instance, euryhaline strains contain more genes encoding Na+/H+ antiporter that is important for adjusting cellular iron concentrations, an important

adaptation for growing in an environment with a large variation of salinity. One S5.2 isolate (HK05) grew faster at low salinity, possibly by reducing its biosynthesis of organic osmolyte activity and increased its photosynthetic activity, which allowed it to enhance its assimilation of inorganic carbon and nitrogen. We suggest that estuaries are hot spots of *Synechococcus* diversity and euryhaline strains have developed ability to deal with salinity fluctuation, high turbidity and pollutants in estuarine environments.

S1-invited talk 3

Comparative and ecological genomics of marine picocyanobacteria

Hugo Doré[1], Gregory K. Farrant[1], Uysse Guyet[1], Théophile Grébert[1], Morgane Ratin[1], Antoine Bisch[1], Loraine Brillet-Guéguen[3], Mark Hoebeke[2], Erwan Corre[2], David J. Scanlan[4], Frédéric Partensky[1], Laurence Garczarek[1]

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The marine picocyanobacteria *Synechococcus* and *Prochlorococcus* harbor a wide genetic and pigment diversity and thus constitute pertinent biological models to study the genomic bases of niche partitioning in the ocean. To tackle this question, we generated Clusters of Likely Orthologous Genes (CLOGs) using the numerous picocyanobacterial genomes available in public databases or recently generated by our group [1] and used the Cyanorak v2 information system [2] to manually refine their functional annotation. Extensive comparison of these genomes allowed us to i) re-evaluate the size of the picocyanobacterial core and pan-genome, ii) highlight the fairly low number of ecotype-specific genes fixed in the long term in both genera, and iii) assess the relative weights of gene gain/loss vs. genetic divergence in the adaptation of (sub)clades to environmental niches. Altogether, these analyses enlightened the evolutionary history of this radiation [1]. To validate these observations, to examine the distribution of specific functions and to identify new genes and/or metabolic pathways potentially involved in the adaptation to various environmental niches, these genomes were then used as references to recruit Tara Oceans meta-omic reads covering a large variety of oceanic biomes. For instance, recruitment of three genetic markers was used to establish the first global distribution map of all *Synechococcus* pigment types, showing that type IV chromatic acclimaters, which can dynamically modify their pigmentation to maximally absorb green or blue light, were the most abundant pigment type oceanwide [3]. Whole genome recruitment also revealed that each assemblage of picocyanobacterial ecotypes, as defined based on the high-resolution marker gene petB [4], possesses a distinct gene repertoire and correlation network analyses led us to identify the most representative individual genes of each ecological niche and/or taxonomic assemblage. Furthermore, integration of these data with knowledge on the gene synteny in reference genomes using a network approach was used to unveil clusters of adjacent genes in reference genomes (CAGs), which display a differential distribution in situ and thus are potentially involved in the same metabolic pathway. Altogether, these studies provide important insights into the complex interactions between vertical phylogeny, pigmentation and environmental parameters that shaped the community structure and evolution of these organisms.

References:

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S1-invited talk 4

Faster growth of the major prokaryotic versus eukaryotic CO_2 fixers in the oligotrophic ocean

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Because maintenance of non-scalable cellular components, membranes and chromosomes, requires an increasing fraction of energy as cell size decreases, miniaturization comes at a considerable energetic cost for a phytoplanktonic cell. Consequently, if eukaryotes can use their superior, organelle-derived energetic resources to acquire inorganic nutrients with more or even similar efficiency compared with prokaryotes, larger unicellular eukaryotes should be able to achieve higher growth rates than smaller cyanobacteria. To test this hypothesis, we directly compare the intrinsic growth rates of phototrophic prokaryotes and eukaryotes using a flow sorting 14CO2-tracer approach. At the ocean basin scale, cyanobacteria double their biomass twice as frequently as the picoeukaryotes indicating that the prokaryotes are faster growing CO_2 fixers, better adapted to phototrophic living and nutrient acquisition in the oligotrophic open ocean, the most extensive biome on Earth.

S1-invited talk 5

Ecological controls of *Prochlorococcus* and *Synechococcus* global distributions now and in the future

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The geographical distributions of *Prochlorococcus* and *Synechococcus* are distinct with, in particular, in-situ measurements of *Prochlorococcus* cell abundance suggesting that its population size declines dramatically poleward of 40°N and 40°S. Often this decline is suggested to occur where sea surface temperatures drop below 13°C. However, recent data collected over 30 cruises in the subtropical gyres by continuous flow cytometry (SeaFlow), show that the decline in *Prochlorococcus* populations occurs between 13°C to 18°C depending on location and the season. Here we hypothesize an ecological mechanism for the decline: top down control by grazers (and/or viruses). Traditional ecological theory suggests the ubiquity of small phytoplankton, with larger size classes only co-existing in regions of higher productivity. In- situ observations support these theoretical predictions for most phytoplankton, but not for *Prochlorococcus* (and to a lesser extent, also not for *Synechococcus*.) We develop an additional theoretical framework to explore the decline of *Prochlorococcus* and test in a complex 3-D biogeochemical/ecosystem global computer model. We find that the increase in abundance of heterotrophic bacteria of similar size to *Prochlorococcus* can explain the decline of *Prochlorococcus* population. In the oligotrophic subtropical gyres there is a potentially mutualistic relationship between *Prochlorococcus* and bacteria, with *Prochlorococcus* supplying organic matter which the bacteria consume, and bacteria remineralizing the organic matter and supplying inorganic nutrients to *Prochlorococcus*. In

the transition from subtropical to subpolar waters with higher nutrient supply, higher productivity and larger size classes are increasingly supported. Higher productivity subsequently leads to greater supply of organic matter and thus increased bacterial abundance. Grazing pressure by species that prey on both *Prochlorococcus* and bacteria also increases until *Prochlorococcus* are no longer able to sustain its population growth and collapses, while bacteria in contrast are still sustained. In this transition region, increased bacteria populations negatively affect *Prochlorococcus* because of this shared grazing. We show that this mechanism re-creates the *Prochlorococcus* collapse in the global 3-D model, while also potentially explaining *Synechococcus* increases and subsequent decreases in these regions. Our study shows the importance of both the bottom-up (nutrient supply rate) and top-down (size or species specific grazing/losses) control in the geographical distribution of *Prochlorococcus* and *Synechococcus* populations.

S1-invited talk 6

Seasons of *Syn*: Insights into the annual patterns of a coastal *Synechococcus* population from in-situ flow cytometry

Kristen Hunter-Cevera[1], Michael Neubert[2], Robert Olson[2], Alexi Shalapyonok[2], Andrew Solow[2], Heidi Sosik[2]

1 Marine Biological Laboratory, 2 Woods Hole Oceanographic Institution

Understanding the dynamics of any phytoplankter requires observations at the space and timescales relevant to their ecologies and physiologies. For most phytoplankton, this is on the order of hours to days and poses a significant observational challenge. Development of automated flow cytometers and imaging flow cytometers have allowed for high resolution, species-specific measurements, and their long term deployments have yielded time series that enable the separation of short-term, seasonal and annual variation of cell dynamics. Flow cytometry not only provides information of cell counts, but also observations of cell size and fluorescence properties, which can provide additional insight into the physiological state of a population. In particular, diel patterns of cell volume coupled to a matrix population model that represents size transitions can provide accurate estimates of daily population division rates for picophytoplankton. Division rate is a critical metric for understanding abundance patterns as it allows separation of the contribution of cell growth to abundance changes, and relationships between division rate and environmental factors can identify variables that limit growth. At the Martha's Vineyard Coastal Observatory on the New England Shelf, hourly observations of the *Synechococcus* population have been obtained with the automated flow cytometer, FlowCytobot since 2003. We analyze a 16-year time series of cell concentration, cell properties, and division and loss rates, to understand the annual abundance cycle of *Synechococcus* at this location. We find that the drivers of growth vary over the annual cycle; cells are temperature limited in winter and spring, but light limited in fall. These differences in growth dynamics are reflected in seasonal changes of cell size and PE fluorescence. Loss processes strongly mediate features of *Synechococcus* abundance and also systematically vary with season. We present a working, testable framework for understanding *Synechococcus* dynamics in a temperate, coastal system that can be used to further explore the ecophysiology of this organism.

S1-invited talk 7

Understanding ocean biogeochemical cycles through the lens of *Prochlorococcus* and *Synechococcus*

Adam Martiny
University of California, Irvine

The evolution of *Prochlorococcus* and *Synechococcus* are characterized by extensive gene gain and loss leading to clear genome divergences within closely related clades. In the first part of my presentation I will review how the processes of gene gain and loss are important for adaptation to phosphorus, nitrogen, and iron limitations. Using different field experiments, we can then quantify how genomic shifts have direct implications for key ocean biogeochemical features such as nutrient assimilation or cellular C:N:P ratios. In the second part of my presentation, I will flip the approach by demonstrating how population genomics analyses of *Prochlorococcus* and *Synechococcus* can identify patterns of nutrient (co)limitation in poorly characterized ocean regions. Based on a global metagenomic survey, we find previously unknown regions of phosphorus, nitrogen, or iron limitation. This approach reveals how we can apply the mechanism of adaptation within *Prochlorococcus* or *Synechococcus* as a sensitive 'biosensor' of nutrient limitation across the global ocean.

S1-invited talk 8

Exploring the diverse functional attributes of marine cyanobacteria in natural populations

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Marine cyanobacteria modulate their proteomes in response to environmental cues in order to optimize their fitness. Metaproteomic analyses allows proteome composition of abundant marine cyanobacterial populations to be examined over broad oceanographic spatial and temporal dimensions. Although the picocyanobacteria with their minimal genomes are limited in their ability to sense and regulate their expression, large shifts in *Prochlorococcus* proteome composition are observed in the oceans. Transporters and enzymes related to for nitrogen, phosphorus, and iron acquisition or sparing vary geographically, vertically, and temporally, in patterns that are consistent with modeled nutrient limitation regimes. In many cases, the abundance (or lack thereof) of nutrient responsive proteins is surprising and contrary to expectations based on culture experiments and provides impetus for the study and modeling of use of alternate chemical species for nutrition. Correlations of RNA transcripts and proteins tends to be significant for these nutrient responsive proteins, and can be explained by use of a cellular model. Metaproteomic results also have the ability to discern to subspecies resolution, if peptides uniqueness is examined using tryptic databases such as Metatryp within the Ocean Protein Portal. This talk will describe some of the surprises and large scale observations from metaproteomic analyses of picocyanobacteria populations in the Atlantic and Pacific Oceans basins.

S1-invited talk 9

Data driven models of *Prochlorococcus* and *Synechococcus* abundance distributions

Ginger Armbrust[1], Mohammad Ashkezari[1], Francois Ribalet[1], Raphael Hagen[1], Annette Hynes[1], Michael Dnholz[1], Jarred Swalwell[1], Chris Berthiaume[1]
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Simons Collaborative Marine Atlas Project (Simons CMAP) is designed as a portal for the dynamic retrieval, integration and visualization of public data sets generated from research cruises, multiple decades of satellite products, global-scale numerical models, and compilations of physics and biogeochemistry data such as the World Ocean Atlas and Argo. I will illustrate how we have used Simons CMAP to co-localize ~125,000 independent observations of surface abundances of *Prochlorococcus* and *Synechococcus* with over 50 environmental variables derived from global models, biogeochemical climatologies and satellite products. The abundance data includes a compilation of global flow cytometry data, with a majority of observations from the surface North Pacific Ocean. We employed simple statistical metrics to identify those environmental variables highly correlated with *Prochlorococcus* and *Synechococcus* abundances and then used these variables to train machine-learning regressor models. I will demonstrate how we used these approaches to predict picocyanobacterial distributions throughout the North Pacific Ocean over the seasonal cycle. This approach can be readily expanded to the global scale with increasing availability of flow-cytometry-based abundance data from other oceans and is equally applicable to distributions of other organisms for which there is sufficient data.

Session 2: Physiology, gene regulation and metabolism

S2-invited talk 10
From glutamine synthetase to glucose uptake: 25 years working on N and C metabolism in marine picocyanobacteria.

José Manuel García-Fernández[1], Guadalupe Gómez-Baena[1], Antonio López-Lozano[1], María Agustina Domínguez-Martín[1], María del Carmen Muñoz-Marín[1], José Ángel Moreno-Cabezuelo[1], Yessica Melero-Rubio[1], Jesús Díez[1]
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We started working on glutamine synthetase in *Prochlorococcus* when little was known about nitrogen assimilation in this microorganism. This was the first of a series of pioneering studies on nitrogen metabolism, which were progressively widening the topics, to include glutamate dehydrogenase, isocitrate dehydrogenase and lack of nitrate reductase. We demonstrated the progressive loss of sensitivity of the transcriptional regulator NtcA to 2-oxoglutarate, observed in *Prochlorococcus* strains, which is a clear example, at the molecular level, of the streamlining of regulation mechanisms in this cyanobacterium. We carried out as well gene expression and quantitative proteomic studies. Our contributions have shown a series of differential features of the nitrogen and carbon metabolisms in marine cyanobacteria, which have simplified both their metabolic pathways and their regulation to adapt to the environment they inhabit. In the latest years, we have established a new research line focused on the effects of low nitrogen concentrations in marine *Synechococcus*. Our results indicate that *Synechococcus* is enabled to detect and take up nitrate at nanomolar concentrations. We hypothesize that

high affinity transporters might be also part of the physiological responses of marine picocyanobacteria to the scarcity of nutrients in the oligotrophic oceans.

Additionally, more than 10 years ago our team discovered that *Prochlorococcus* takes up glucose. Later, we demonstrated that the gene *glcH* encodes a biphasic, very high affinity glucose transporter and that natural *Prochlorococcus* populations take up glucose in the ocean. Comparative studies in *Prochlorococcus* and *Synechococcus* showed a high degree of diversity in the glucose uptake kinetics. Finally, studies of *glcH* expression in *Prochlorococcus* and *Synechococcus* cultures subjected to different glucose and light conditions indicate that the transcriptional control of this gene has been modulated during the diversification of these cyanobacterial genera. Glucose addition in the dark was early shown to induce a decrease in the expression of photosynthetic genes in *Prochlorococcus*, in good agreement with recent proteomic results from our team. Our studies have provided evidence to demonstrate that marine picocyanobacteria have a mixotrophic behaviour, being capable of using molecules devoid of essential elements (as glucose) when they are available in the environment.

S2-invited talk 11

Evolution and diversity of *Synechococcus* phycobilisomes

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The light-harvesting complexes of marine *Synechococcus*, called phycobilisomes (PBS), are composed of a conserved allophycocyanin core from which radiates six to eight rods with variable phycobiliprotein and chromophore content. Seven distinct pigment types or subtypes have been identified so far in this taxon, based on the phycobiliprotein composition and/or the proportion of the different chromophores in PBS rods. Most genes involved in their biosynthesis and regulation are located in a dedicated genomic region called the PBS rod region. Here, we examined the variability of gene sequences and organization of this genomic region in a large set of sequenced isolates and natural populations of *Synechococcus* representative of all known pigment (sub)types. All regions start with a tRNA-PheGAA and some possess mobile elements including tyrosine recombinases, suggesting that a tycheposon-like mechanism may have played a role in the evolution of phycobilisomes. Comparison of the phylogenies obtained for PBS and core genes revealed that the evolutionary history of PBS rod genes differs from the rest of the genome and is characterized by the co-existence of frequent allelic exchange. We propose a scenario for the evolution of the different pigment (sub)types and highlight the importance of lateral transfers in maintaining a wide diversity of pigment types in different *Synechococcus* lineages despite multiple speciation events.

S2-invited talk 12

Chromatic acclimation in *Synechococcus*: Mechanisms, regulation, and evolution

Bo Chen[1], Allissa Haney[1], Joseph Sanfilippo[2], Adam Nguyen[3], Théophile Grébert[5] , Laurence Garczarek[5], Frédéric Partensky[5], Wendy Schluchter[3], Jonathan Karty[6],David Kehoe[1]

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Marine *Synechococcus* thrives in various light niches in part due to its varied photosynthetic light harvesting pigments. Approximately 40 percent of *Synechococcus* cells worldwide use a process that is maximally responsive to blue and green light called Type 4 chromatic acclimation (CA4) to optimize the ratio of two chromophores, green-light absorbing phycoerythrobilin (PEB) and blue-light absorbing phycourobilin (PUB), within their light harvesting complexes or phycobilisomes. A mechanistic understanding of how *Synechococcus* cells tune their PEB to PUB ratio within the phycobilisomes during CA4 has not yet been obtained, nor has the regulation of this system been determined. We are using molecular tools, including CRISPR-based mutagenesis, biochemical approaches, comparative and functional genomics, and phylogenetics to determine the CA4 mechanism, its regulation by blue and green light, its evolution, and ecological role.

Our results show that of the three cysteine residues that shift their associated chromophore between PUB and PEB during CA4, one is explained by the blue light induction of a lyase-isomerase called MpeZ that attaches PEB and isomerizes it to PUB (1). The other two positions are not apparently controlled by the induction of any additional lyase-isomerase(s) and the mechanism controlling these changes has not been identified. We have discovered and will present new findings on a 107 amino acid protein with no sequence similarity to known lyase-isomerases whose expression is directly correlated with the addition of PUB at these two cysteines.

We are also examining how the CA4 response is regulated by blue and green light. In addition to identifying two CA4 master regulators, FciA and FciB (2), we have recently discovered and will discuss a third regulator called FciC that controls this process at least in part at the transcriptional level and is absolutely required for the CA4 response.

Finally, a second form of CA4 has been identified and we have conducted initial experiments to test the hypothesis that these two forms exist to convert two other pigment types of *Synechococcus*, PEB-rich, green-light specialists and PUB-rich, blue-light specialists, to the CA4 lifestyle. These findings will be provided.

1. Shukla A, et al. (2012) Phycoerythrin-specific bilin lyase-isomerase controls blue-green chromatic acclimation in marine *Synechococcus*. Proc Natl Acad Sci USA 109(49):20136-20141.

2. Sanfilippo JE, et al. (2016) Self-regulating genomic island encoding tandem regulators confers chromatic acclimation to marine *Synechococcus*. Proc Natl Acad Sci USA 113(21):6077-6082.

S2-invited talk 13

Gene regulation with non-coding RNAs in *Prochlorococcus*

Claudia Steglich[1]
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Prochlorococcus, the smallest and the most abundant photosynthetic organism on Earth, has a very streamlined genome. On average, these cells have about 2,000 genes and very few regulatory proteins. The limited capability of regulation is thought to be a result of selection imposed by a relatively stable environment in combination with a very small genome. Unlike its reduced suite of regulatory proteins, the number of small non-coding RNAs (sRNAs) relative to genome size in *Prochlorococcus* is comparable to that found in other bacteria, suggesting that RNA regulators likely play a major role in regulation in this group. sRNAs are found within all domains of life and can modulate the expression of target genes at the level of translation initiation by blocking or exposing ribosomal binding sites. In the same way sRNAs can influence the expression of target genes on post-transcriptional level by masking or de-masking ribonuclease cleavage sites. I will provide an overview of what is known about gene regulation through non-coding RNAs in *Prochlorococcus*.

S2-invited talk 14
Feeling Blue? Feeling Low? *Prochlorococcus*, light & oxygen
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In the upper ocean picophytoplankton move through large fluctuations in irradiance, under O2 saturation. In contrast, low, spectrally biased light prevails deeper in the photic zone, sometimes concurrently with low O_2. Photosystem II is subject to photoinactivation via multiple light-, spectral- and O_2-dependent mechanisms. Some picocyanobacteria counter photoinactivation using just in time repair, with high investments in protein metabolism. Others use a warehouse strategy with higher costs in protein investment. These strategies limit the light levels, which are tenable for different strains, and impose differing costs of growth. O_2 interacts with light to predict the prevalences of these strategies across strains and environments. We are now uncovering alternate picophytoplankton photophysiologies under low O_2 and low light.

S2-invited talk 15
HOW DOES LEACHATE FROM PLASTIC POLLUTION AFFECT MARINE PRIMARY PRODUCERS?
Lisa Moore, Indrani Sarker, Amaranta Focardi, Liam Elbourne, Ian Paulsen, Sasha Tetu
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Plastic pollution, a growing global threat to marine ecosystems, leaches a variety of substances into marine environments. However, little is known regarding how leachate affects marine microorganisms, particularly those at the base of the marine food web. An initial culture-based study of the effect of plastic leachates from HDPE plastic bags and PVC plastic matting on two *Prochlorococcus* strains, HLII ecotype MIT9312 and LLI ecotype NATL2A, showed negative impacts on growth and photophysiology in addition to genome-wide transcriptional changes (Tetu et al 2019; https://doi.org/10.1038/s42003-019-0410-x). The two strains also showed distinct differences in the extent and timing of

their responses to leachate, with MIT9312 being more sensitive than NATL2A. In order to further understand the effects of plastic leachate, we tested leachates from these same plastics that were weathered in estuarine waters for 17 and 112 days. These plastics continued to leach substances, including metals, causing significant impairment in *Prochlorococcus* growth, photophysiology and membrane integrity consistent with the previous study but to a lesser extent (Sarker et al 2020; doi: 10.3389/fmars.2020.571929). Zinc, a known component of many plastic additives, showed the highest degree of enrichment in both leachates even after weathering, and likely plays a major role in the toxicity. Culture-based and mesocosm experiments of the impacts of high zinc concentrations on marine primary producers showed declines in the growth rate and photophysiology, with a significant effect on natural populations of eukaryotic phototrophs. The culture-based studies showed differences in timing and degree of responses to elevated zinc levels, with *Prochlorococcus* strains showing declines in growth and photophysiology at significantly lower zinc levels than the *Synechococcus* strains tested (Sarker et al 2021; DOI 10.1099/mic.0.001064). The dose-dependent photophysiological toxicity curves for zinc reflected the response to plastic leachate. However, zinc is not the only culprit causing toxicity; PVC plastic leachate filtered through Sep Pak®Plus C18 cartridges to remove hydrophobic organics showed less toxicity for *Prochlorococcus*. Additional experiments of toxicity from organic components leached from plastics will also be presented.

S2-invited talk 16
Ecophysiology of marine picocyanobacteria, one cell at a time.
Solange Duhamel[1]
1 Lamont-Doherty Earth Observatory of Columbia University, 2 The University of Arizona

Because of their wide metabolic and genetic diversity, marine picocyanobacteria have a substantial role in mediating many of the global biogeochemical reactions that govern the flow of energy and material in the ocean. However, due to their minute size, overlapping with other prokaryotes (i.e. bacteria) and picoeukaryotes, in situ measurements of picocyanobacteria contribution to biogeochemical rates and processes remain a challenge. Recent applications of flow cytometry cell sorting combined with other techniques have greatly advanced our understanding of the role of *Prochlorococcus* and *Synechococcus* in ocean biogeochemistry and shade light on their nutritional plasticity in the wild. I will present recent results that take advantage of these approaches to tease apart the contribution of *Prochlorococcus* and *Synechococcus* to primary production, and to phosphorus and nitrogen cycling, and to improve our understanding of their role in the cycling of organic matter.

S2 (cont´d)-invited talk 17
Indigenous *Prochlorococcus* from oxygen-depleted oceanic waters
Osvaldo Ulloa
Instituto Milenio de Oceanografía

The ecology and evolution of *Prochlorococcus* are understood in terms of light, temperature and nutrients. However, uncultured *Prochlorococcus* lineages thrive also in the dimly sunlit layer of oxygen-depleted oceanic waters, but their genetic repertoires are unknown. We analyzed 130 single-amplified genomes (SAGs) of *Prochlorococcus* generated from water samples collected from the oxygen-depleted waters of the Pacific Ocean off northern Chile and off Mexico. We found that members of all the indigenous

uncultured phylogenetic lineages, including a novel one, present complementary genes or gene variants for several microaerobic or anaerobic metabolisms, particularly for pigment biosynthesis. Additional genetic features, including those for the photosynthetic apparatus and nutrient metabolisms, will be presented.

S2-invited talk 18

The emerging complexity of nitrogen cycling in *Prochlorococcus* populations

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Since the discovery of *Prochlorococcus*, our understanding of its role in the nitrogen cycle has continued to be shaped and reshaped as more is learned about the functional diversity encompassed by this group. In particular, over the years since our community gathered for the first Prochlorofest in 2008, research has revealed many new facets of *Prochlorococcus* ecology and evolution in the context of nitrogen acquisition. Since nitrogen is known to be the proximal limiting nutrient in much of *Prochlorococcus* habitat, studying how *Prochlorococcus* makes use of this scarce resource can provide valuable information on the assembly of *Prochlorococcus* populations and their functional role within the broader microbial community. The past decade has witnessed a rapid increase in the availability of genomics data, which has provided a lens through which to view the nitrogen assimilation features found within the global *Prochlorococcus* collective. Notably, the vast majority of nitrogen transporters and assimilation pathways are encoded by the accessory gene pool; i.e. they are not part of the universally shared core genome. While all *Prochlorococcus a*ppear to use ammonium as a nitrogen source, pathways that enable the assimilation of other nitrogen containing molecules - e.g. nitrite, nitrate, urea, cyanate, and amino acids - are only found in a subset of *Prochlorococcus* cells. The distribution of these functions across the *Prochlorococcus* collective has provided information on the forces that have shaped the evolution and assembly of *Prochlorococcus* populations in the wild. These advances have opened up new avenues of inquiry, particularly with regard to intra- and inter-species interactions as well as the emergent properties of *Prochlorococcus* populations.

S2-invited talk 19

Identifying the role of membrane transport in open ocean cyanobacterial symbionts by expression in model heterologous systems

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In the sunlit region of the world's oceans where dissolved nutrients are largely limited, several lineages of heterocyst-forming cyanobacteria form stable relationships with a few genera of eukaryotic cells, specifically diatoms. Diatoms are most renowned for their beautifully ornate siliceous cell walls (frustules), high abundances and major contribution to primary production. A remarkable character in these diatom-cyanobacteria symbioses is the degree of symbiont cellular integration, which is reflected in the symbiont genome size, content, and age of the partnership. In two diatom genera, the symbionts are endobionts, and therefore reside internal, either penetrating the cell membrane of the host

(true endobiont), or residing in the interstitial space between the frustule and the cell membrane of the host (partial endobiont). In the third partnership, the symbionts are external, and live attached to their respective hosts. These diatom symbioses are broadly distributed in the world's oceans, and contribute substantially to global N and C cycles due to high fixation and sinking rates. Yet, our understanding of the intimate nature between the partners remains largely unknown, including the very nature of metabolite transport between the partners.

Given the observed continuum of cellular integration in the various diatom symbioses, we expected to identify differences related to transporters in the symbiont genomes. Using a comparative genomics approach, mainly with the model heterocyst-forming cyanobacterium, *Anabaena* sp. PCC 7120, we identified the types and numbers of transporters (for C, N, P, Fe, S) in each of the symbiont strains. Transport also appeared directly related to the symbiont cellular location. Internal symbionts are more similar in the number, types and predicted affinity of their transporters, while the external symbiont possesses a higher number and wider array of transporters that favor life in a dilute nutrient environment. Additionally, we found evidence for a higher dependence on the host for reduced substrates by the internal symbionts. Recently, we have begun to test gene functionality for several identified homologues by heterologous expression in available cyanobacterial mutants. After cell complementation, the gain of function is tested by growth tests, tracer assays, including quantification and visualization of transport for reduced substrates by secondary ion mass spectrometry. Results from our recent work have begun to identify several intriguing aspects for these planktonic symbioses while also allowing a circumvention to the (currently) uncultivable nature of the diatom symbioses.

Session 3: Predation and trophic interaction

S3-invited talk 20
Coexistence and Resistance among Cyanobacteria and their Phages in the Oceans

Debbie Lindell[1], Michael Carlson[1], Sophia Zborowsky[1], Noor Mruwat[1], Francois Ribalet[2], Nava Baran[1], Sveta Goldin[1], Sara Ferron[3], David Karl[3], Angelicque White[3], Joshua Weitz[4], E. Virginia Armbrust[2]
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Viruses are globally abundant and highly diverse. They influence the abundance, diversity and evolution of their hosts as well as biogeochemical cycles in the oceans. However, the lack of methods to measure virus populations and the extent of viral infection at taxonomically meaningful levels have precluded a quantitative understanding of their impact on these processes. Towards this end, we have developed the polony and iPolony methods, single molecule solid-phase PCR techniques, to quantify discrete cyanophage families and the extent to which they infect the unicellular marine cyanobacteria, *Synechococcus* and *Prochlorococcus*. Using these methods, we found dramatic differences in cyanophage distribution and infection patterns across environmental gradients in the North Pacific Ocean. A hotspot of cyanophage infection was observed in the transition zone between the subtropical and subpolar gyres, that at times, was high enough to limit the geographic range of *Prochlorococcus*. However, in the vast North Pacific Subtropical Gyre cyanophage infection was low despite high cyanophage abundances, raising the possibility that resistance mitigates infection and allows for coexistence. Mechanisms of resistance differ against specialist and generalist cyanophages, with resistance being primarily at the stage of adsorption against specialist cyanophages, yet intracellular against generalist cyanophages. Our results suggest that novel mechanisms of intracellular resistance are present in marine picocyanobacteria, one of which is the adaptive loss of cellular components essential for phage reproduction. Combined our findings highlight how changing environmental conditions and coevolutionary processes affect the ecology of cyanophages and their cyanobacterial hosts.

S3-invited talk 21
Prochlorococcus and *Synechococcus*: Friends, enemies, frenemies?

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Prochlorococcus and *Synechococcus* coexist in the euphotic zone of the ocean. As members of the marine phytoplankton community they have overlapping niches; however, the extent to which these genera interact is largely unknown. We have begun to explore the nature of their co-existence, with the goal of uncovering positive and negative interactions that contribute to phytoplankton community assembly in the oligotrophic ocean. We will present our findings where co-culture outcomes are influenced by resource (N) limitation, the presence of toxins including hydrogen peroxide, and the co-occurrence of heterotrophic bacteria. Our results will present an argument that interactions between microbes within the same trophic group are not as black or white as may have been assumed.

S3-invited talk 22
Cyanobacterial extracellular vesicles: origins, contents, and community interactions
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Interspecies interactions play important roles in shaping the physiology, ecology, and evolution of *Prochlorococcus* and *Synechococcus*. Our understanding of the mechanisms mediating these interactions has been expanded in recent years by the discovery that picocyanobacteria and most, if not all, marine microbes release small (50-100 nm diameter), membrane bound structures known as extracellular vesicles into the surrounding ecosystem. Vesicles are abundant in both coastal and open-ocean waters, where they can transport a compounds including lipids, nucleic acids, proteins, and small molecules between cells. The diversity of vesicle contents suggests that these structures could play a variety of roles for cyanobacteria, including mediating horizontal gene transfer, contributing to food web interactions, modulating host-phage dynamics, and providing a platform for exoenzyme activity.

I will provide an overview of our current understanding of vesicle biogenesis and discuss recent findings revealing the remarkable diversity of biomolecules that *Prochlorococcus* exports within vesicles. I will also present data describing advances in determining the degree to which *Prochlorococcus* vesicles can interact both with different *Prochlorococcus* strains as well as other groups of marine microbes. Preliminary data further highlight the potential for vesicles to mediate trophic interactions, ranging from nutrient exchange to delivering cyanobacterial proteins onto heterotrophic cells. These studies reveal that some of the material released by *Prochlorococcus* into the ecosystem is found in the form of locally structured, discrete packets of nutrients and bioactive compounds which may be differentially accessible to different groups of cells. Our knowledge of extracellular vesicle biology is still in its relative infancy, and new findings continue to raise more questions than they answer. However, it is becoming increasingly apparent that these structures could mediate a vast and complex network of export, transport and exchange of compounds within marine microbial communities. Many challenges await us in exploring this 'new' component of marine ecosystems, but future research into vesicles has the potential to transform our knowledge of the nature and consequences of cellular exchange between *Prochlorococcus*, *Synechococcus*, and the marine ecosystem.

S3-invited talk 23
Experimental Evolution of a Simple *Prochlorococcus* Community
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Despite the fact that researchers have often used them in studies, axenic phytoplankton cultures offer poor representations of the behavior of these organisms in nature. Heterotrophic bacteria are more than passive consumers of photosynthate and competitors over limiting resources; in many cases they engage in complex commensalistic and mutualistic exchanges both with each other and with phytoplankton. This phenomenon is

clearly visible in algal cultures that have been maintained in nonaxenic conditions for long periods, where dozens of different heterotrophic taxa stably coexist with a single phototroph, presumably participating in a simple, stable ecosystem. Cultures of *Prochlorococcus*, in particular, are exceptionally dependent on contributions from heterotrophic reactive oxygen scavengers for stable growth and tolerance of transient stress. Despite the importance of algal:heterotroph interactions, little is known about the specificity of these interactions or their
evolutionary stability. Here we present results from a 500-generation evolution experiment where cocultures of *Prochlorococcus* MIT9312 and *Alteromonas* EZ55 were adapted to projected year 2100 pCO_2 conditions. The ancestral strains exhibited positive interactions at modern pCO2, but appeared to switch to antagonism under elevated pCO2, resulting in reduced Prochlorococcus production and culture instability. However, after 500 generations at elevated pCO_2, these negative effects no longer obtain and *Prochlorococcus* growth is not significantly affected by pCO2 within the range expected to exist between 2019 and 2100. Evolutionary repair of the helper interaction between *Prochlorococcus* and *Alteromonas* appears to have occurred through different genetic mechanisms in replicate lineages, and we consider evidence that these adaptations may not be exchangeable between communities, suggesting a kind of interspecies epistasis influencing the evolution of these simple communities.

SHORT TALKS

Session 1: Ecology, distribution and dynamics: past, present and future

S1-short talk 1

Novel constraints improve divergence time estimates for picocyanobacteria, suggesting a history marked by mass extinctions and recoveries

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The evolutionary history of marine cyanobacteria has been shaped by environmental changes across planetary history. In the oceans today, the dominant marine phototrophs are picocyanobacteria; however, these groups represent a relatively recent evolutionary diversification with respect to the ancient origin and history of Cyanobacteria. Several studies have attempted to estimate the divergence and diversification times for groups within Cyanobacteria, using molecular clocks calibrated by the cyanobacterial fossil record and other constraints. However, picocyanobacteria have not left any known diagnostic microfossils or clear lipid biomarker record, and so age estimates for these groups have remained poorly constrained, and highly sensitive to choices of evolutionary models. Most previous studies estimate the last common ancestor of marine *Synechococcus* and *Prochlorococcus* groups to have existed in the late Neoproterozoic or early Phanaerozoic; however, the large uncertainties in these age estimates prevent attributing their evolution and diversification to environmental or ecological events or processes.

Here, we show that phylogenetic analysis of families of chitin-degrading enzymes within cyanobacteria provide a novel older-bound age constraint on the marine SynPro clade, greatly improving the precision of age estimates within this group. Specifically, we show that the common ancestor of marine SynPro acquired a gene encoding a chitinase enzyme in their common ancestor, which was subsequently vertically inherited in descendant lineages. Pelagic marine chitin is almost entirely produced by molting arthropods, which first appear and reach abundance in the fossil record in the Cambrian. This constrains the last common ancestor of marine SynPro as diversifying after the early Cambrian. Including this older-bound constraint on molecular clocks using conserved proteins and RNA effectively doubles the precision of divergence time estimates for lineages within SynPro clade, and surmounts the challenges posed by the lack of a fossil record for these groups. The resulting divergence time estimates are consistent with 'modern' marine SynPro evolving from non-marine ancestors after the Neoproterozoic glaciations, suggesting these events may have caused a mass extinction or evolutionary bottleneck of pelagic cyanobacteria. Furthermore, the recent radiation of high-light *Prochlorococcus* groups is dated conspicuously close to the Cretaceous-Paleogene mass extinction event. The impact causing this event is predicted to have been sterilizing for the high-light marine photic zone on a global scale, consistent with a scenario in which extant high-light *Prochlorococcus* groups became established as part of the recovery following the event.

S1-short talk 2

Thermal acclimation and nutrient availability control *Prochlorococcus* growth in surface waters

Francois Ribalet[1], Stephanie Dutkiewicz[2], Kristof Glauninger[1], Christopher Follett[2], Mick Follows[2], E Virginia Armbrust[1]
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Future oceans will have warmer sea surface temperatures and, in many locations, lower macro-nutrient supplies. How will the two potentially competing processes impact *Prochlorococcus* growth rates? Here we applied a Bayesian size-structured matrix population model using time series flow cytometry data to estimate growth rate in surface waters during 40 cruises in the North Pacific. We showed that *Prochlorococcus* growth rates increased from the subpolar into the subtropical gyre, leading to a positive linear relationship with sea surface temperature from 13 to 22 degrees. Above 22 degrees, the growth rates exhibit high variability and there is no relationship with temperature. Using a combination of numerical model and resource competition theory, we showed that the linear relationship results from the anti-correlation between temperature and nutrient concentrations found in surface waters of the open ocean such that growth is limited by temperature rather than nutrient availability. At the higher temperatures, where the growth rate is no longer correlated with temperature, low and variable nutrient availability can result in sudden and dramatic decreases in growth rates. Understanding this play-off between nutrient and temperature limitation will be crucial to predicting *Prochlorococcus* response to a future warmer, more oligotrophic ocean. We suggest that the combination of both warmer temperatures and lower nutrient supplies will create wider fluctuations in *Prochlorococcus* growth rates and lead to more stochastic population dynamics.

S1-short talk 3

***Synechococcus* tolerance to environmental stressors in the warm Red Sea**

Susana Agusti[1], Alexandra Coello-Camba[1], Sebastian Overmans[1], Sreejith Kottuparambil[1]
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The Red Sea is one of the warmest seas on earth where seawater temperatures range from 22°C to 32°C, but maxima of 35.7°C was recorded for coastal waters. In the context of global ocean warming, the Red Sea is a model to study the functioning and adaptation of organisms to a future, warmer, ocean. The Red Sea waters are also highly transparent and oligotrophic where picocyanobacteria represent a dominant component of the pelagic primary production, and where *Synechococcus* is ubiquitous across the nutrients and temperature gradients of the Red Sea. The Red Sea is receiving substantial inputs of oil pollution, from natural and anthropogenic sources, and is also experiencing warming at a rate faster than the global ocean. It is expected that organisms are adapted to their environment and microorganisms, because of their fast growth and large population size, have a large adaptation capacity. However, multiple environmental stresses, and a fast changing environment may force organisms to their limits. In a series of studies we are testing the tolerance and limits of Red Sea *Synechococcus* populations to environmental stresses. We are testing growth thermal performance, responses to nutrients inputs, and the tolerance to UVB radiation and pollutants on Red Sea *Synechococcus*. Nutrients, UVB radiation and pollutants tolerance was tested on natural populations, generally at different locations across the Red Sea, although the thermal capacity was tested at the laboratory in cultures of *Synechococcus* strains isolated from the Red Sea (Roscoff culture collection) and corresponding to four different clades including clade IIA, the dominant clade in Red Sea waters. Our results indicated that *Synechococcus* is well adapted to the environmental extremes of the Red Sea. Red Sea *Synechococcus* tolerates higher levels of toxic hydrocarbons than those reported for populations growing in other oligotrophic regions; also tolerates extreme levels of UVB radiation. The thermal response of the dominant clade IIA strain suggests it is a generalist that will be able to experience maximum growth rates

at a broad range of temperatures. The join results indicated that Red Sea *Synechococcus* is well adapted to the environmental conditions suggesting will be a winner tolerating future changes, within the limits, however, imposed by competition with other photosynthetic plankton for nutrients and light.

S1-short talk 4

When are Niche Models Predictive?: Reconciling Opposing Predictions for *Prochlorococcus* in a Warming Ocean

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Statistical niche models are increasingly used to predict how species like *Prochlorococcus* and *Synechococcus* will shift on a warming planet. However, determining their predictive power can be difficult. Here, we argue that these models are more likely predictive if they, or their dependent variables, can independently explain population fluctuations across multiple, distinct, spatial-temporal scales. We apply this idea to a well-established niche model for *Prochlorococcus*, exploring whether the dependent variables in this model (temperature and light) correlate with either temporal fluctuations from the Hawaii Ocean Timeseries dataset or the spatial-temporal location of sharp changes in the species' abundance from global transect data. We find that both temporal fluctuations in surface waters at station ALOHA and the location of sharp spatial transitions correlate weakly with temperature and light. However, all spatial transitions occur at temperatures greater than the laboratory observed viability threshold for the species. A two-state model based on this observation explains the majority of the variance contained in the original, but by definition has no predictive capability on abundance changes within the species range. This result reconciles recent work demonstrating that niche and global computational models for *Prochlorococcus* predict opposing trends as the ocean warms. Testing the skill of niche models as a function of spatial-temporal scale is a powerful mechanism to determine under what conditions their predictions are accurate, and will lead to better predictions in a present and warming sea.

S1-short talk 5

How will ocean environmental change affect the resource limitation of marine microbes in the low latitude oceans?

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In the sunlit low latitude oceans where *Prochlorococcus* and *Synechococcus* thrive, resource availability exerts a major control on their rates of growth and biomass accumulation. In the Pacific Ocean low latitudes, the micronutrient iron, which is required for photosynthesis, respiration and acquisition of other resources, is often scarce due to limited external input. Understanding the impacts of a changing ocean, with altered availability of iron and other resources, on P*rochlorococcus* and *Synechococcus*, requires global ocean models that integrate impacts on biological and biogeochemical processes. However, such models tend to focus solely on the external availability of resources to quantify their impact on microbial growth and biomass accumulation, which ignores feedbacks associated with changes to cellular physiology and biochemistry. Here we present a new framework for evaluating the impact of changing resource levels on marine microbes that deals explicitly with resource acquisition, including luxury uptake and storage

of iron, and the individual cellular physiological costs in a climate change context. We embedded this framework within a state of the art global ocean circulation and biogeochemistry model, which considers the external supply and internal cycling of multiple resources (including iron, nitrogen, phosphorus, cobalt and zinc), and phytoplankton, zooplankton and particulate matter dynamics. By conducting simulations over the period 1801 – 2100 under the RCP8.5 scenario, we quantify the influence of environmental variability over different space (horizontal and vertical) and timescales (seasonal to interannual) on the cellular costs associated with photosynthesis and respiration, as well as the implications arising from sensitivity experiments that make different assumptions about the prevalence of iron storage and the dominant biological nitrogen source. Combining these results with those from culture experiments with *Prochlorococcus* and field data concerning natural populations, allow us to explore the role of iron alongside other important micronutrients such as cobalt and zinc in shaping *Prochlorococcus* resource limitation, now and in the future ocean.

S1-short talk 6

***SYNECHOCOCCUS* spp. LIVING IN THE MESOPELAGIC ANOXIC REALM OF THE BLACK SEA**

Cristiana Callieri, Pedro J. Cabello-Yeves, Andrea Di Cesare

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We describe the recovery, isolation, and whole-genome characterization of four novel strains belonging to the picocyanobacterial genus *Synechococcus*, two of them (BS56D, BS55D) from inthe mesopelagic realm of the Black Sea and two (BSF8, BSA11) isolated from the superficial layer of itsnorth-west coast. We demonstrated the possibility for the deep strains to perform heterolactic acidfermentation, to assimilate ammonium by different pathways, and to accumulate chlorophyll *a* in thedark, thanks to the presence of light-independent oxidoreductase. Tests on the *Synechococcus* sp.BS56D revealed its adaptability to dark anoxic conditions, accumulating chlorophyll *a*, andphotosynthesizing when re-exposed to light. Deep and superficial *Synechococcus* strains-specific primers targeting the *rpoC1* gene were designed and used in qPCR assays on 22 Black Sea samplesalong horizontal and vertical profiles. The coastal strains were never found in deep water layers,whereas the strains isolated in the deep were present also in surface waters. The presence of*Synechococcus* in mesopelagic profiles of the Black Sea, provides new evidence to link the origin of the“deep red fluorescence” signal to viable picocyanobacteria populations in deep dark anoxic waters.

S1-short talk 7

Genomic mosaicism underlies the adaptation of marine *Synechococcus* ecotypes to several, distinct oceanic iron niches

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Iron (Fe) limits the growth of phytoplankton across ~40% of the world's oceans including High-Nutrient Low-Chlorophyll (HNLC) regions. While low-Fe adaptation has been well-studied in large eukaryotic diatoms, less is known about Fe adaptation among ecotypes of picocyanobacteria, small prokaryotic phytoplankton. Using biogeographic and comparative genomic analysis, we identify key genomic differences underlying adaptation of marine *Synechococcus* ecotypes to several distinct Fe niches. Ecotype CRD1 strains possess an expanded repertoire of Fe transporter, storage, and regulatory genes compared to other ecotypes consistent with their prevalence in low-Fe HNLC regions. The other HNLC ecotype CRD2 interestingly possesses a different repertoire of Fe-adaptive genes from CRD1 genomes, including siderophore uptake genes, highlighting how co-existing ecotypes have evolved independent approaches to life in low-Fe habitats. Metagenomic analysis reveals that *Synechococcus* genes encoding ferritin, flavodoxin, Fe transporters, and siderophore uptake genes are more abundant in low-Fe waters, mirroring paradigms of low-Fe adaptation in diatoms. Genomic, biogeographic, and metagenomic data support that the oligotrophic ecotypes II and III occupy distinct Fe niches, with the latter found in regions with notably higher levels of Fe. *Synechococcus* and *Prochlorococcus* HNLC ecotypes exhibit independent, genome-wide reductions of predicted Fe-requiring genes, presumably to reduce their Fe quota. The Fe-related gene content of HNLC ecotype CRD1 and CRD2 were interestingly most similar to coastal ecotypes I and IV respectively, suggesting populations from these different biomes experience similar Fe-selective conditions, namely period of low Fe availability. This work supports an improved perspective that phytoplankton are adapted to several, more nuanced Fe niches in the oceans than previously implied from mostly binary comparisons of low- vs. high-Fe habitats and populations.

S1-short talk 8

Picocyanobacteria in Inland Seas

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As a suite of vast inland seas encompassing strong biogeochemical gradients, the Laurentian Great Lakes are a unique freshwater system for exploring fundamental questions about microbial adaptations and evolution across the salinity divide. Here we focus on picocyanobacteria, whose phylogenetic and physiological diversity has been well characterized in marine systems but much less so in freshwater. We used single-cell and metagenome-assembled genomes to construct a genome atlas of picocyanobacteria across the Laurentian Great Lakes. Genome diversity was structured into five distinct clades (GLI-GLV) affiliated with two subclusters: *Cyanobium/Synechococcus* subcluster 5.2 and *Synechococcus* subcluster 5.3. These major groups were distinguished by genome properties (e.g. GC content) and gene content; for example, genomes affiliated with subcluster 5.3 lacked genes for nitrate reductase, nitrite reductase, and cyanase. Predicted pigment composition also varied across clades, reflecting a history of lateral

gene transfers and gene losses of pigment biosynthesis genes over the course of picocyanobacterial evolution. With respect to spatial distribution, some lineages were cosmopolitan while others showed enrichment in the lower-productivity upper lakes (Superior, Michigan, Huron) or the higher-productivity lower lakes (Erie, Ontario). Notably, genomes with type 2 and type 3 pigments coexisted in each sample, providing evidence for niche complementarity. Our findings, together with the ecotype framework developed for marine picocyanobacteria, highlight common evolutionary and ecological patterns across the salinity divide, as well as features unique to freshwater and marine systems.

Session 2: Physiology, gene regulation and metabolism

S2-short talk 9

Molecular mechanisms of thermal acclimation in *Prochlorococcus* marinus MIT9301

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Temperature is a major driver of the oceanic distribution of the key global primary producer *Prochlorococcus*, which cannot proliferate above 40 degrees latitude. Yet, we lack fundamental knowledge about the underlying molecular mechanisms of thermal acclimation in this cyanobacterium. Here, we analysed the thermal response of an ecologically representative strain (*Prochlorococcus* marinus MIT9301) at the transcriptional level in long-term experimental acclimations. MIT9301 cells were able to maintain the concentration of transcripts related to major pathways such as the Photosystem II core proteins, carbon fixation and the oxidative pentose phosphate pathway along the thermal niche. However, under cold conditions, an accumulation of mRNA transcripts and marked changes in the expression of selected genes were observed. Components of the Photosystem (PS) I and the oxygen-evolving complex (psbO) were strongly downregulated under cold conditions, mirroring the decreased *Prochlorococcus* growth rates. Additionally, cold temperature induced an impairment in the tightly regulated daily transcriptional patterns in MIT9301 cells. Under warm acclimation, the impact on global transcriptomic patterns was low, but the transcript inventories became streamlined in MIT9301 shrinking cells. Our results unveil a transcriptional basis behind the cold-temperature growth restriction in *Prochlorococcus*, which may be key to explain their inability to thrive in high-latitude waters.

S2-short talk 10

***Prochlorococcus* strains employ different photosynthetic strategies under distinct conditions of oxygen concentration and light availability: Mining the Ocean Protein Portal**

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The photosynthetic physiology and biochemistry of select *Prochlorococcus* strains have been explored in lab grown cultures. In previous publications we have demonstrated that different strains grown under identical conditions of light and nutrient availability have different photosynthetic parameters, photosystem stoichiometries and susceptibilities to photoinactivation. In the oceans, however, the many known *Prochlorococcus* strains occupy different niches of irradiance, nutrient availability and oxygen concentration among other parameters. To determine whether natural populations use photosynthetic strategies similar to those observed in *in vitro* studies, we have used data in the Ocean Protein Portal to probe protein expression patterns at different depths and measured oxygen concentrations. We have examined data from the KM1128 cruise in the South Pacific using R scripts to process, analyze and visualize the raw data, focusing on core photosynthetic processes and related metabolism. Thirteen strains of *Prochlorococcus* are represented in the database, seven high light (HL) strains and 6 low light (LL) strains. All strains express Photosystem II (PSII) and the ATP Synthase complex. Some, but not all, strains express detectable levels of other complexes of the core photosynthetic apparatus including Photosystem I (PSI), the Cytochrome b6f complex and Rubisco. These findings suggest alternate strategies employed by different strains for survival in these niches. Some differences in expression are strain specific, however, some are ecotype specific. For example, HL strains express NAD (P) H:Quinone oxidoreductase (Ndh-1) but only at higher oxygen concentrations, suggesting the capacity for cyclic electron flow around PSI for the generation of a proton motive force for ATP production, while LL strains do not express Ndh-1 under any of the conditions. Similarly, most HL strains express the FtsH isoforms responsible for PSII repair following photoinactivation, while LL strains do not despite encoding the genes for these isoforms.

S2-short talk 11
Small things reconsidered: The dark matter of genetics in Cyanobacteria
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Advances in the number of available complete genome sequences and metagenomic datasets and recently developed experimental approaches suggest the presence of hundreds of additional small genes even in the most compact cyanobacterial genomes. We have established ribosome profiling with stalled initiation complexes in the model cyanobacterium *Synechocystis* 6803 leading to the identification of numerous novel initiation sites and small ORFs (smORFs) in intergenic regions but also in antisense transcripts and within sRNAs previously considered to be non-coding. One of these smORFs encoding an only 48 amino acids small protein showed already 10 min after transfer into darkness a striking upregulation. After 6 hours the corresponding mRNA was the most abundant mRNA dominating the dark transcriptome. Homologs of this smORF are ubiquitous in cyanobacteria, but were not found in any other phylum. Although these homologs share in case of *Prochlorococcus* MED4 only six (12.5%) identical amino acids with the *Synechocystis* 6803 protein, we found the dark-induced expression as a shared characteristic. Translational fusions to the green fluorescent protein revealed targeting to the thylakoid membrane, while immunoprecipitation assays followed by mass spectrometry and far Western blots identified the ATP synthase complex as target. ATP hydrolysis assays with isolated membrane fractions as well as purified ATP synthase complexes demonstrated that this smORF encodes a novel inhibitor of the futile ATP synthase reverse

reaction, therefore named as AtpΘ. These data suggested that AtpΘ is recruited during unfavorable conditions as an inhibitor that prevents the hydrolysis of ATP. Small peptide inhibitors exist also for the ATP synthase of eukaryotic mitochondria suggesting AtpΘ as an example of convergent evolution. The characterization of several further small proteins show that these are too small for enzymatic functions but frequently function in acclimation processes to changes in the environmental conditions.

S2-short talk 12

Redefining the paradigm: Are substrate binding proteins in cyanobacteria scavenging diverse nutrients'

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Unicellular marine cyanobacteria are abundant primary producers that are fundamental players in global biogeochemical processes, fuelling marine ecosystem productivity. They encode a diverse range of predicted nutrient uptake systems that are highly conserved. Some of these transport systems contribute to their global distribution and success in diverse marine ecosystems, while others are poorly characterised. We aim to integrate biochemical, structural and evolutionary studies with (meta)genomics and ecology to define the full repertoire of genes encoding substrate binding proteins (SBPs) in cyanobacteria. SBPs are high-affinity binding components that facilitate the uptake of compounds (e.g. inorganics, carbohydrates, amino acids), generally through partnership with specific membrane transporters. We identified putative SBP genes from model *Synechococcus* and *Prochlorococcus* cultured strains as well as analysed their occurrence in 353 metagenome datasets across Australian waters, using an innovative approach combining Hidden Markov Models with 3D SBP protein structure data. Our phylogenomic analyses provide an in-depth assessment of their diversity and potential function. Based on >1500 putative SBP genes identified, ~45% of the SBPs are predicted to bind ligands such as Fe+2, Fe+3, Pi or different organic forms such as phosphonate, NO2-, NO3- , all likely to be important for cyanobacterial growth in a marine setting. Still, ~30% SBP genes are predicted to bind organic substrates such as aromatic carbon compounds and saccharides. The ecological/environmental significance of many of these predicted compounds in marine habitats is unknown. Also, no potential ligand could be predicted for ~25% putative SBPs. Novel approaches are therefore warranted for functional characterisation of these predicted transport systems. To investigate the potential ligands and molecular structure of marine picocyanobacterial SBPs, we cloned and heterologously expressed over 300 predicted SBP genes. Recombinant proteins produced in high-throughput formats are being subjected to thermal shift assays and crystallisation screening, for substrate identification and structure determination, respectively. We report on likely substrates identified for a range of predicted cyanobacterial SBPs, including functions not previously known. We are in the process of establishing a genome foundry at Macquarie University which would enable the extension of this approach to investigate a diverse range of protein families in cyanobacteria. Our integrative science will provide a

molecules-to-ecosystems understanding of cyanobacterial nutrient acquisition, serving as a prototype extendable to other marine microbes.

S2-short talk 13

The evolution of siderophore use in *Prochlorococcus*

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We recently showed that some *Prochlorococcus* populations have adapted to iron scarcity by scavenging exogenous siderophores - small iron-binding secondary metabolites produced by other microbes to capture and uptake iron. However, the evolutionary mechanisms underlying the biogeographic and genomic distributions of this trait are unknown. Focusing on a collection of low-light adapted LLI genomes isolated at Station ALOHA in the N. Pacific subtropical gyre, we find that the seven-gene siderophore transport cluster has been horizontally acquired via Tycheposon-mediated integration. Furthermore, the presence of siderophore transport clusters within extracellular vesicles hints at a mechanism for horizontal transmission. Genes from the exterior edges of the cluster are conserved, while interior genes show very high rates of evolution. The outer membrane siderophore receptor sequences contain an excess of nonsynonymous mutations on outward-facing loops and are under some of the strongest diversifying selection in the LLI pangenome. These high rates of evolution at the outer membrane receptor appear to be driven by alternating bouts of recombination and selection: Recombination between distantly related sequences generates novel receptor classes, while positive selection produces nonsynonymous changes at key protein residues. We argue that cycles of antagonistic coevolution between siderophore producers and scavenging non-producers prevent selective sweeps of a single receptor morphology in *Prochlorococcus* populations at Station ALOHA. We expect this evolutionary conflict to be broadly representative of siderophore public good dynamics and provides a simple explanation for the extensive chemical diversity of siderophores and their receptors in the ocean.

S2-short talk 14

Arsenic biotransformation by *Prochlorococcus*: comparative physiology, protein expression and production of methylarsenicals

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Prochlorococcus is the numerically dominant primary producer in oligotrophic subtropical gyres. When phosphate concentrations in these surface waters become extremely low, the mol: mol availability of phosphate relative to the chemically similar arsenate molecule is

reduced, potentially resulting in increased cellular arsenic exposure. *Prochlorococcus* employ two main strategies for arsenic detoxification, a methylation pathway, which is nearly ubiquitously present in global *Prochlorococcus* populations, and an efflux pathway, present only in populations which experience extremely low PO4:AsO4, such as parts of the tropical and subtropical Atlantic. Here we examine the comparative arsenic physiology of two high-light adapted strains that exemplify these two populations: AS9601 which possesses genes for the methylation pathway: an arsenate reductase (*arsC*) and arsenite S-adenosylmethionine methyltransferase (*arsM*) and MED4, which has these two genes as well as an arsenite-specific efflux pump (*acr3*), and an arsenic related repressive regulator (*arsR*). We grew both strains axenically under P-limiting conditions and subjected them to a range of arsenate concentrations (0.025, 0.25, and 2.5 uM) the lowest of which approximated natural surface water concentrations of arsenate (~10-15 nmol L-1). Growth rate and final cell yield of AS9601 declined in higher As treatments relative to the no-As treatment. In contrast, for MED4, there was relatively little difference in growth rate or final cell yield between any of the three arsenate concentrations and the no-As treatment. We suggest the relative resistance of MED4 to concentrations of arsenate that are damaging to AS9601 is a result of its ability to remove arsenic from the cell through the efflux pathway. Consistent with this idea, proteomic analysis of MED4 treated with arsenic revealed the expression of the arsenate reductase (ArsC) and arsenite-specific efflux pump (ACR3) under these conditions. Both the axenic MED4 and AS9601 cultures produced the simple organoarsenicals monomethylarsonic acid (MMA) and dimethylarsinic acid (DMA), the first two steps in the methylation pathway, confirming that both these strains are capable of arsenic methylation. These results have implications for understanding the role of *Prochlorococcus* in the generation of organoarsenicals, which can be biomagnified in the marine food web.

S2-short talk 15

Electron transport and carbon metabolism in low-light and high-light ecotypes of *Prochlorococcus* marinus

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Our goal was to understand the physiological mechanisms of niche-partitioning of *Prochlorococcus* populations in the water-column. We first studied electron transport and carbon metabolism in contrasting *Prochlorococcus* ecotypes: the high-light strain PCC9511 and the low-light strain MIT9313 grown under constant irradiance and nutrient replete conditions. The kinetics of electron donation to Photosystem I indicates that in the high-light strain PCC9511, a significant cyclic electron flow (40% of all electrons) around Photosystem 1 operates, while in the low-light ecotype MIT9313, the dominant pathway is the DCMU sensitive linear electron flow from Photosystem 2. In both strains, the Photosystem 2 has excess capacity of 60%, meaning that 60% of PS2 can be deactivated without influencing the linear flow to PS1. Then we compared the growth-rate dependent carbon allocation dynamics. The cells were cultured in balanced growth in nitrogen-limited continuous chemostat cultures. Chl-specific gross (GPCb) and net (NPCb) carbon productions were independent of nitrogen-limited growth rate. However, the time-dependent catabolism of newly fixed carbon was significantly different between these two strains reflecting its rapid respiration and probable use for ATP regeneration in LL strain.

Such different carbon processing could be result of higher demands for energy in the form of ATP which these LL ecotypes might needed. Obtained results provide key insights into the cell characteristics and to the efficiency of carbon conversion to the biomass between LL and HL adapted *Prochlorococcus* ecotypes.

S2-short talk 16
Stabilization of extensive fine-scale diversity by dispersal and ecologically driven spatio-temporal chaos
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Prochlorococcus exhibits remarkably extensive genetic diversity that coexists at the same time and spatial location, as well as being mixed throughout the oceans. Coexistence of extensive fine-scale diversity has more recently been found within many other terrestrial and human-commensal bacterial species. Although a variety of ecological niches surely causes some of this diversity, is it reasonable to invoke a huge multitude of micro- and even pico-niches to explain this diversity' Or are there other potential mechanisms for stabilizing coexistence for far longer than natural time scales of ecological dynamics and effects of even-very-small selective differences' In this work, we explore simple generalized Lotka-Volterra models of many interacting strains without any niche-like structure. Interactions --- competitive or otherwise --- between siblings are not larger, statistically, than between distant cousins. Although the results are more general, a natural realization is the interactions between a phage species with multiple generalist strains, and multiple strains of a host bacterial species, with slight, effectively random, differences in infectability among the strains We show (using statistical physics methods) that with spatial dispersal a highly robust ecologically driven spatio-temporally chaotic 'phase' can exist in which extensive diversity stably coexists. Some strains will go globally extinct, but many strains with negative average birth-minus-death rates will survive. At any time and location, most strains will have tiny or zero local abundance. But all the long-term surviving strains will occasionally -- at some times and locations -- bloom to high abundance. And dispersal from these blooms will sustain their global populations. One of the predictions is local abundance distributions that look almost identical to those from neutral theory of ecology, but the latter has much slower dynamics and is implausible quantitatively for huge planktonic microbial populations. The possibility of such a state evolving, suggestions for potential future genomic studies, and open questions will be discussed.

Session 3: Predation and trophic interaction

S3-short talk 17

Temporal transcriptional patterns of cyanophage genes suggest synchronized infection of cyanobacteria in the oceans

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Based on the peak expression times during infection, early, middle, and late genes have been characterized in viruses (cyanophages) that infect the unicellular cyanobacterium *Prochlorococcus*. Laboratory experiments show that some cyanophages can only replicate in the light and thus exhibit diurnal infection rhythms under light-dark cycles. Field evidence also suggests synchronized infection of *Prochlorococcus* by cyanophages in the oceans, which should result in progressive expression of cyanophage early, middle, and late genes. However, distinct temporal expression patterns have not been observed in cyanophage field populations. We reanalysed a previous metatranscriptomic dataset collected in the North Pacific Subtropical Gyre. In this dataset, it has been found that aggregate transcripts from cyanophage scaffolds display diurnal transcriptional rhythms with transcript abundances decreasing at night, which was also observed in laboratory cultures and was shown to be due to decreased photosynthetic activity of the cyanobacterial host cells in the dark. By mapping metatranscriptomic reads to individual viral genes, we identified periodically expressed genes from putative viruses infecting the cyanobacteria *Prochlorococcus* and *Synechococcus*, heterotrophic bacteria, and algae. Of the 41 cyanophage genes, 35 were from cyanomyoviruses. We grouped the 35 periodically expressed cyanomyovirus genes into early, middle, and late genes based on the conserved temporal expression patterns of their orthologs in cyanomyovirus laboratory cultures. We found that the peak expression times of late genes in cyanophage field populations were significantly later than those of early and middle genes, which were similar to the temporal expression patterns of synchronized cyanophage laboratory cultures. The significantly later peak expression times of late genes in cyanomyovirus field populations suggested that cyanophage infection of *Prochlorococcus* is synchronized in the North Pacific Subtropical Gyre. The night-time peak expression of late genes also suggested synchronized lysis of *Prochlorococcus* at night, which might result in synchronized release of dissolved organic matter to the marine food web.

S3-short talk 18

Extracellular vesicles secreted by *Synechococcus*: effect of stress in their production and content

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Membrane vesicles are thought to be formed when regions of the outer membrane are pinched off and released. This still little understood phenomenon is believed to allow the

interaction of bacteria with their environment. Secretion of membrane vesicles could thus have large and diverse ecological impact on the marine microbial community. To date, there are no studies carried out on marine Synechococcus vesicles, apart from observations in a single axenic culture(1) and further work is critical to clarify their exact roles in the marine environment.

To advance our understanding of marine extracellular vesicles, we characterized vesicle production in marine *Synechococcus*. We first showed that vesicles are produced in cultures of three marine *Synechococcus* strains (WH8102, WH7803, BL107). We measured vesicle production and abundance in these strains, finding that vesicles are 40 times more numerous than *Synechococcus* cells in both exponential and stationary phase growth. Our results suggest that, on average, *Synechococcus* release more vesicles per cell than *Prochlorococcus* when grown under similar conditions. Currently, we are studying the effects of extreme nitrogen and phosphorus limitation, frequent features of the oligotrophic oceans, on vesicle secretion by this organism trying to answer new questions concerning how cells allocate resources into vesicles, and whether vesicle secretion contributes to stress responses.

Moreover, we are using fluorescent proteins to investigate whether vesicle proteins are transferred between cyanobacterial strains. For that, we produced plasmids encoding periplasmic versions of the Green Fluorescent Protein. *Synechococcus* sp. strain PCC 7002 was transformed with these plasmids, and the obtained strains are currently being analyzed to observe how vesicles are formed in this cyanobacterium.

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1. S. J. Biller et al., Bacterial vesicles in marine ecosystems. Science 343, 183-186 (2014).

S3-short talk 19

Integrative elements abundant in marine vesicles shape *Prochlorococcus* genomic plasticity

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Horizontal gene transfer is a key accelerant of microbial evolution promoting rapid diversification and adaptation through genomic plasticity. Highly abundant open ocean microbes such as *Prochlorococcus* and *Pelagibacter* possess heavily streamlined genomes and extensive pangenomes reflecting extensive gene exchange. Surprisingly,

these streamlined organisms lack the capacity for conjugation and transformation, two of the most common modes of horizontal gene transfer.

Leveraging over 600 genomes - mostly of single cells - we revealed that *Prochlorococcus* harbors a diverse system of mobile genetic elements that appear to shape its considerable genomic plasticity. 70% of its flexible genes are predominantly organized within distinct genomic islands that have formed around a small set of tRNAs. The latter serve as integration hotspots for the majority of the mobile genetic elements. The elements themselves exhibit a high degree of mosaicism, and their hallmark genes suggest that they are integrative, self-replicating and regulated through stress signals. Moreover, many of the elements carry additional cargo including operons for the acquisition of nutrients and genes associated with viral defense underpinning their potential role in niche adaptation. We also found that many of these elements are highly enriched in either extracellular vesicle and viral fraction metagenomes, indicating that they can be efficiently transmitted between cells through these extracellular particles. As evidence of gene mobilization via these elements grows and if their dispersal through viral capsids and vesicles is found to be prevalent among free-living marine microbes, it could add a significant piece to the puzzle of what governs microbial evolution in the planet s largest habitat.

S3-short talk 20

Biochemical characterization of cyanophage encoded auxiliary metabolic proteins give insights into their role during infection

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Viruses and bacteriophages (viruses that infect bacteria) are the most abundant biological entities on our planet. In the marine environment, cyanophages (viruses infecting cyanobacteria of the genera *Proclorococcus* and *Synechococcus*) have significant impact on ecology, evolution and biogeochemical processes. Their self-replication relies on the molecular machinery of a host bacterium to generate viral progeny. Bacteriophages may have a lysogenic or lytic cycle, the latter ultimately resulting in the lysis of the host cell. Infection by a lytic phage transforms the host bacterium into a so-called virocell. The virocell represents the intracellular state of the phage's life cycle whose sole function is to produce virions. Phage infection induces a dramatic change in various host metabolic pathways, which is further expanded by the introduction of auxiliary metabolic genes (AMGs). Cyanophage encoded AMGs are often related to photosynthesis and are suggested to modulate und supplement the host bacterium's metabolism to satisfy the elevated metabolic demand. We have biochemically characterized a number of AMG encoded proteins, specifically those involved in light harvesting pigment biosynthesis and assembly. While the AMG encoded proteins often mimic the respective host activities, the pigment biosynthesis gene *pebS* encodes an enzyme catalyzing a reaction that requires two consecutive enzymes in uninfected host cells. PebS sequences often co-occur with genes encoding CpeT phycobiliprotein lyases, enzymes facilitating the assembly of light harvesting phycobiliproteins. Here, we will present a combination of biochemical characterization and structural analysis and will discuss the role of these proteins during infection.

S3-short talk 21

Pico-parachutes; marine picocyanobacteria use pili as a strategy to retain optimal positions in the water column and evade predation

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Planktonic microorganisms have evolved a range of strategies to stay afloat e.g. via intracellular gas vesicles or swimming via flagella. In marine ecosystems where energy and nutrients are scarce, planktonic organisms had, to date, no known mechanisms to avoid sinking and were thought to remain buoyant thanks to their reduced size and passive transportation within currents. Here, we identify for the first time the production of a type IV pili which allows both *Synechococcus* and *Prochlorococcus* strains to increase drag and remain suspended in optimal positions within the water column, i.e. nutrient and light. This seemingly costly mechanism also provides evasion from predation by grazers. The downside of producing these long pili is, possibly, a higher chance of encountering phage. The evolution of this sophisticated floatation mechanism in these purely planktonic streamlined microorganisms has important implications for our current understanding of microbial distribution in the oceans and predator–prey interactions which ultimately will need incorporating into future models of marine carbon flux dynamics.

S3-short talk 22
Macroplanktonic pelagic tunicates prey on picocyanobacterial
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Top-down controls play a critical role in the ecology and biogeochemical contributions of marine picocyanobacteria *Prochlorococcus* and *Synechococcus*. Protists, viruses, and small zooplankton are accepted as a significant source of mortality. However, pelagic tunicates (salps, doliolids, pyrosomes, appendicularians) are one group of grazers with high potential for predation on marine picocyanobacteria that is rarely considered or quantified as a source of mortality. This group of grazers is understudied due to their patchy distributions and extreme fragility in standard sampling approaches. We examined interactions of salps, pyrosomes, and doliolids with marine picocyanobacteria to understand how these enigmatic animals prey on abundant microbial members of ocean food webs. Using a combination of approaches including SCUBA-based sampling and incubation, sequencing, qPCR, microscopy, and flow cytometry we tested the susceptibility of *Prochlorococcus* and *Synechococcus* to grazing by these large fragile organisms. We revealed the retention of picocyanobacteria by all groups of pelagic tunicates tested, but quantified preference for larger phytoplankton taxa such as diatoms in some taxa (pyrosomes) and picocyanobacteria in other taxa (salps). The work shows that large gelatinous zooplankton are an important source of mortality for picocyanobacteria and suggests an underappreciated facet to the carbon cycle facilitated by gelatinous zooplankton.

S3-short talk 23
With a little help from my friends: *Prochlorococcus* survival and growth under low nutrient and energy conditions through metabolic interactions with heterotrophs.
Daniel Sher[1]

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With their small size, unique photopigments and streamlined genomes, *Prochlorococcus* are expected to be well adapted to the conditions where they thrive: the nutrient-poor, oligotrophic ocean, often at the deep chlorophyll maximum (DCM). However, axenic *Prochlorococcus* cannot survive extended nutrient or light starvation alone – they require the presence of heterotrophic "helper" bacteria. Here, we present the results of lab and field experiments, statistical analysis of large genomic data and mathematical models, aiming to characterize and quantify the role of microbial interactions in the survival of *Prochlorococcus* under long-term starvation conditions. We show that the requirement for microbial interactions to support long-term nitrogen starvation is conserved across *Prochlorococcus* ecotypes, and many (but not all) clades of heterotrophic bacteria enable such survival. On the short term (days-weeks), interactions with a model clade of heterotrophic bacteria, Alteromonas, stabilize the morphology, physiology and metabolic profile of *Prochlorococcus*, reducing its mortality rate and likely allowing cells to acclimate to nutrient stress. These interactions likely include the exchange of nitrogen-containing metabolites. On the longer term (weeks-months), such interactions may facilitate genetic adaptations to long-term nutrient starvation, for example through modifying nitrogen metabolism, compatible solute production and/or intracellular stress signaling. In the oceans, metabolic interactions with members of the microbial community through the exchange of carbon-containing metabolites (i.e. mixotrophy) support up to 25% of vertically-integrated carbon assimilation by *Prochlorococcus*, including the growth of essentially all low-light adapted cells at the base of the photic zone. We propose that reliance on metabolic exchange with co-occurring microbes underlies the ecological success of a large fraction of the global *Prochlorococcus* population and its collective genetic diversity.

S3-short talk 24

Proteomics of nitrogen incorporation in *Synechococcus*, *Prochlorococcus* and their phages

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Nitrogen assimilation by marine microbes and *Prochlorococcus/Synechococcus* in particular is one of the largest fluxes in the global N cycle. Most N in microbial cells resides in proteins, making quantitative understanding of how microbes use nitrogen anabolically to build which proteins under which circumstances, and for which metabolic purposes central to marine N biogeochemistry. We have developed novel quantitative and isotope-tracking proteomics techniques to assay these processes in both cultured isolates and field populations.

First, we determined the uptake of extracellular nitrogen during phage infection and its biosynthetic incorporation into both virus and host proteins in *Synechococcus* WH8102 infected by lytic cyanophage S-SM1. We find that proteins in progeny phage particles can be comprised of significant amounts of extracellularly derived nitrogen, while proteins of the infected host cell show almost no isotope incorporation, demonstrating that de novo amino acid synthesis continues during infection and contributes specifically and substantially to phage replication. The source of nitrogen for phage protein synthesis shifts over the course of infection, from mostly host-derived in the early stages to more medium-derived later on. The phage-encoded photosystem II reaction center proteins D1 and D2

are produced de novo during infection in an apparently light-dependent manner. We also identified a small set of host proteins that do continue to be produced during infection; notably, most of them are homologues of AMGs in S-SM1 or other viruses, suggesting selective continuation of host protein production during infection that may be related to relief of metabolic chokepoints to viral replication. The continued acquisition of nutrients by the infected cell and their utilization for phage replication is significant for both the evolution of viruses and biogeochemical impact of virus-driven nutrient flows.

Second, we tracked incorporation of five different isotopically-labeled N substrates and protein-level gene expression responses to addition of those substrates in the surface water community at the Hawaii Ocean Time-series station ALOHA. Over short-term incubations during light and dark periods, we found substantial incorporation of label into peptides that map to a wide range of taxa and metabolic functions. Individual taxa showed a spectrum between specialization and generalism with regard to substrate use and functional allocation. These findings provide direct connections between nutrient biogeochemistry and cellular allocation in specific members of the microbial community.

S3-short talk 25
Cyanovirocell infochemicals driving microbial interactions
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Marine microbes interact with one another through exudation and sensing of dissolved chemical compounds or infochemicals. During infection, viruses manipulate host metabolism, stimulating the release of dissolved chemical cues from intact phytoplankton (virocells), which can elicit chemotactic responses from neighboring microbes, potentially generating biological hotspots around infected cells. We used an interdisciplinary approach combining mass spectrometry, microfluidic chemotaxis assays, and high-resolution video microscopy to identify cyanovirocell derived chemical cues that attract model heterotrophic bacteria. Microfluidic experiments with the marine bacterium Vibrio alginolyticus revealed that the strongest positive chemotactic responses occurred not to cell lysis products but rather to exudates derived from *Synechococcus* during early stages of cyanomyovirus infection. Polar and non-polar metabolite analyses revealed a suite of compounds released from cyanovirocells during early infection and subsequent chemotaxis assays using target compounds enabled identification of virocell-derived chemoattractants. These data provide new insights into cyanovirocell metabolism and consequent impacts on microbial interactions.

POSTER SESSIONS

Session 0 (Workshop): Databases

S0P01: Simons CMAP an interconnected oceanic data portal. Mohammad Ashkezari[1], E. Virginia Armbrust[1], Norland Raphael Hagen[1], Michael Denholtz[1]

S0P02: "CyanoMarks, a reference database of genetic markers to study the diversity and ecology of marine picocyanobacterial" Gregory K. Farrant[1], Jade Leconte[1], Patricia Kembia[1], Ulysse Guyet[1], Francisco M. Cornejo-Castillo[3], Hugo Doré[1], Morgane Ratin[1], Martin Ostrowski[2], Mark Hoebeke[2], Erwan Corre[2], David J. Scanlan[4], Laurence Garczarek[1]

S0P03: Planet Microbe: a cyberinfrastructure for integrating oceanographic omics, environmental and physiochemical data layers. Alise Ponsero[1], Kai Blumberg[1], Matthew Bomhoff[1], Ken Youens-Clark[1], Edward Delong[2], Elisha Wood-Charleson[3], Bonnie Hurwitz[1]

S0P04: A Curated *Synechococcus elongatus* Pathway/Genome Database at BioCyc.org. Peter Karp[1], Ron Caspi[1]

S0P05: Comparative Genomics for Gene Discovery using Integrated Microbial Genomes (IMG). Rekha Seshadri

S0P01: Simons CMAP an interconnected oceanic data portal

Mohammad Ashkezari[1], E. Virginia Armbrust[1], Norland Raphael Hagen[1], Michael Denholtz[1]
1 University of Washington

Simons Collaborative Marine Atlas Project (Simons CMAP) is an open-source data portal interconnecting data sets across Oceanography disciplines. It enables scientists and the public to dive into the vast and often underutilized ocean datasets to retrieve custom subsets of data, create data visualizations and run analyses. The ever-growing CMAP database is approaching 50 TB in size hosting global multi-decade remote sensing (e.g. satellite temperature, chlorophyll), several decades of global biogeochemical models (e.g. MIT Darwin, Mercator-Pisces), and decades of field measurements (e.g. ~100 cruise expeditions, Argo floats, Hawai'i Ocean Time series).

The linking chain between CMAP datasets is space-time: every single datum is annotated and indexed by space and time making it possible to cross-reference the underlying datasets automatically regardless of their sizes and resolutions. Later on, during this conference, Prof. Armbrust will showcase this feature by co-localizing approximately 125,000 independent observations of *Prochlorococcus* and *Synechococcus* abundances with over 50 environmental variables derived from satellite observations and biogeochemical models to capture the spatio-temporal variations of *Prochlorococcus* and *Synechococcus* abundances and to identify the dominant environmental determinants using machine learning algorithms.

Nearly all CMAP data functionalities are embedded at the database layer making CMAP ecosystem [programming] language-agnostic. Currently, the CMAP application layer involves a web app under active development, an R package (cmap4r) and a python package (pycmap). More programming languages will be supported in the near future.

Today I will first present the CMAP underlying architecture and then we will review a selected list of tutorials using the pycmap package. Pycmap can be run on cloud-based Jupyter notebooks and does not require local installation. If you wish to run the tutorials on your local machine please pre-install a python 3 distribution, preferably Anaconda.

S0P02: CyanoMarks, a reference database of genetic markers to study the diversity and ecology of marine picocyanobacteria.

Gregory K. Farrant[1], Jade Leconte[1], Patricia Kembia[1], Ulysse Guyet[1], Francisco M. Cornejo-Castillo[1], Hugo Doré[1], Morgane Ratin[1], Martin Ostrowski[2], Mark Hoebeke[3], Erwan Corre[3], David J. Scanlan[4], Laurence Garczarek[1]

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Metabarcoding and metagenomics are currently the most widely used approaches to study the diversity, ecology and dynamics of marine microbial communities. However, detection of significant changes in community composition is often limited by the lack of curated reference sequence databases for reliable taxonomic assignments. For marine picocyanobacteria, several core phylogenetic markers, which display different advantages and drawbacks, are commonly used: i) 16S rDNA, a universal marker for prokaryotes encoding the small ribosomal RNA subunit, ii) *petB*, encoding the b6 subunit of the photosynthetic complex cytochrome b6/f [1,2], iii) *rpoC1*, encoding the gamma subunit of RNA polymerase [3] and iv) the 16S-23S internal transcribed spacer (ITS; [4]). However, the correspondence between the taxonomies inferred from these different markers remains to be achieved for a number of environmental taxa.

In this context, we developed CyanoMarks, a curated collection of marker genes for marine cyanobacteria with an emphasis on Prochlorococcus, Synechococcus and Cyanobium. This database takes part of the RoskoBaz initiative, which aims at unifying the four banks of reference markers developed at the Station Biologique de Roscoff (CyanoMarks, PR2 & EukRibo for protists 18S rDNA, MicRhoDE for microbial rhodopsins and PhytoRef for chloroplastic 16S rDNA) to ensure their quality and longevity using semi-automated update and curation tools. CyanoMarks gathers most published cyanobacterial sequences for 16S rDNA, *petB* and *rpoC1* as well as representative eukaryotic sequence homologs to be used as outgroups, which have been annotated using a unified and curated taxonomy. This database also includes extensive contextual data such as sequence origin (strain, clone, etc.), sampling location and physico-chemical parameters of their isolation site. CyanoMarks was notably used to compare the taxonomic resolution of these markers over their whole length and to reliably match taxonomies of the different markers using the numerous genomes, SAGs and MAGs available for marine picocyanobacteria. This database, regularly updated with newly available sequences (e.g. from NCBI/EBI) using a semi-automatic pipeline based on BLAST, phylogenetic placement and Bayesian inference, constitutes a valuable tool to analyze the wealth of omic data available for marine ecosystems.

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[4] Rocap G. et al., 2002. AEM 68: 1180–1191.

S0P03: Planet Microbe: a cyberinfrastructure for integrating oceanographic omics, environmental and physiochemical data layers

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Oceanographic research cruises provide abundant physical, chemical, and biological data, using a plethora of methods and equipment, and involving collaborative efforts from diverse scientists and disparate disciplines. These research endeavors are critical to investigating questions related to the interplay between biological, geological, and chemical processes in ocean systems over space and time. The advent of sequencing technologies allows for an analysis of gene expression in a variety of environmental settings, to measure the distribution and significance of metabolites and lipids in organisms and the environment. Importantly, although scientists carefully curate and share their data with collaborators, no systematic, unifying framework currently exists to integrate 'omics data with physiochemical and biological datasets for the broader geoscience community. The development of resources to promote the publication of oceanic 'omics datasets along with their physio-chemical information is critical.

Here, we present Planet Microbe, a federated resource to enable data discovery and open data sharing for historical and on-going oceanographic sequencing efforts. In this project, several historical oceanographic 'omics datasets (Hawaii Ocean Time-series (HOT), Bermuda Atlantic Time-series (BATS), Global Ocean Sampling Expedition (GOS), C-DEBI) are integrated into Planet Microbe and reconnected to their physiochemical measurements. New oceanic large-scale datasets such as the Tara Ocean Expedition and Ocean Sampling Day (OSD) are also integrated into the platform. To systematically integrate these data, we make use of and extend existing ontologies to provide a unique search interface for data discovery based on physiochemical parameters and disparate features. Moreover, modern data publication requires the user to meet FAIR data standards to ensure data are Findable Accessible, Interoperable, and Reusable (FAIR), however, few tools are currently available for the user to ensure the data are meeting these important standards. Planet Microbe aims to provide tools to evaluate private datasets against these standards and promote the integration of new data in data repositories.
Finally, Planet Microbe provides researchers with the infrastructure required to describe and store sequence data, discover and link data sets by important contextual metadata, and save and share project outputs. Members of the research community can integrate tools and pipelines using National Science Foundation (NSF) funded Cyberinfrastructure (CyVerse and XSEDE) to provide users with free access to large-scale computing power to analyze and explore their datasets. These cyberinfrastructure components are broadly useful to any research community.

Observations:
First unified system to make microbial omics, cruise, and physiochemical data sets interoperable with common ontologies, and systematically searchable in a single unified platform that enables global ocean studies and promote FAIR data standards.

S0P04: A Curated *Synechococcus elongatus* Pathway/Genome Database at BioCyc.org

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BioCyc.org is an extensive web portal for microbial genomes and metabolic pathways. BioCyc contains 14,700 microbial genomes including 66 *Synechococcus* genomes, 8 *Synechocystis* genomes, and 18 *Prochlorococcus* genomes. The first step in the creation of BioCyc databases is to run prediction algorithms for metabolic pathways, operons, PFam domains, and orthologs. We next execute programs that import data from related databases including regulatory network data, protein features, subcellular locations, and Gene Ontology assignments. Curated databases next receive intensive review and updating by a Ph.D. biologist that includes reviewing the computationally predicted metabolic pathways, entering new gene functions and metabolic pathways from the experimental literature, and defining protein complexes. The resulting databases are high-quality reference sources for the latest gene and pathway information. BioCyc contains a curated database for *Synechococcus elongatus* PCC 7942 [1] that contains 197 metabolic pathways, 1190 enzymatic reactions, 41 transcriptional regulatory interactions, 2,182 protein features, and one gene essentiality dataset.

The BioCyc website provides an extensive set of bioinformatics tools for searching and analyzing these databases, and leveraging them for analysis of omics datasets. Genome-related tools include a genome browser, sequence searching and alignment, and extraction of sequence regions. Pathway-related tools include pathway diagrams, a tool for navigating zoomable organism-specific metabolic map diagrams, and a tool for searching for metabolic routes that connect metabolites of interest. Regulation tools depict operons and regulatory sites, as well as showing full organism regulatory networks. Comparative analysis tools enable comparisons of genome organization, of orthologs, and of pathway complements. Omics data analysis tools support enrichment analysis and painting of transcriptomics and metabolomics data onto individual pathway diagrams and onto zoomable metabolic map diagrams. A new Omics Dashboard tool enables interactive exploration of omics datasets through a hierachy of cellular systems. SmartTables enable users to construct and store tables of genes, metabolites, or pathways, and to perform analysis such as transforming a set of pathways to all genes within the pathway set.

[1] https://biocyc.org/organism-summary’object=SYNEL

S0P05: Comparative Genomics for Gene Discovery using Integrated Microbial Genomes (IMG)

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Genome sequencing is widely accessible these days, but there is still a wide gap between sequence and biological discovery. The Integrated Microbial Genomes & Microbiomes (IMG/M) system helps bridge that gap by serving as a resource for gene discovery and comparative analysis of genomic and metagenomic datasets. The IMG data warehouse includes data generated by the U.S. DoE Joint Genome Institute (JGI) as well as publicly available datasets sourced from GenBank and more. Within IMG/M, ProPortal serves as a dedicated data mart for genomic, metagenomic and population data from both cultivated

strains and wild populations of cyanobacteria and phage. The system's capabilities will be presented through a biological use case scenario employing various tools to browse data in the context of environmental metadata, perform and visualize comparisons, and interpret results. Learn more about IMG/M's capabilities by watching an introductory video: https://youtu.be/Li8YFk0vhUE

Session 1: Ecology, distribution and dynamics: past, present and future

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S1P12: Mar Marine *Synechococcus* and Ocean Color: Multiscale analysis of the adaptive advantage conferred by chromatic acclimation. Louison Dufour, Morgane Ratin, Jonathan A. Karty, Stéphanie Dutkiewicz, Anna E. Hickman, Wendy M. Schluchter, David M. Kehoe, Julia Uitz, Laurence Garczarek, Frédéric Partensky

S1P13: Assessing *Synechococcus* diversity across gradients of abiotic conditions in the oceans. Noam Eshed, Michael C. G. Carlson, Irena Pekarski, Lilach Pnueli, Margalit Peleg1, Debbie Lindell

S1P14: Introducing monitoring of picophytoplankton in the Baltic Sea proper. Hanna Farnelid

S1P15: Phenology, diversity and protozoan grazing of *Synechococcus* at a long-term time series within the Gulf of Maine, USA. Nicole Poulton, Michael Sieracki, Laura Lubelczyk, Peter Countway

S1P16: Comparative study of the ammonium transporter amt1 in *Prochlorococcus* and *Synechococcus*. Ramón Quiles-Bernabéu, Carmen Torres-Granados, Jesús Díez, José Manuel García-Fernández, Antonio López-Lozano, Guadalupe Gómez-Baena

S1P17: **A meta-analysis of different methods to estimate the contribution of *Prochlorococcus* to global marine primary production.** Junyao Gu, Zackary Johnson.

S1P18: Phototroph-heterotroph interactions during growth and long-term starvation across *Prochlorococcus* and *Alteromonas* diversity. Osnat Weissberg, Dikla Aharonovich, Daniel Sher

S1P19: Picocyanobacteria in hypertrophic waters reach high numbers and variability. Jitka Jezberova, Anna Matousu, Karel Simek, Jiri Nedoma, Jaroslav Vrba

S1P20: Heterotroph diversity in enrichment cultures of marine picocyanobacteria. Sean Kearney, Elaina Thomas, Allison Coe, Sallie Chisholm

S1P21: A comparative analysis of the temporal variability of *Synechococcus* and SAR11 across phylogenetic ranks at a temperate coastal ocean site
Junyao Gu, Zackary Johnson

S1P22: Phylogenomic analysis of novel *Prochlorococcus* and *Synechococcus* genomes. Jade Leconte, Ulysse Guyet, Hugo Doré, Gregory K. Farrant, Jukka Siltanen, Loraine Brillet-Guéguen, Mark Hoebeke, Martin Ostrowski, Frédéric Partensky, Laurence Garczarek

S1P23: HTS screening for *Prochlorococcus* and *Synechococcus* in sea water and sediment impacted by mariculture. Anamarija Kolda, Zrinka Ljubesic, Ana Gavrilovic, Jurica Jug-Dujakovic, Kristina Pikelj, Damir Kapetanovic.

S1P24: Co-existence of *Prochlorococcus* and *Synechococcus* sustained by their different uptake affinity of ammonium and nitrate. Takako Masuda[1], Keisuke Inomura[2], Taketoshi Kodama3 Takuhei Shiozaki, Takato Matsui, Koji Suzuki, Shigenobu Takeda, Curtis Deutsch, Ondřej Prášil, Ken Furuya

S1P25: Distribution of *Prochloroccocus* and *Synechococcus* in the Spanish Mediterranean (RADMED Time Series). Francisca Moya Ruiz[1], María del Carmen García-Martínez, Manuel Vargas-Yáñez, María Muñoz Muñoz, Laurencia Guzmán Pocaterra

S1P26: The effect of co-culture remodels the transcriptomes of Prochlorococcus, Synechococcus and co-cultured ?helper? bacterium Alteromonas during elevated CO2 growth. Marcelo Barreto Filho

S1P27: Characterization of novel *Synechococcus* isolates representing key ecotypes in the Baltic Sea. Anabella Aguilera, Javier Alegria-Zufia, Laura Bas Conn, Leandra Gurlit, Sylwia Wilczewska, Jarone Pinhassi, Catherine Legrand, Hanna Farnelid

S1P28: Reevaluation of the distribution of *Synechococcus* and *Prochlorococcus* in marine phytoplankton communities. Juan Jose Pierella Karlusich, Eric Pelletier, Fabien Lombard, Tara Oceans Coordinators, Chris Bowler

S1P29: A proposal of a genome based taxonomy of *Synechococcus*. Vinicius Salazar, Cristiane Thompson, Diogo Tschoeke, Jean Swings, Marta Mattoso, Fabiano Thompson.

S1P30: Independent and interactive effects of copper and irradiance on coastal *Prochlorococcus* and *Synechococcus* communities. Katherine Mackey

S1P31:Trans-domain comparison reveals unexpected abundance of picocyanobacteria in large size fractions. Pierella Karlusich JJ, Pelletier E, Zinger L, Lombard F, Zingone A, Colin S, Gasol JM, Dorrell RG, Scalco E, Acinas SG, Wincker P, de Vargas C, Bowler C

S1P32: Annual dynamics of cyanophages and their hosts in the Sargasso Sea. Camelia Shopen Gochev, Debbie Lindell

S1P33: Phage infection of a nutrient-deplete *Synechococcus* host. Alberto Torcello-Requena, Richard Puxty Andrew D. Millard Yin Chen, David J. Scanlan

S1P34: The island mass effect drives shifts in *Prochlorococcus*:*Synechococcus* ratios from nearshore Oahu, Hawaii to oceanic waters. Christina M Comfort, David M Karl, Chris E Ostrander, Mariam Moreno, Margaret A McManus

S1P35: Identification of Ecologically Important *Synechococcus* Clade II Isolates That Exhibit Genome Reduction Without GC Decrease. Michael Lee, Nathan Ahlgren, Joshua Kling, Nathan Walworth, Gabrielle Rocap, Mak Saito, David Hutchins, Eric Webb

S1P36: Impact of diverse nutrient regimes on cyanophages. Julia Weissenbach, Yotam Hulata, Debbie Lindell

S1P37: Modeling ocean color niche selection by *Synechococcus* chromatic acclimaters. Raisha Lovindeer, Lucas Ustick, Francois Primeau, Adam Martiny, Katherine Mackey

S1P38: Cyanobacterial sources of free DNA in the North Pacific Subtropical Gyre. Morgan D. Linney, John M. Eppley, Anna E. Romano, Elaine Luo, Edward F. DeLong, David M. Kar

S1P39: *Synechococcus* extracellular vesicles: characterization and response to stress inducers. Elisa Angulo-Cánovas, Rodrigo Jiménez-Ulloa, Steven J. Biller, Jesús Díez, José Manuel García-Fernández, María del Carmen Muñoz-Marín

S1P40: Pro. and Syn. in the subtropical North Atlantics P cycle *Prochlorococcus* and *Synechococcus* and their role in phosphorus cycling in the subtropical North Atlantic. Lukas Marx, Sarah Reynolds, Michelle Hale, B. B. Cael, Edward Mawji

S1P41: Diverse light responses of coastal *Synechococcus*. Emily Stone

S1P42:Exploring the Relationships between *Prochlorococcus* and *Synechococcus* Barbara Duckworth, Stephanie Dutkiewicz, Christopher L. Follett

S1P43: Ecological significance of *Prochlorococcus* diversity across four orders of magnitude of genomic divergence. Alana Papula, Daniel Fisher

S1P44: Biofilm Formation in *Prochlorococcus.* Nikolai Radzinski, Lin Hou, Allison Coe, Eli Salcedo, Sallie Chisholm

S1P45: Exploring the diversity, dynamics and distribution of picocyanobacteria in oligotrophic and eutrophic New Zealand lakes using eDNA metabarcoding. Lena A. Schallenberg, Carolyn W. Burns, Susie A. Wood

S1P06: Hanging from wires, marine picocyanobacteria use type IV pili as a strategy to remain suspended in the water column as well as avoid predation

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Planktonic microorganisms have evolved a range of strategies to stay afloat e.g. via intracellular gas vesicles or swimming via flagella. In marine ecosystems where energy and nutrients are scarce, planktonic organisms had, to date, no known mechanisms to avoid sinking and were thought to remain buoyant thanks to their reduced size and passive transportation within currents. Here, we identify for the first time the production of a type IV pili used by both *Synechococcus* and *Prochlorococcus* strains as a strategy to remain suspended in positions within the water column where conditions, i.e. nutrient and light levels, are optimal. This, seemingly costly mechanism also provides evasion from predation by grazers and, possibly, avoids phage dispersion amongst the population.

S1P07: Seasonal shifts in picocyanobacterial abundance and clade composition at an offshore and coastal station in the Baltic Sea

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Picocyanobacteria (free-living, <2 µm in diameter) in the Baltic Sea are highly diverse and significant contributors to total phytoplankton biomass. Picocyanobacterial abundances peak during summer, under high temperature and low nutrient conditions. Generally, monitoring of picocyanobacteria in the Baltic Sea have been limited to summer months and they are often overlooked in ecological models. In this study, we monitored phycoerythrin rich (PE-rich) and phycocyanin rich (PC-rich) picocyanobacterial abundance and

community composition at a coastal (K-station) and an offshore station (Linnaeus Microbial Observatory; LMO; ~10 km from land) weekly and biweekly over three consecutive years (2018-2020). Additionally, we evaluated the effect of in situ nutrients (NO_3, PO_4, SiO_2), temperature, salinity, stratification, presence of N_2-fixers and total phytoplankton biomass on picocyanobacterial dynamics. Cell abundances of picocyanobacteria were positively linked to temperature and negatively linked to NO_3 concentrations. PE-rich abundance was linked to the presence of N_2-fixers, but reached similar peak abundances at both stations (up to 3.8×10^5 cells mL^{-1}) from spring to summer at the K-station and from summer to autumn at the LMO, albeit the absence of N2-fixers at the K-station. PC-rich abundances were generally higher at the K-station (3 m depth, up to 2.1×10^5 cells mL^{-1}) compared to the LMO (39 m depth, up to 4.8×10^3 cells mL^{-1}) and was linked to salinity and stratification. This is probably due to its high adaptation to red light harvesting. The picocyanobacterial targeted amplicon sequencing provided a high number of amplicon sequence variants (ASVs; 2169 picocyanobacterial ASVs in total). The picocyanobacterial community was dominated by clade A/B, except during summer when low NO_3, high PO_4 and warm temperatures promoted S5.2 dominance. At the LMO, S5.2 had low relative abundance during summer, probably due to the low PO_4 concentration. The study revealed a high physiological diversity and shows differences in the dynamics and community composition at a coastal and offshore location in the Baltic Sea Proper. The results also show that there is a direct relationship between the environmental conditions and the community clade composition. Consequently, a deeper knowledge of picocyanobacterial community composition could help us understand its dynamics under different environmental conditions in a changing world.

S1P08*:* Mapping and modelling cyanobacteria across a hotspot of climate change

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Climate-change driven shifts in ocean currents will likely alter the dynamics of planktonic microbes that s ocean productivity, The effects of these changes are poorly understood, particularly in regions that span transition zones, for example between temperate and tropical waters, where the consequences of even subtle shifts may be amplified, In order to address this knowledge gap we examined the diversity and dynamics of cyanobacteria across the East Australia Current (EAC) system. The EAC is the major Western Boundary Current (WBC) of the Pacific, similar to other major WBCs, the EAC has intensified in strength and influence over the past decades, resulting in a 300km southward range expansion, and is therefore an interesting model system to study the consequences of climate change.

Phytoplankton communities were observed over four oceanographic voyages and in the context of three coastal time series over a period of six years. The abundance of *Synechococcus* and *Prochlorococcus* varied seasonally across the region and the major lineages of each partitioned across a broad transition zone below ~35˚S, i.e *Synechococcus* clades I and IV were present in the south, while clade II and *Prochlorococcus* HLII were abundant in the north. Two distinct *Synechococcus* lineages (sub clades IIe and IIh) characteristically appeared in transition zones, suggesting that these lineages have specifically adapted to the conditions along ocean fronts.

Flow cytometry, dilution incubations and elemental carbon analyses were used to characterise the standing stocks and the rates of viral lysis and grazing on cyanobacteria. Estimates calibrated with cellular carbon measurements on environmental populations show that cyanobacteria make a significant contribution to the carbon (C) standing stocks in the EAC. Distinct differences were observed for C flux through viral and grazing pathways, with up to 50% of the population being removed by viral lysis each day in the EAC. Carbon transfer to higher trophic levels via grazing was observed in all samples, however, C "recycling" due to viral lysis was significantly higher in the EAC in comparison to the cooler Tasman sea. These data were used to generate regional models and predictions that show where and when changing conditions will significantly alter the dynamics at the base of the food web, with consequences for the flow of C and energy through the ecosystem.

S1P09: Alpha-cyanobacteria possessing form IA RuBisCO globally dominate aquatic habitats

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Cyanobacteria are key players in the planet's carbon cycle with the vast majority of the phylum possessing a β-carboxysome and a form IB RuBisCO, including most cyanobacteria used as models in laboratories. The exception are the exclusively marine *Prochlorococcus* and *Synechococcus* genera that numerically dominate open ocean systems and possess α-carboxysomes and a form IA RuBisCO. To date, the reason why marine systems favour an α-form has remained elusive. Here, we report the genomes of 58 cluster 5 picocyanobacteria, closely related to marine *Synechococcus* that were isolated from freshwater lakes across the globe. Remarkably, we find all these isolates possess α-carboxysomes accompanied by a form 1A RuBisCO. Moreover, we demonstrate α-cyanobacteria dominate freshwater lakes worldwide. Hence, the paradigm of a separation in carboxysome type across the salinity divide does not hold true, and instead the α-form dominates all aquatic systems. We thus question the relevance of β-cyanobacteria as models for aquatic systems at large and pose several hypotheses for the reasons for the success of the α-form in nature.

S1P10: Picocyanobacteria community and cyanophage infection responses to nutrient enrichment in a mesocosms experiment in oligotrophic waters

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Prochlorococcus and *Synechococcus* are pico-sized cyanobacteria that play a fundamental role in oceanic primary production, being particularly important in warm, nutrient-poor waters. Their potential response to nutrient enrichment is expected to be contrasting and to differ from larger phytoplankton species. Here, we used a metagenomic approach to characterize the responses to nutrient enrichment in the community of picocyanobacteria and to analyse the cyanophage response during a mesocosms experiment in the oligotrophic Red Sea.

S1P11: Multiple Biofuel Producing *Synechococcus* strains from Himalayan Mountains

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Himalayan Mountain range is a fascinating landscape having some of world's highest peaks. In Pakistan it is an unexplored region for microbial diversity. Cyanobacteria is a class of autotrophic microbes that have great potential of economically important metabolites. In search of alternate energy sources, there is extensive research regarding production of microbial metabolites that can be used as fuel. Current study aims to explore the northern areas of Pakistan and isolate high biofuel producing cyanobacteria. More than 20 cyanobacterial strains were isolated from different locations of Himalayan range and among them, Synechococcus strains (SM-CUF-2 and SM-CUF-14) from two different locations i.e., Jalkhand (more than 10,000 feet above sea level) and Balakot (more than 3,000 feet above sea level) were selected. Production potential of three different kind of biofuels i.e., biodiesel, biohydrogen and bioethanol were analyzed from these isolates. Strains were cultures under different environmental and nutrient conditions and the production of biofuels was measured by gas chromatography. Usually, the cyanobacteria have the ability to store lipids in their cells to a great extent, even more than 60% of their biomass. So, they can be excellent source of lipids that can be converted to biodiesel. These strains showed a total lipid content up to 40% of their biomass whereas the maximum biodiesel productivity was up to 190 mg L^{-1} day^{-1}. Hydrogen is an important alternate fuel. The strain SM-CUF-14 showed hydrogen gas production up to 182 µmol mg Chl *a*-1 h^{-1} in presence of light and nitrate supplemented BG11 media. Most of the earlier studies showed ethanol production from microalgae through fermentation of its biomass. In current study, we measured the direct secretion of ethanol by the cyanobacteria in the culturing medium. The strain SM-CUF-14 showed ethanol production up to 165 mg L^{-1} in presence of light and nitrate supplemented BG11 media. The isolated strains have multiple biofuels production ability that can have extensive application in the biotechnology. These can be optimized and grown for production of biofuels at large scale.

S1P12: Marine *Synechococcus* and Ocean Color: Multiscale analysis of the adaptive advantage conferred by chromatic acclimation.

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The ongoing global change notably caused by the sharp rise in atmospheric CO2 is predicted to have numerous impacts on the physico-chemical properties of the ocean, among which the rapid expansion of warm nutrient-poor areas is known to impact the ocean color and thus to modify the underwater light niches of phytoplankton cells. As the second most abundant phytoplanktonic organism of the ocean and the most diversified one with regard to its pigmentation, *Synechococcus* constitute a very
good model to evaluate the consequences of changes in oceanic water optical properties on the distribution, dynamics and composition of marine phytoplanktonic communities. To date, seven *Synechococcus* pigment types (PT) have been identified, based on the composition of their antenna complexes, called phycobilisomes. While some strains have a fixed pigmentation, others are capable to change their pigmentation to capture either blue or green photons according to the dominant color
of the underwater environment. These type-IV chromatic acclimaters (CA4), which display two genetically distinct forms (CA4-A and CA4-B), were recently found to be the most abundant PT in the ocean [1], but the reasons for their prevalence in natural *Synechococcus* populations remain obscure.

The first objective of this study is to better understand the fitness advantage conferred by the CA4 process and the differences between CA4-A and -B. For this purpose, six strains were grown under different temperatures (18°C and 22°C), light intensities (15 µE and 75 µE) and colors (variable blue/green ratios). Several parameters (growth rate, chromophores and phycobiliproteins ratio, side scatter as a proxy of biovolume, photosystem II quantum yield as a proxy of photosynthetic efficiency)
were measured in each condition. The second objective is to highlight the seasonal variations of *Synechococcus* PTs over two years at two oceanographically different sites (SOMLIT-Astan, English Channel and Boussole, Mediterranean Sea), and to highlight potential depth partitioning of the different PTs. Data collected include *Synechococcus* cell abundance, phytoplankton pigments, metagenomic samples as well as contextual physico-chemical parameters such as temperature,
salinity and color and intensity of the light field as measured using a multi-spectral radiometer (C-OPS). All these data will ultimately be integrated into the global ocean Darwin model (http://darwinproject.mit.edu/research) to simulate the present global spatial and temporal distribution of *Synechococcus* PTs and predict the effect of global change on this population structure over the forthcoming decades.

S1P13: Assessing *Synechococcus* diversity across gradients of abiotic conditions in the oceans

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Synechococcus populations are very diverse, changing in their clade composition and abundance with depth, season and geographic location. Environmental conditions such as light, temperature and nutrient availability are likely to be the main drivers of these changes. Our aims are to understand how the diversity and composition of *Synechococcus* populations vary with environmental conditions and the underlying genomic features that enable microbes from each clade to succeed in specific environmental conditions. The Gulf of Aqaba is characterized by an annual convectional deep mixing event in the winter followed by stratification beginning in spring. The nutrient, light and temperature gradients throughout the euphotic zone vary quite significantly in the transitional period. Water samples from 20, 60 and 100 meter depths were collected bi-weekly from February to June during the spring bloom and once more in September in 2015 and 2016. From these samples we isolated ~1800 *Synechococcus* cultures to examine the genomic features of individual genotypes that composed the *Synechococcus* populations over changes in abiotic conditions. Isolates were identified based on two marker genes, *rpoC1* and *petB*, and approximately 85% were found to belong to clades II and X. Interestingly, these clades exhibit opposite trends, clade II being isolated from the whole mixed water column in winter and more often from the top 20 meters in spring while most clade X members were isolated in winter and for the ones isolated in spring a larger proportion were from 100-meter depth. To evaluate whether these patterns of *Synechococcus* isolates are also found using non-culture dependent methods, we sequenced metagenomes from the same samples. Here we will use them to assess the relative abundance of *Synechococcus* clades using a semi-quantitative, phylogenetically-aware read classification method. We will then compare the trends observed in the Red Sea to trends along similar abiotic gradients in the North Pacific Ocean, from the warm, oligotrophic Subtropical Gyre to the cold, nutrient rich Subpolar Gyre. Comparing the distribution and diversity of the different clades to the environmental gradients in these two distinct geographical locations will determine how *Synechococcus* genotypes shift as a function of abiotic factors and provide insights that transcend a particular geographic region.

S1P14: Introducing monitoring of picophytoplankton in the Baltic Sea proper

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The Baltic Sea is one of the planet's largest bodies of brackish waters. It is situated in a temperate climate zone and is characterized by large phytoplankton blooms during spring and summer. Currently, regular monitoring of picophytoplankton (<2 µm cell size) is largely absent in the Baltic Sea proper. Baltic Sea picocyanobacteria, primarily *Synechococcus* are known to be highly diverse and are thought to be dominated by unusual brackish adapted genotypes during summer. The dynamics of photosynthetic picoeukaryotes (PPEs) are largely unknown. To investigate the seasonal patterns of picocyanobacteria and PPEs, a coastal and an offshore station (~10 km from land) were sampled weekly and biweekly over two consecutive years (2018 and 2019). Picophytoplankton abundances were assessed using flow cytometry and isolates of PPEs and *Synechococcus* with diverse pigmentation to investigate the physiology of brackish picophytoplankton strains were obtained. In 2018, a cold winter was followed by an unusually warm summer allowing for consistently high abundances of *Synechococcus*. In the coastal station peaks in PPE abundances were observed during spring and late summer but these patterns were not as clear at the offshore station. During winter and fall, PPE reached similar or higher

abundances compared to that of *Synechococcus*. Overall, when compared with *Synechococcus*, the abundances of PPEs were more dynamic. During 2019, there were large shifts in abundances of both populations, especially at the coastal site, indicating that even weekly sampling did not provide high enough resolution to be linked with environmental variables. Preliminary results suggest that temperature is the main driver for picophytoplankton populations and that picophytoplankton blooms occurred earlier and reached higher abundances at the coastal station. This study provides the first long-term monitoring of picophytoplankton in the Baltic Sea proper and demonstrates that both *Synechococcus* and PPEs are present at significant abundances. Including picophytoplankton in future monitoring will be an important step towards a deeper understanding of this unique ecosystem.

S1P15: Phenology, diversity and protozoan grazing of *Synechococcus* at a long-term time series within the Gulf of Maine, USA

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Synechococcus is a genus of marine cyanobacteria that is ubiquitous across most marine waters. It is a primary producer that accounts for a significant proportion of primary productivity in the oceans and play a critical role in the global carbon cycle. *Synechococcus* cells have been counted weekly as part of a time-series in Booth Bay, Maine. For over 18 years (2001-2019), *Synechococcus* has bloomed annually in late summer, with rapid onset of the bloom over 1-2 weeks whereas the decline typically lasts many weeks. A strong seasonal pattern emerges over the annual cycle that correlates with temperature. Over the last decade, a trend has emerged where by the timing of the peak *Synechococcus* abundance occurs earlier in the year. These changes in the phenology between trophic levels due to changes in seasonal timing may alter recruitment success of higher trophic level species. *Synechococcus* are a highly diverse group of phytoplankton, yet little is known about the temporal distribution of different clades in the Gulf of Maine. The genetic variability of *Synechococcus* was examined at the Booth Bay site using rpoC1 and 16S rRNA gene sequencing. Clades I & IV were the dominant ecotypes, accounting for most of the *Synechococcus* abundance. Using qPCR the ecotype distribution of Clade I and IV was examined and demonstrated similar abundance estimates as determined by flow cytometry. DNA sequences showed a greater proportion of clade I rpoC1 sequences relative to clade IV. The impacts of grazing on *Synechococcus* were also determined using dilution experiments over the bloom period for two years, using 0.45 µm and 30 KDa filtered seawater in order to determine protozoan and viral-based mortality, respectively. The annual decline of *Synechococcus* was associated with significant grazing by small protists (<20 µm) over the course of the bloom period. Using ITS qPCR assays clade specific mortality estimates were also determined. Overall grazing pressure on the two clades was similar (~0.2-0.3 per day). Identification of the putative grazers was determined using cytometry (phycoerythrin incorporation) and the protists were selectively bulk sorted, followed by whole genome amplification, and next-gen sequencing. Resulting 18S rRNA gene sequences identified many ciliates, choanoflagellates, telonema, and cercozoans as the potential protist grazers of *Synechococcus*. This data set contributes to our understanding of grazers and *Synechococcus* diversity and ecology in the Gulf of Maine.

S1P16: Comparative study of the ammonium transporter amt1 in *Prochlorococcus* and *Synechococcus*

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The photosynthetic marine picocyanobacteria *Prochlorococcus* and *Synechococcus* inhabit broad regions of the oceans contributing significantly to the global primary production. They are also pivotal in the nitrogen and carbon biogeochemical cycles. Nitrogen, in particular, has been considered as one of the main driving forces of evolution in cyanobacteria due to its limited availability in the ocean. Furthermore, cyanobacteria have evolved a fine control of the nitrogen metabolism based on the regulation of key enzymes and the assimilation of various forms of nitrogen.
The gene *amt1* encodes for a permease of high affinity for ammonium. In the current study we have analyzed the *amt1* gen and promoter region and have identified binding sites for NtcA (a transcription factor involved in the regulation of nitrogen metabolism) in all the studied strains, suggesting that the *amt1* gene expression could be regulated by nitrogen availability. Gene expression studies confirmed that amt1 expression responds to nitrogen variations in both groups of cyanobacteria (*Prochlorococcus* and *Synechococcus*). Both nitrogen starvation and an augmented concentration of ammonium in the media, promoted a significant increase in the expression of the *amt1* gene.
Finally, we have started the production of a recombinant *Synechococcus elongatus* PCC 7942 strain expressing the amt1 gene from *Synechococcus marinus* WH7803. This strain will allow a more detailed characterization of the transport kinetics and regulation of the AMT1 transporter.

S1P17: A meta-analysis of different methods to estimate the contribution of *Prochlorococcus* to global marine primary production

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Marine cyanobacteria have been estimated to contribute ~25% of global marine net primary production. *Prochlorococcus* dominates the marine cyanobacteria community in many ocean regions, especially in the warm oligotrophic oceans, and as such it makes a significant contribution to global ocean primary production. Some studies have directly estimated, via filter fractionation or pigment labeling, this contribution in some oceanographic regions such as parts of the Pacific Ocean, Indian Ocean, and Atlantic Ocean. Other studies have calculated the global contribution by combining abundance data and estimated growth rate and carbon content. However, direct measurements are often limited to local regions and global estimates are poorly constrained because of variability in input parameters. To address this gap, here we performed a meta-analysis using available literature containing estimates of the contribution of *Prochlorococcus* to primary production. The goal of this work is to identify sensitivities in global estimates to available data and parameters to chart a path towards combining existing datasets (across measurement approaches) and identifying regions that require additional measurements. The long term goal is to provide a global map and integrated assessment of the primary production done by *Prochlorococcus.*

S1P18: Phototroph-heterotroph interactions during growth and long-term starvation across *Prochlorococcus* and *Alteromonas* diversity

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Interactions among microorganisms are ubiquitous, yet to what extent strain-level diversity affects interactions is unclear. Phototroph-heterotroph interactions in marine environments have been studied intensively, due to their potential impact on ocean ecosystems and biogeochemistry. Here, we characterize the interactions between five strains each of two globally abundant marine bacteria, *Prochlorococcus* (a phototroph) and Alteromonas (a heterotroph), from the first encounter between individual strains and over more than a year of subsequent co-culturing. *Prochlorococcus-Alteromonas* interactions affected primarily the dynamics of culture decline, which we interpret as representing cell mortality and lysis. The shape of the decline curve and the carrying capacity of the co-cultures were determined by the phototroph and not the heterotroph strains involved. Comparing various models of culture mortality suggests that death rate increases over time in mono-cultures but decreases in co-cultures, with cells potentially becoming more resistant to stress. During 435 days of co-culture, mutations accumulated in one *Prochlorococcus* strain (MIT9313) in genes involved in nitrogen metabolism and the stringent response, indicating that these processes occur during long-term nitrogen starvation. Our results suggest potential mechanisms involved in long-term starvation survival in co-culture, and highlight the information-rich growth and death curves as a useful readout of the interaction phenotype.

S1P19: Picocyanobacteria in hypertrophic waters reach high numbers and variability

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Although picocyanobacteria of the *Synechococcus-Cyanobium* group have been studied intensively in oligotrophic waters, they are heavily underestimated and neglected in eutrophic and hypertrophic waters. On a large set of hypertrophic fishponds, that are used for intensive aquaculture, we have demonstared that picocyanobacteria are very abundant and important here. In some cases they reached up to 10^7 cells per ml and played a key role in the trophic chain. Each living form had different predators and different function in the trophic chain. The single-cell form in the spring was grazed by protozoa (especially by several different kind of cilliates) and the colonial in summer was heavily grazed by zooplankton (especially by *nauplius* and *Daphnia*). Microbial and picocyanobacterial genetic diversity was investigated by next-generation-sequencing. This research is very important for understanding of the complex view on functioning of hypertrophic fishpond systems and the nutrient flow from sediment to upper trophic layers.

S1P20: Heterotroph Diversity in Enrichment Cultures of Marine Picocyanobacteria

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Prochlorococcus and *Synechococcus* together are responsible for around 10% of global net primary productivity, placing them firmly at the base of marine microbial food webs. Heterotrophic bacteria are often co-isolated with the cyanobacteria when enrichment cultures are established from seawater samples. These enrichments contain no added organic carbon, so the heterotrophs must sustain their growth using organic carbon fixed by the autotrophs. The diversity of heterotrophic communities in these enrichments is generally unknown. By virtue of environmental selection and serial passage for 100s to 1000s of generations in the lab, these cultures present ideal mesocosms for examining the selective pressures exerted by *Prochlorococcus* and *Synechococcus* on heterotroph communities. Here we examine the diversity of heterotrophs - using 16S rDNA sequencing - in 74 enrichment cultures of *Prochlorococcus* (n=56) and *Synechococcus* (n=18) obtained from the global oceans. Heterotroph communities tended to be more similar among cultures from the same genus than between. Surprisingly, *Prochlorococcus* strains belonging to the deepest branching clade, LLIV, exhibited the most distinct heterotrophic community compositions, that is, dissimilar both from other clades of *Synechococcus* and *Prochlorococcus* and dissimilar from each other. These findings suggest that selective pressures are not uniform across picocyanobacterial cultures. Specific heterotrophic genera, including Alteromonas and Marinobacter, were present and abundant in 31 and 50 of 74 cultures, respectively, highlighting their capacity for opportunistic growth. Indeed, the majority of heterotrophs present in the cultures are rare in bulk seawater, implying that enrichment conditions favor the opportunistic outgrowth of rare (and the die-off of abundant) bacteria from oligotrophic waters. Further, several heterotrophic genera were only present in single cultures, suggesting that stochastic effects from bottlenecking lead to unique heterotrophic communities, even in enrichments obtained from the same water samples and under the same culturing conditions. The artificial association of rare and minimally abundant marine heterotrophs in picocyanobacterial cultures may introduce additional selective pressures not seen in the oligotrophic ocean, and we consider the implications of these now frequent interactions on evolution in the laboratory.

S1P21: A comparative analysis of the temporal variability of *Synechococcus* and SAR11 across phylogenetic ranks at a temperate coastal ocean site

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Synechococcus sp. are important cyanobacterial primary producers in the global ocean and dominate many coastal ecosystems. SAR11 are numerically dominant heterotrophic bacteria across these same locations. There is evidence that these major taxa each harbor substantial fine scale phylogenetic diversity that structures along environmental gradients. While there is a growing understanding of the diversity of marine microbiomes, it remains difficult to disentangle the effects of different environment variables and processes in driving the composition and functioning of these communities. Here we take a comparative analyses approach, using co-occurring dominant autotrophic and heterotrophic bacteria to identify similarities and differences of response to the environment across taxa and taxonomic ranks. Using observations from a temperate coastal ocean observatory (Pivers Island Coastal Observatory – Beaufort, NC, USA) that has substantial and repeatable environmental variability, we show strong and repeatable annual patterns of both *Synechococcus* and SAR11 communities, with the highest compositional dissimilarities

found ~6 months apart. However, when compared to the SAR11 community, *Synechococcus* has a larger magnitude of seasonal change, but reduced differences between taxonomic ranks (oligotype-level vs. OTU-level). Specific populations can be clustered in response groups across taxa and linked to environmental drivers. These broad differences among SAR11 and *Synechococcus* communities as well as similarities among specific populations help to tease apart the ecological (and evolutionary) mechanisms structuring populations in response to changing environments.

S1P22: Phylogenomic analysis of novel *Prochlorococcus* and *Synechococcus* genomes

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The rapidly growing number of available microbial genomes and metagenomes provides new opportunities to investigate the ecology and functioning of marine microbial communities. In this context, a particularly high number of picocyanobacterial genomes have been recently released both from strains (WGS) and environmental samples (SAGs and MAGs), providing an unprecedented source of information for omic analyses. Genome data from Cyanobacteria Cluster 5 (sensu[1]), including *Prochlorococcus*, *Synechococcus* subcluster (SC) 5.1 and 5.3 and *Cyanobium* (SC 5.2) genera were retrieved from Genbank and JGI and those lacking gene predictions were annotated using Prokka[2]. To benefit from the rich, manually curated functional annotation of the Cyanorak information system[3], CDS, tRNA and rRNA were assigned to Cyanorak Clusters of Likely Orthologous Genes (CLOGs) using reciprocal Blast and HMM profiles. Furthermore, all genomes were taxonomically assigned based on their k-mer composition using kraken-Uniq and on the *petB*, *rpoC1* and 16S rDNA gene markers available within the Cyanomarks database (poster by Farrant et al.). These numerous novel genomes greatly improved the genetic diversity coverage within Cluster 5, in particular for some little or not yet represented taxa, such as *Synechococcus* clades EnvA or EnvB (a.k.a. as CRD2) and *Prochlorococcus* HLIII and IV. Still, many of the retrieved genomes were tiny, while some were either redundant with available genomes or even chimeric, as shown by inconsistencies between taxonomic assignments based on different genetic markers, high contamination level and/or highly divergent kmer composition. Based on this information, a selection of subsets of genomes was made for further omic and phylogenetic analyses. All marker genes were integrated into CyanoMarks and were used to refine the taxonomy of picocyanobacteria either by reconciling existing taxa obtained based on different marker genes or by identifying new taxa. Some of these genomes were also integrated into Cyanorak for comparative genomic analyses and visualization (e.g. genomic context, phyletic pattern, etc.). Finally, these genomes were also used as references for metagenomic recruitment in order to identify specific genes or metabolic pathways potentially involved in the adaptation to the main marine ecological niches. Altogether, this wealth of novel, manually curated genomic information constitutes a considerable asset to investigate the ecology and evolution of these important components of the marine ecosystem.

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S1P23: HTS screening for *Prochlorococcus* and *Synechococcus* in sea water and sediment impacted by mariculture

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Cyanobacterial indicators are less represented in marine environment than in more eutrophic freshwater ecosystems, where *Cyanobacteria* often form extensive blooms. Furthermore, they are more scarce when investigating mariculture impacted sites, even though aquaculture is the fastest growing food industry and generates additional pressure on coastal areas. Sampling of sea water and sediment was conducted seasonally in 2017 and 2018, on the fish farm of European sea bass and nearby control site. Sampling sites are situated in the southern Eastern Adriatic Sea, in semi-enclosed bay under the influence of the freshwater river. Sea water parameters were measured in situ (oxygen, temperature, conductivity, turbidity, pH, TDS) and in laboratory (total P, total N and SiO2). Sediment granulometric analysis was performed. eDNA was extracted from 16 sea water and 16 sediment samples. High-throughput sequencing was performed targeting V1-V3 variable region of 16S rRNA gene. Data was processed in QIIME 2 2019.4, using taxa filtering to include only cyanobacterial ASVs and excluding chloroplast and mitochondrial sequences. Phylogenetic tree was constructed in iTOL 4.4.2., and statistical analysis performed in RStudio 1.2.1335. In sea water was revealed predominance of eutrophic, coastal strain *Synechococcus* CC9902 (20-70% ASVs) in all seasons during both years. *Synechococcus* CC9902 was recorded as potential bloom-forming strain which can furthermore negatively impact fish behavior. *Prochlorococcus* was found to be low-light adapted ecotype *Prochlorococcus* MIT9313. Moreover, this strain is known to utilize organic nitrogen compounds (urea and amino acids) generated by the fish in the farming process. *Prochlorococcus* MIT9313 had inconsistent presence in samples of different years (up to 29% in 2017 and 1.4%-10.1% in 2018, respectively), although totally absent from winter samples 2017 and summer 2018. In sediment, strain adaptable to light/oxygen deprivation was found to be *Synechococcus* PCC-7336. It was mostly present in sandy gravel type of sediments on control site, in relative abundances of 1.6 - 14.6% in 2017 and 2.3 - 18% in 2018. Other ever-present genus in water samples was Cyanobium PCC-6307, whereas in sediments total of 13 genera was found in 2017 sampling and 9 genera in 2018. Predominant in all sampling points were benthic genera Pleurocapsa PCC-7319 and Xenococcus PCC-7305. Discovered *Prochlorococcus* and *Synechococcus* strains are indicating more eutrophic conditions then implied by water quality parameters and sediment type. This opens new questions in monitoring eutrophication indicators in the mariculture.

S1P24: Co-existence of *Prochlorococcus* and *Synechococcus* sustained by their different uptake affinity of ammonium and nitrate

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Prochrolococcus and *Synechococcus* are the two major pico-sized phytoplankton in the ocean dominating the oligotrophic gyres. The basis for their co-existence in these biomes is not understood, but has been hypothesized to result from different grazing pressure. Here we combine laboratory experiments and an ecological model to show that this co-existence can instead arise from specialization in the uptake of distinct N substrates. In field incubations, the response of both *Prochlorococcus* and *Synechococcus* to nanomolar N amendments demonstrates N limitation in both populations, but *Prochlorococcus* showed a higher affinity to NH4+ whereas *Synechococcus* is more adapted to NO3- uptake. A simple ecological model suggests that this different uptake capacity of these organisms for NO3- and NH4+ sustain their co-existence. Due to the predicted increase in stratification leading to reduced NO3- supply in the future climate, the model predicts that the relative abundance of *Synechococcus* will decrease. The existence of nitrogen fixer may favor *Prochlorococcus* since their exudation of NH4+ increases the relative abundance of NH4+ to NO3-. Our study suggests that resolving these different nitrogen species and distinct uptake capacities are essential in predicting co-existence of these organisms. Our results show a mechanism for species co-existence based on the uptake of the same element (here N) that may be common in microbial resource competition.

S1P25: Distribution of *Prochloroccocus* and *Synechococcus* in the Spanish Mediterranean (RADMED Time Series).

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In this study we propose a global analysis of the temporal (seasonal) and spatial *Synechococcus* and *Prochlorococcus* distribution in the Spanish Mediterranean. Data were obtained by flow cytometry of the samples collected in the RADMED surveys. The RADMED project is a monitoring program funded by the Instituto Español de Oceanografía. This program was launched in 2007 and it is devoted to the implementation and maintenance of a monitoring system around the continental shelf and slope around the Spanish Mediterranean waters. Therefore, we analyzed more than 10 years time series of *Synechococcus* and *Prochlorococcus* abundances that allow us to distinguish both spatial and temporal distribution patterns of the populations of these microorganisms.

S1P26: The effect of co-culture remodels the transcriptomes of *Prochlorococcus*, *Synechococcus* and co-cultured helper bacterium *Alteromonas* during elevated CO2 growth.

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Prochlorococcus and *Synechococcus* depend on heterotrophic bacteria for accomplishing essential functions. The effect of elevated CO_2, however, can break such interactions. We aimed to understand the effects of co-culture on cyanobacteria and the 'helper' bacterium *Alteromonas* during elevated $pC0_2$ growth. We have co-cultured one strain of *Prochlorococcus* (P. marinus MIT9312) and two different strains of *Synechococcus* (sp. CC9311 and sp. WH8102) along with the heterotrophic bacterium *Alteromonas* sp. EZ55 at either current (400 ppm) or year 2100 CO_2 concentrations (800 ppm). We have extracted and sequenced RNA from each co-cultured cyanobacterium and EZ55, as well as EZ55 in axenic culture, and performed a differential gene expression analysis using the Rsubread/edgeR pipeline. Our data showed that *Prochlorococcus* significantly decreased expression of RUBISCO, carboxysome and high-light inducible (HLI) genes while *Synechococcus* WH8102 increased RUBISCO and carboxysome genes. The response of *Synechococcus* CC9311 was unclear. In response to elevated pCO_2, EZ55 had 116 and 17 downregulated and upregulated, respectively. Remarkably, the effect of co-culture resulted in a much higher number of up- and down-regulated genes (580 and 564, respectively). Pathway analysis revealed cyanobacterial strain-specific significantly differently expression of TCA cycle, glycolysis, glyoxylate and dicarboxylate metabolism, glycine, serine and threonine metabolism carbon metabolism, proponoate metabolism, valine, leucine and isoleucine degradation, fatty acid degradation, bacterial chemotaxis among others. The impact of year 2100 pCO_2 is expected to greatly affect the availability of organic and inorganic nutrients and in determining physiological stress states of marine microbial communities.

S1P27: Characterization of novel *Synechococcus* isolates representing key ecotypes in the Baltic Sea

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Among picocyanobacteria, *Synechococcus* is increasingly recognized as a significant contributor to primary productivity in coastal and estuarine systems. *Synechococcus* is a relevant player in the Baltic Sea, one of the largest brackish water areas in the world, characterized by gradientes in salinity and nutrients and strong seasonality. The dominance and high diversity of *Synechococcus* in the Baltic Sea has been recently recognized. Until now only a few of the key strains have been characterized which limits inference with sequencing data and our understanding of the role of the multiple co-occurring ecotypes in the ecosystem.
In this study, we present 17 novel strains of *Synechococcus* isolated from the Baltic Sea. The strains were identified using fragments of the 16S rRNA, and phycocyanin and phycoerythrin genes. In addition, we assessed their relative contribution to picocyanobacterial amplicon sequence libraries from a coastal and offshore monitoring station.
The strains varied in terms of pigment composition, and grouped together with estuarine and freshwater strains belonging to subcluster 5.2, and separated from marine strains. Four isolates (KAC 102, KAC 106, KAC 108, KAC 114) were selected for further

physiological characterization based on their diverse phylogeny and contribution to the *Synechococcus* community. The four selected strains were able to grow in a wide range of light (10, 100, 190 µmol photons $m^{-2}s^{-1}$), temperature (15, 20, 25, 30°C), and salinity (2, 6, 10, 14 PSU) conditions, but responded differently to the treatments. For instance, phycoerythrin-rich KAC 108 was susceptible to increases in salinity while phycocyanin-rich KAC 102 outgrew the rest under the highest temperatures and irradiances. In the field, temperature and salinity were the main drivers of *Synechococcus*, and the dynamics of the ecotypes were in concordance with the laboratory assays. Overall, the assays with the isolates in combination with patterns of ASV evidenced the diverse physiological capacities of co-occurring *Synechococcus* populations in the Baltic sea. These results contribute to better predicting the distribution of *Synechococcus* under future scenarios of global change.

S1P28: Reevaluation of the distribution of *Synechococcus* and *Prochlorococcus* in marine phytoplankton communities

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Marine picocyanobacteria (*Prochlorococcus* and *Synechococcus*) are widespread and are the most abundant phytoplankton in the global ocean, but their relative importance in comparison to other phytoplankton groups (photosynthetic eukaryotes, photosymbionts, and other cyanobacteria) is still unclear. In addition, most studies are focused on the free-living representatives of these two genera, while the role of aggregation and symbiosis remains poorly described. Here, we explored the metagenomic and high-throughput imaging datasets from size fractionated samples (0.22-3, 0.8-5, 5-20, 20-180, and 180-2000 µm) of plankton communities obtained by *Tara* Oceans at a global scale. Using a set of photosynthetic marker genes, we were able to estimate the relative abundances and biogeographical patterns of the main prokaryotic and eukaryotic photosynthetic groups, and determined the environmental factors that shape these patterns. Unexpectedly, picocyanobacteria were found to be abundant in high size fractions, and numerous images of picocyanobacterial aggregates and symbionts were found among the high-throughput confocal microscopy image dataset. Overall, this work implies the need to reevaluate our current appreciation of the roles of picocyanobacteria in marine phytoplankton communities.

S1P29: A proposal of a genome based taxonomy of *Synechococcus*.

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The genus *Synechococcus* is a major contributor to global primary productivity. It is found in a wide range of aquatic ecosystems, such as freshwater, seawater and brackish water

environments. In contrast to the related group of *Prochlorococcus*, which is characteristic of high temperature, oligotrophic waters, *Synechococcus* is more metabolically diverse, with some species thriving also in polar and nutrient-rich locations. Although many studies have discussed the ecology and evolution of *Synechococcus*, only some very few have addressed its taxonomical status, and this issue still remains largely ignored. Using 144 publicly available *Synechococcus* genomes and based on recent advances on genomic taxonomy, we present a review of the taxonomy of *Synechococcus* based on genomic features such as GC content, genome size, pairwise AAI and ANI values, core-genome and MLSA phylogenies and in silico phenotypes, which include characters like carbon fixation methods and light-utilization strategies. Our study enhances taxonomic support to previously proposed genera within the *Synechococcus* group. These genera are broadly divided into two main evolutionary lineages: (1) seawater and (2) freshwater and thermal environments. The former encompasses the two genera with the highest number of representative genomes, the previously proposed *Parasynechococcus* and *Pseudosynechococcus* (with 28 and 27 genomes, respectively), and other marine genera. The latter lineage represents the type species for *Synechococcus* elongatus, the PCC 6301 strain, along with other strains e.g. the thermophilic proposed genus *Leptococcus*, isolated from hot spring microbial mats. Type strains were chosen based on the literature when possible and by empirical criteria such as genome completeness, submission date or whether the genome is derived from single-cell sequencing instead of being a metagenome-assembled genome. This new taxonomic division reflects the ecological characteristics and evolutionary relationships of the group, with each newly proposed genus being distinct in terms of niche adaption, geographical distribution and ecological characteristics. It is also aligned with the current paradigm of genomic taxonomy which is becoming ever more prevalent between microbiologists, thus providing a practical and theoretical framework for the description of newly sequenced organisms contained within or related to the *Synechococcus* group.

S1P30: Independent and interactive effects of copper and irradiance on coastal *Prochlorococcus* and *Synechococcus* communities

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Microbial competition is strongly influenced by resource availability. Resource scarcity limits phytoplankton growth rates and determines whether coexistence between populations is possible. Certain resources can also limit growth rates at high levels through toxicity. Copper (Cu) and light are two well-characterized resources that can limit phytoplankton growth at very low (deficiency) or very high (toxicity) levels. In this study, four field incubation experiments were conducted with natural phytoplankton assemblages from coastal California during September (experiments A and B) and October (experiments C and D) when the phytoplankton community is dominated by *Synechococcus* and *Prochlorococcus*, two genera of picocyanobacterial known to be sensitive to Cu and irradiance. Experiment A used surface water, B used water from the deep chlorophyll maximum, C used surface water, and D used surface water with added nitrate. The experiments utilized a fully factorial design, with seven Cu concentrations and seven irradiance levels. Interactive effects between Cu concentration and irradiance were assessed using multiple linear regression. In experiments A and B, irradiance had a significant main effect on chlorophyll a, with stronger growth responses observed under low light conditions. No interactive effects with Cu were observed. For experiment C, there

was a significant positive correlation between Cu and chlorophyll a concentration, whereas for experiment D, Cu concentration was significantly negatively correlated with chlorophyll a concentration. Although no interactive effects were identified from the regression, the marginal means for each independent variable in experiment C suggest that antagonistic interactions may be possible for higher Cu concentrations. *Prochlorococcus* and *Synechococcus* displayed different thresholds of tolerance to Cu and irradiance, leading to shifts in their relative abundances. Collectively, these experiments demonstrate that multiple, simultaneous stressors can cause shifts in the phytoplankton community composition that reflect the tolerance thresholds of each group, and provide insight into how stratification, mixing, and upwelling shape picocyanobacterial communities by influencing nutrient, trace metal, and light availability.

S1P31:Trans-domain comparison reveals unexpected abundance of picocyanobacteria in large size fractions.

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Marine picocyanobacteria (*Prochlorococcus* and *Synechococcus*) are widespread and are the most abundant phytoplankton in the global ocean, but their relative importance in comparison to other phytoplankton groups (photosynthetic eukaryotes, photosymbionts, and other cyanobacteria) is still unclear. In addition, most studies are focused on the free-living representatives of these two genera, while the role of aggregation and symbiosis remains poorly described. Here, we explored the metagenomic and high-throughput imaging datasets from size fractionated samples (0.22-3, 0.8-5, 5-20, 20-180, and 180-2000 µm) of plankton communities obtained by *Tara* Oceans at a global scale. Using the universal photosynthetic single-copy gene *psbO*, we were able to estimate the relative abundances and biogeographical patterns of the main prokaryotic and eukaryotic photosynthetic groups, and determined the environmental factors that shape these patterns. Unexpectedly, we detected a high abundance of both *Prochlorococcus* and, in particular, *Synechococcus*, in the large size fractions (>5 µm) across multiple and geographically distinct basins of the tropical and subtropical regions of the world's ocean. We then examined the *Tara* Oceans confocal microscopy dataset, and found many microscopy images evidencing colony formation and symbiosis in the same size-fractionated samples. These results suggest that we should move from the traditional view of *Synechococcus*/*Prochlorococcus* as being exclusively part of picoplankton communities, and instead we should consider them as part of a broader range of the plankton size spectrum.

S1P32: Annual dynamics of cyanophages and their hosts in the Sargasso Sea.

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Cyanobacteria of the genera *Prochlorococcus* and *Synechococcus* are the most abundant primary producers in the ocean. They are widely distributed across different geographic regions and both genera display seasonal dynamics due to changes in environmental

conditions. Cyanophages that infect cyanobacteria are made up mainly of the T4-like myoviruses and T7-like podoviruses. To understand cyanobacterial-cyanophage population dynamics in the Sargasso Sea, we are sampling monthly at the Bermuda Atlantic Time-series (BATS) station. BATS is located in the northwest Sargasso Sea and it is an example of an oligotrophic gyre system in which cyanobacterial abundances vary quite dramatically over an annual cycle, with *Synechococcus* most abundant in the spring and *Prochlorococcus* most abundant in the summer-fall. Data on cyanophages abundances and distribution is still very limited for nearly all parts of the oceans and is non-existent in this region. In this project our aim is to evaluate the seasonal effect on cyanophage abundances over depth profiles and to assess how seasonality affects the extent of viral infection of cyanobacteria. Here I will show annual dynamics of T4- and T7-like cyanophage abundances in the Sargasso Sea using the solid-phase PCR-based polony method developed in our lab. This research will help to better understand the ecology of cyanophages and their interaction with cyanobacterial host over the seasonal cycle in the Sargasso Sea.

S1P33: Phage infection of a nutrient-deplete *Synechococcus* host

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The cyanobacterial genus *Synechococcus* is one of the most abundant phototrophs in the world ocean. Oligotrophic areas (aka nutrient deserts) represent a challenge for these microbes to thrive, especially limitation for essential macronutrients like phosphate (Pi). In addition, biotic factors like viral infection also reduce productivity and growth. We sought to understand viral infection dynamics under nutrient-deplete growth – a scenario likely to be common in oligotrophic gyres. Using cyanophage S-PM2 and *Synechococcus* sp. WH7803 as the phage-host model we examined infection dynamics following Pi-deplete host growth. We observed a delay in cell lysis, and when lysis occurred, a reduction in the burst size (i.e. the number of progeny phage released). Transcriptomics analysis showed the specific upregulation of several cyanophage genes, including a cluster of genes that contain upstream Pho boxes suggesting regulation by the host PhoBR two-component system. Using purified PhoB and electrophoretic motility shift assays (EMSAs) we demonstrate PhoB regulation of a sub-set of these genes including the cyanophage DNA polymerase. Thus, whilst phage productivity is reduced under infection of a Pi-deplete host we provide a molecular basis for how some phage progeny are produced via hijacking the host regulatory machinery to prioritize its replication under low Pi.

S1P34: The island mass effect drives shifts in *Prochlorococcus*:*Synechococcus* ratios from nearshore Oahu, Hawaii to oceanic waters.

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Islands in oligotrophic ocean regions act as local sources of nutrients. These nutrients originate from land-based inputs such as rivers, lagoons, and groundwater, and from the upwelling of oceanic nutrients driven by the abrupt bathymetric features of islands interacting with ocean tides and currents. Together, these processes create the island

mass effect, a very common feature observed throughout oligotrophic ocean basins where increased concentrations of chlorophyll-a are found in coastal island waters compared to nearby oceanic waters. While the island mass effect has been thoroughly described via satellite chlorophyll-a observations, the features of the phytoplankton community that are influenced by the enhanced nutrient availability are not well documented. From 2012-2020, chlorophyll, nutrient, and picoplankton samples were collected from multiple depths on quarterly to bimonthly cruises at sites 1.6 km, 2 km, and 7 km south of O'ahu, Hawai'i. *Prochlorococcus* and *Synechococcus* were enumerated using flow cytometry. We compare data from these nearshore coastal sites to an oceanic site 100km offshore of O'ahu (The Hawai'i Ocean Time Series data from Station ALOHA). Consistent with the expected island mass effect, extracted chlorophyll-a concentrations were significantly enhanced at all coastal sites compared to Station ALOHA. *Prochlorococcus* concentrations increased modestly with greater distance from shore at all depths; *Synechococcus* concentrations in the mixed layer showed a significant decrease with greater distance from shore. However, comparisons of cell count profiles with chlorophyll-a depth profiles show that the increase in *Synechococcus* populations near the island does not solely drive the increased chlorophyll-a observations in the nearshore environment. Seasonal patterns in *Synechococcus* abundance are evident, with an increase in abundance during the summer (dry) season. While land-based nutrient input during the dry season may actually decrease due to lower rain amounts, wind conditions may favor upwelling of oceanic nutrients on south O'ahu during this time, when trade winds are stronger and more consistent. This study is a step towards characterizing the cross-shore shift in phytoplankton community distribution due to the island mass effect, which has implications for carbon fixation, nutrient cycling, and food web dynamics in oligotrophic island ecosystems.

S1P35: Identification of Ecologically Important *Synechococcus* Clade II Isolates That Exhibit Genome Reduction Without GC Decrease

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Synechococcus spp. are globally abundant photoautotrophs and large drivers of the Earth's carbon cycle. Recently, we presented 12 new, high-quality draft genomes of marine *Synechococcus* isolates spanning several clades. We used phylogenomic and pangenomic methods with previously available reference genomes and ~100 environmental metagenomes (largely sourced from the TARA Oceans project) to assess the global distributions of these currently available genomic lineages. This provided heretofore-unattained resolution into genus-level variability both within and across samples. We find that the newly sequenced clade II isolates are by far the most representative of recovered in situ populations. These genomic lineages possess the smallest genomes yet recovered in *Synechococcus* (2.14 ± 0.05Mbps; mean ± 1SD), while concurrently hosting some of the highest GC contents (60.67 ± 0.16%). This is in direct opposition to *Prochlorococcus* wherein decreasing genome size via genomic streamlining coincides with a strong decrease in GC content suggesting this sub-clade of *Synechococcus* appears to have convergently undergone genomic streamlining, but via a fundamentally different evolutionary trajectory. Regarding *Synechococcus* ecology, we find

that selection across the marine environment plays a larger role than dispersal, and we demonstrate the value of utilizing the entire genetic complement identified in the current pangenome (~85,000 genes) to functionally characterize in situ populations.

S1P36: Impact of diverse nutrient regimes on cyanophages

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Although phytoplankton biomass is less than 0.5 % of the total mass in photosynthetic organism on earth, they contribute approximately half of the global productivity due to their fast turnover. The marine unicellular cyanobacteria of the genus *Synechococcus* and *Prochlorococcus* dominate vast oceanic areas. Their abundance and activity are regulated by abiotic factors as well as by biotic factors such as grazing, competition and infection by phages. With an estimated abundance of 107 viruses ml-1 seawater, marine bacteriophages are numerically the most abundant DNA-containing entities in the open ocean. Infection by phages has a profound impact on bacterial populations and on the biogeochemical cycling of matter. Current estimates suggest that 1023 infections occurring every second. Amongst others, cyanobacteria are strongly impacted by these infections. Between 0,005 % and 30 % of the cyanobacterial population is estimated to be killed by phages on a daily basis. Even though several studies have focused on viral diversity, phage host specificity and viral abundance in the marine system, the specific impact of cyanophages remains unclear. Here, we focus on the infection dynamics of the two major cyanophage families: T7-like and T4-like phages. To get a better understanding of host-virus interaction it is crucial to get new insights in the virus production and decay in different nutrient regimes. Such virus production assays enable us to assess virus population dynamics as well as to provide community level estimates of virus-induced microbial mortality rates. Previous studies have already shown that virus-like-particle (VLP) production varies over time and space in the open ocean. Virus production assays for total viruses revealed significantly higher production in nutrient rich cyclonic compared to a more nutrient deplete anticyclonic eddy in the North Pacific Ocean. Nonetheless, there was no significant difference in bacterial standing stock in these two eddies, suggesting a higher turnover rate of bacteria in the cyclonic eddy. Yet, specific cyanophage infection properties in response to environmentally relevant conditions, such as changes in nutrient availability, remains to be explored. The recently adapted solid-phase single-molecule PCR polony method for cyanophages enables us to quantify the abundance of the different cyanophage families and the extent to which they infect cyanobacteria. Combining this methodology with the virus production approach will provide new insight into the host-virus-environment interaction specifically for cyanophages

S1P37: Modeling ocean color niche selection by *Synechococcus* chromatic acclimaters

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Light penetrating the ocean creates colorful underwater niches for photosynthesis, and the selection pressure for pigment types adapted to these colors within *Synechococcus* is not well understood. We investigated ocean conditions that led to high proportions of *Synechococcus* blue-green acclimaters (generalists) over

other *Synechococcus* pigment types that do not acclimate (specialists) in a model ocean column. We then compared the output of our simulations to the percentage of generalists distributed throughout the global ocean using *in situ* metagenomic markers from Bio-GO-SHIP cruises, supplemented with GEOTRACES, and *Tara* Oceans, and coupled with physical oceanographic data. We found that large mixing depths selected for generalists in simulated *Synechococcus* competition for light, but that deep mixing did not correlate with generalists in situ. Instead, oceanographic signatures for upwelling and possibly ocean frontal zones saw higher percentages of generalists in the *Synechococcus* population.

S1P38: Cyanobacterial sources of free DNA in the North Pacific Subtropical Gyre

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Over half of the DNA in the open ocean is present outside living organisms. This exocellular DNA that passes through a membrane filter (0.1 or 0.2 µm) is dissolved (D-DNA) and is comprised of three known pools: viruses, extracellular vesicles, and “free” DNA (F-DNA). Using a method that provides separation of these three fractions, we compared open ocean depth profiles of DNA associated with each. Pelagibacter-like DNA dominated the vesicle fractions for all samples examined over a depth range of 75 - 500 m. The viral DNA fraction consisted predominantly of myovirus-like and podovirus-like DNA and contained the highest proportion of unannotated sequences. Of these viruses 17-59% are predicted to infect Cyanobacteria. Euphotic zone F-DNA (75-125 m) contained primarily bacterial and viral sequences, with bacteria dominating samples from the mesopelagic zone (500-1000 m). A high proportion of mesopelagic zone F-DNA sequences appeared to originate from surface waters, including a large amount of DNA contributed by high-light *Prochlorococcus* ecotypes. 30-53% of annotated F-DNA was from *Prochlorococcus*, and 23-68% from cyanophages. Throughout the water column, but especially in the mesopelagic zone, the composition of F-DNA sequences was not always reflective of co-occurring microbial communities that inhabit the same sampling depth. These results reveal the composition of F-DNA in different regions of the water column (euphotic and mesopelagic zones), with implications for dissolved organic matter cycling and export (by way of sinking particles and/or migratory zooplankton) as a delivery mechanism of small phytoplankton DNA to the mesopelagic.

S1P39: *Synechococcus* extracellular vesicles: characterization and response to stress inducers

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Synechococcus is one of the most important cyanobacteria in marine ecosystems alongside *Prochlorococcus*. Recent findings have shown the ability of this cyanobacteria to produce vesicles and release them into the ocean (1). However, further work is critical to clarify their exact roles in the marine environment.
For this purpose, we have examined the growth of *Synechococcus* sp. WH8102 and *Synechococcus* sp. WH7803 cultures under different stress conditions: nitrogen and phosphorous starvation and light shock. We measured the vesicle concentration in each culture by nanoparticle tracking analysis (*NanoSight*), observed these samples by transmission electron microscopy and characterized their protein content.
Our results showed an increase of vesicles per *Synechococcus* cell in both strains under light shock. Furthermore, we have shown that nitrogen starvation causes growth decrease in *Synechococcus* sp. WH8102. On the other hand, phosphorous starvation does not affect culture growth but it does cause an increase in vesicle release.
We have also carried out proteomic studies of the content of vesicles from *Synechococcus* sp. WH7803 after a light shock. Preliminary results showed and increased concentration of proteins related to photosynthesis, secretion/transport and stress, but delving into these results is still necessary.
Moreover, in order to study how these vesicles are formed, we are currently analyzing *Synechococcus* cells at different stages of growth by transmission electron microscopy; as well as vesicle concentrates from this cyanobacterium.

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S1P40: Pro. and Syn. in the subtropical North Atlantics P cycle *Prochlorococcus* and *Synechococcus* and their role in phosphorus cycling in the subtropical North Atlantic

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Primary producers are a major link in the biogeochemical cycling of vital nutrients in the marine environment. Conversely, nutrient limitation governs primary production in many regions of the surface ocean. *Prochlorococcus* and *Synechococcus* dominate the phytoplankton community in the oligotrophic ocean and thus, play a critical role in the cycling of nutrients. Here we present the role of *Prochlorococcus* and *Synechococcus* on the phosphorus cycling in the oligotrophic subtropical North Atlantic on a west-east transect along the nominal latitude of 24°N. At 38 stations, samples for dissolved organic phosphorus (DOP), particulate phosphorus and phytoplankton community composition were collected from 6 depths within the euphotic zone. Flowcytometric analysis revealed that *Synechococcus* only occupied the top 100 m of the water column, with higher abundances (> 50000 counts/ml) closer to coastlines, *Prochlorococcus* was generally more abundant (> 100000 counts/ml), occupying the whole euphotic zone, with a pronounced maxima around ~130 m water depth. As the subtropical North Atlantic is

depleted in nitrate and phosphate, DOP becomes a vital alternative source of phosphorus for the cyanobacteria. DOP across the transect revealed higher concentrations in the western basin (> 0.1 µM) and depleted surface waters (< 0.01 µM) in the eastern basin. Particulate phosphorus was found mainly in the upper 100 m and increased in concentration towards the eastern boundary (> 0.03 µM). To investigate anthropogenic nutrient perturbation in the North Atlantic, nutrient amendment bioassays were conducted at seven location along the transect, inducing enhanced P-limitation on the natural planktonic communities. These amendments revealed not only a consistent drawdown of DOP, but also enhanced conversion of dissolved phosphorus into particulate pools. These findings coincide with increased abundance of *Synechococcus*, specifically at stations with adjacent coastlines and therefore suggest that *Synechococcus* is highly involved in the oceanic phosphorus cycling and to a higher degree than the smaller cell sized and less adaptive *Prochlorococcus*.

S1P41: Diverse light responses of coastal *Synechococcus*

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Marine *Synechococcus* populations are typically diverse, and comprised of genetically different clades, which vary in distribution patterns as well as physiological responses to nutrient utilization, temperature, light and other variables. At the Martha's Vineyard Coastal Observatory, the population is comprised of at least 14 different clades, with clade I types being most dominant. These clades follow regular patterns of relative abundance, believed to be driven by seasonal changes in environmental variables, such as light and temperature. To better understand how changes in light contribute to observed diversity patterns, we investigated the growth response of 13 different MVCO clade isolates to different light levels. Cultures were grown at 5 different light levels, and monitored daily via flow cyotmetry. In vivo, absorbance and fluorescence excitation and emission spectra were also measured for each clade at each light level. Clades differed markedly in their growth responses to light. Clade representatives from CB5, II, III, VI, and 5.2MV1 were able to reach maximum growth rate at low-medium light levels (> 150 µmol/m2/s), while I, IV, VII, VIII, XV, and WPC1 appeared to reach maximum growth at only higher light levels (> 300 µmol/m2/s). Some clades also demonstrated stark differences in cell size (as measured by side scatter) and PE fluorescence in response to changing light levels. Excitation, emission and absorbance spectra did not appear to change with light level, except for a clade VI representative, with PUB only detected at light levels > 50 µmol/m2/s. We find that clade I strains, while dominant at MVCO, did not have the highest average growth rates at any given light level suggesting that other variables (or combination of), such as temperature will be important to investigate for a full understanding of clade dynamics at this temperate location.

S1P42:Exploring the Relationships between *Prochlorococcus* and *Synechococcus*

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Prochlorococcus and *Synechococcus* are globally important phytoplankton of similar size but the relationships between them are poorly understood. In order to gain insights into the driving factors and relative importance of apparent competition between these two species, we look for correlations between them as a function of spatial scale. Current theories predict that population growth will occur according to size class, with *Prochlorococcus*

appearing first, followed by an increase in *Synechococcus* as a function of increasing nutrient supplies. High resolution transect data from SeaFlow shows a diverse range of positive, negative, and no correlations between changes in *Prochlorococcus* and *Synechococcus* populations as a function of spatial scale. Here we explore patterns in both the scaling relationships in both the transect data and from numerical simulations from the Darwin model.

S1P43: Ecological significance of *Prochlorococcus* diversity across four orders of magnitude of genomic divergence.

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Clades and clusters of *Prochlorococcus* have been delineated over genomic divergences spanning four orders of magnitude. At the highest levels, several high-light (HL) and low-light (LL) ecotypes are based on measured physiological differences. Based on ITS phylogeny, increasingly fine-scale clusters have been described, such as Kashtan et al's ribotypes defined by 1% ITS diameter. Using extensive single-cell data, we study levels of diversity ranging from the entire *Prochlorococcus* species, to ecotypes such as HLII, to sub-ecotype cliques such as CN2 within HLII, to sub-CN2 ribotypes, to close cliques within these ribotypes, and finally anomalously-close cell pairs within these cliques. How is this fine-scale diversity established and maintained? At what level are these ITS-based clades representative of distinguishable ecological niches? Presumably this cannot continue down until each genetically-distinct cell occupies its own ecological pico-niche. An alternative scenario is a continuum-like `ecospectrum' of multiple subtle differences between a huge number of strains.

Diversity within *Prochlorococcus* is generated through mutation and recombination. We identify groups of closely-related cells which retain a large fraction of their clonally-inherited genome and estimate the number and origin of successful recombination events in the history of these samples. In increasingly diverged cell groups, the clonal frame decays and by the level of HLII it is overwritten completely. Examining the entire species, we analyze ~1000 genes with homologs in HL and LL cells. We find that typically ~1% of a cell's genes have best hits with cells from the opposite HL/LL ecotype and that there are genes that have recently swept across the entire Prochlorococcus population. We find many examples of gene flow between the most disparate cells in the dataset, far beyond the divergence typically thought to inhibit homologous recombination.

If *Prochlorococcus* were divided into distinguishable ecotypes, one would expect to find genome-wide linkage between alleles and/or in flexible gene content. Are such linkage patterns evidenced in the data and can we assign putative functions to sets of linked genes? Alternatively, are *Prochlorococcus* genomes a more `random' assortment of alleles and flexible genes that might be better-characterized as quasi-sexual, an extreme example of which is the quasi-sexual thermophilic *Synechococcus* population studied by Rosen et al?

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S1P44: Biofilm Formation in *Prochlorococcus.*

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Prokaryotic organisms often form biofilms, a lifestyle defined by spatial coordination into organized assemblages – an alternative to purely planktonic living. Organization into biofilms allows for emergent properties compared to a free-living lifestyle, such as stress tolerance, cell specialization within the population, and more efficient nutrient transfer. However, to our knowledge, biofilm formation by *Prochlorococcus* has not yet been observed or characterized. Recent evidence, such as the observation of *Prochlorococcus* in the particle fraction of marine samples, the presence of biofilm-related genes in *Prochlorococcus* genomes, and biofilm formation by freshwater *Synechococcus* together suggested some *Prochlorococcus* may possess the capability as well. We have identified likely axenic biofilm-formation by *Prochlorococcus* using a series of indirect assays including crystal violet staining, macroscopic imaging, and microscopy, with further support from bioinformatic analysis. The phenotype is widespread among observed high-light and low-light strains, and variation is much greater between clades than the individual strains within clades, suggesting a role for this function in adaptation to each strain's local environment.

S1P45: Exploring the diversity, dynamics and distribution of picocyanobacteria in oligotrophic and eutrophic New Zealand lakes using eDNA metabarcoding.

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Picocyanobacteria (Pcy) play a vital role at the base of the aquatic microbial food web, yet relatively little is known about the diversity, distribution and dynamics of Pcy genotypes in freshwater systems, particularly eutrophic waterbodies. Here, we present two studies utilizing a combination of epifluorescence microscopy and environmental DNA (eDNA) metabarcoding to explore these knowledge gaps. First, we determined the spatio-temporal diversity and dynamics of Pcy communities in two oligotrophic and three eutrophic New Zealand lakes over the course of one year. Cell abundances and community structure were correlated with environmental variables relating to water quality within each lake. The results indicate that lacustrine Pcy communities are highly diverse and dynamic across all study lakes. Temporal shifts in Pcy community structure correlated with shifts in water quality, suggesting strain-specific responses to environmental change. High Pcy cell abundances were often maintained through significant disruptive events including marine intrusion and lake destratification in some lakes, alongside a significant adaptive restructuring of genotypes within the community. Second, we assessed the distribution of Pcy genotypes across 128 New Zealand lakes covering wide geographic, environmental and trophic gradients. Over 409 genotypes were found, revealing a high diversity of freshwater Pcy across the lakes. No single genotype inhabited all lakes, while 34% of genotypes were restricted to single lakes, resulting in a strongly unimodal lake occupancy distribution. Some genotypes appeared to have more restricted distributions and were found only in lakes at certain altitudes or ends of the trophic spectrum, while others occupied lakes across these large gradients. This suggests that some genotypes may be more resilient to environmental change and broad tolerances may allow for their success across a range of contrasting conditions. Together, both studies highlight the dynamic and adaptive nature of freshwater Pcy while illustrating the benefits of using molecular methods to determine genotype responses.

Session 2: Physiology, gene regulation and metabolism

S2P38: Coping with darkness: How does *Prochlorococcus* respond to repeated light energy deprivation?
Allison Coe, Aldo Arellano, Elaina Thomas, Rogier Braakman, Steven Biller, Christina Bliem, Konstantinos Boulias, Keven Dooley, Anna Rasmussen, Sean Kearney, Eric Greer, Sallie Chisholm

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S2P38: Coping with darkness: How does *Prochlorococcus* respond to repeated light energy deprivation?

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Prochlorococcus is found throughout the euphotic zone, but deep mixing can move cells below this, depriving them of light for extended periods of time. In an earlier study, we showed that *Prochlorococcus* strains could regrow following 3 days in the dark when grown with Alteromonas macleodii, but not when they are axenic. Surprisingly, we noticed that repeated dark exposures of the same co-culture enhanced the ability of the strains, which were clonal, to regrow following a period of extended darkness. The time it took for the cells to recover from the 3 day dark exposure decreased from 28 days to 1 day over 3 rounds of extended darkness. This 'dark-adapted phenotype' was stable, retaining the ability to recover from extended darkness in 1 day following 7 serial transfers under a standard 13:11 diel light:dark cycle. Multiple lines of evidence further indicate that the dark-adapted phenotype represents a change within *Prochlorococcus*, and not within *Alteromonas*. Next, we compared the physiological and metabolic processes in the parent

culture and the dark-adapted cultures when growing on a standard diel light-dark cycle. The dark-adapted phenotype cell cycle is less synchronized to the light:dark cycle than that of the parent cells, even though expression of cell division and replication genes are not significantly different. Gene transcription data further suggests enhanced flow of organic carbon from *Alteromonas* to *Prochlorococcus* in the dark-adapted cultures. Finally, we explored the potential mechanisms underpinning the dark-adapted phenotype in *Prochlorococcus*. Illumina sequencing revealed no genomic mutations or structural variations between the dark-adapted and parent culture, suggesting epigenetic mechanisms might be in play. We thus used PacBio sequencing and found no structural variance, no methylated motifs, and no identifiable methyltransferases between the dark-adapted phenotype and the parent. We used LC-MS/MS to measure concentrations of 6mA, 5mC, and 4mC modifications and found that they were absent in both the parent and dark-adapted *Prochlorococcus* NATL2A. Analysis of other strains of *Prochlorococcus*, that also have the capacity to develop the dark-adapted genotype, revealed 6mA or 5mC modifications in parent cultures. Methylation patterns for those strains were not examined after extended darkness, but the NATL2A data suggests that methylation is not the underlying mechanism for dark-adaptation. Analysis of these data is ongoing, but our results indicate that *Prochlorococcus* utilizes a yet-to-be-unraveled mechanism to develop increased tolerance to repeated rounds of extended darkness.

Observations:
Repeated dark exposures to a co-culture of *Prochlorococcus* and Alteromonas resulted in decreased recovery time from 28 days to 1 day over 3 rounds of extended darkness. This 'dark-adapted phenotype' was stable and these changes occurred within *Prochlorococcus*, but the results suggest that a genetic or epigenetic mechanism is not responsible for an increased tolerance to repeated rounds of extended darkness.

S2P39: Adaptation and acclimation strategies of the marine picocyanobacterium *Synechococcus* to iron temperature gradients and iron depletion.

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Oceans are strongly impacted by global change, which is predicted to cause an increase of sea surface temperature but also an expansion of Fe-poor areas, whilst Fe depletion is already impairing phytoplankton growth in as much as 30 % of the global ocean. In this context, one may wonder if/how marine phytoplankton will be able to adapt to such limitation and what will be the consequences of Fe depletion on the ocean ability to sequester CO_2 via the biological carbon pump. Due to its abundance, ubiquity, the availability of numerous strains and genomes and of genetic tools, *Synechococcus* constitutes one of the most pertinent biological models available nowadays to study the molecular processes underlying phytoplankton adaptability to environmental changes occurring in the ocean. While natural *Synechococcus* populations were long thought to be dominated by four clades (I-IV), the ecological importance of a fifth clade (called CRD1) was recently evidenced in Fe-depleted areas of the world ocean [1-3]. Furthermore, the

CRD1 clade was shown to encompass 3 distinct Ecologically Significant Taxonomic Units (ESTU, CRD1A to C), seemingly occupying different thermal niches in the Ocean [2].
In order to better understand the respective roles of Fe depletion and temperature on *Synechococcus* distribution and genetic diversification, we compared the physiology of representative strains of each of the three CRD1 ESTUs with members of clades I-IV, used as controls for cold (I, IV) or warm (II, III), Fe-replete environments. By determining their temperature growth optimum and boundary limits, we validated the occurrence of three distinct thermotypes within the CRD1 clade. Acquisition of additional physiological parameters (max. photosystem (PS) II quantum yield, D1 content and repair rate, pigment content) on cultures acclimated to various temperatures also revealed specificities of photosynthetic apparatus of CRD1 strains with regard to other clades. Comparative analyses of 70 *Synechococcus* genomes, including 8 CRD1, also suggested that the occurrence of this clade in Fe-depleted areas could rely on a reduction of the number of genes encoding Fe-rich proteins as well as an increase in the number of genes encoding proteins using alternative metals and/or involved in acquisition and storage of iron. Finally, analysis of the *Tara* Oceans metagenomes revealed genes specifically present or absent in Fe-poor niches that constitute good candidates for further understanding the mechanisms of adaptation to iron deficiency and temperature.

[1] Sohm et al., ISME J. 2016, 10: 333?45.
[2] Farrant et al., 2016, PNAS 2016 113: E3365?74.
[3] Ahlgren et al., 2020, Env. Microbiol. 22 : 1801?1815.

S2P40: Assembly of phycoerythrin III in *Prochlorococcus marinus* SS120

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Prochlorococcus lacks phycobilisomes (PBS) but uses divinyl chlorophyll complexes as major light harvesting structures. However, *Prochlorococcus* sp. retained remnants of the PBS in form of single phycoerythrin (PE). In *P. marinus* SS120 it is called PE III and consists of an alpha- and beta- subunit. These subunits carry covalently attached linear tetrapyrrole chromophores termed phycobilins. While the genome of *P. marinus* SS120 possesses genes for the biosynthesis of the phycobilins phycocyanobilin (PCB) and phycoerythrobilin (PEB), only PEB and phycourobilin (PUB) have been observed in vivo. The final step in PE III biosynthesis is the post-translational addition of the phycobilin to the alpha- and beta- subunits. *In vivo*, the correct covalent attachment of most chromophores is catalyzed by specific phycobiliprotein lyases. *P. marinus* SS120 possesses five putative lyase genes and additionally the open reading frame Pro1634. Within this project, we wish to explore the composition of PE III and consequently, how PUB is made. In addition, we are keen to understand the function of the PCB biosynthetic enzyme.
In order to investigate the composition of PE III, we will reconstitute PE III in *Escherichia coli*. Therefore, we will establish the pDuet coexpression system. First, all genes potentially involved in PE III synthesis will be individually cloned in specific pDuet expression vectors. Using this system, we will be able to co-express single or multiple PBP lyase genes together with the alpha- or beta-subunit and phycobilin biosynthesis genes. First data demonstrated a specific transfer of PEB by the phycobiliprotein lyase CpeS to the alpha-subunit. This was visible by a pink color and a strong fluorescence emission at 564 nm (lambda ex=550 nm) indicating that the approach is feasible. Another approach to investigate the chromophorylation state of PE III is the expression of the whole PE cluster

including the chromophore biosynthetic genes in *E. coli*. The approach is based on the TREX system (transfer and expression of biosynthetic pathways). We have successfully cloned the PE cluster, and are in the process of testing it.

S2P41: Juggling genes in *Prochlorococcus* and *Synechococcus*: Discoveries, challenges, and opportunities

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The marine cyanobacterium *Prochlorococcus*' pangenome is estimated to be roughly 80,000 genes, most of which are of unknown function. The lack of a genetic system has hampered progress in understanding the functions embedded in this vast diversity. Efforts to demonstrate efficient transformation have historically been unsuccessful. Here, we report our recent progress in building tools for manipulating *Prochlorococcus.* Using electroporation parameters identified for optimal DNA delivery, we have successfully transformed low-light adapted cells with a replicative plasmid that confers resistance to the antibiotic Spectinomycin. To achieve stable integration of DNA into the chromosome, we are adapting the CRISPR-Cpf1 tool designed in *Synechococcus* that, in our hands, worked well for *Synechococcus* WH7803. We have delivered via electroporation the CRISPR constructs and deleted three different ~3Kb genes. We are now working on modifying the CRISPR-Cpf1 tool to obtain the knock-in of fluorescent proteins in both *Synechococcus* and *Prochlorococcus*. To this end, fluorescent proteins are first tested for their efficacy and detection limits in a modified version of the replicative plasmid that has been shown to transiently transform *Prochlorococcus* cells. The introduction of fluorescent proteins into the *Prochlorococcus* genome will serve as a proof of principle for stable integration and allow further genome modifications. Questions we are interested in addressing with this system include knocking out the production of specific secondary compounds, exploring the numerous genes of unknown function, and determining the absolute minimal photosynthetic cell.

S2P42: Cultivation of *Prochlorococcus* isolates from the oxygen deficient zone of the eastern tropical north Pacific

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Marine Oxygen Deficient Zones (ODZs), defined as waters with O2 less than 10 nM, are predicted to expand as a result of rising global temperatures. ODZs support diverse microbial communities that play an essential role in biogeochemical cycling, including the removal of fixed nitrogen, an essential limiting nutrient in much of the world's ocean. In some portions of the ODZs of the eastern tropical Pacific and Arabian Sea a pronounced secondary chlorophyll maximum exists, where the euphotic zone extends into oxygen-depleted waters. These secondary chlorophyll maxima are dominated by the picocyanobacterium *Prochlorococcus*, which drive a cryptic oxygen cycle in the upper ODZ, as demonstrated by metatranscriptomic evidence of aerobic processes, including nitrification, despite undetectable oxygen concentrations. Prior clone-based and metagenomic approaches suggest ODZ *Prochlorococcus* may form a distinct genetic clade, most closely related to low-light (LL) IV cultivated strains, and have the potential for nitrate, nitrite, urea and ammonia assimilation. Here we report the cultivation of four new

Prochlorococcus isolates from 120m, within the secondary chlorophyll maximum of the eastern tropical north Pacific ODZ. Three cultures were isolated on nitrite and the fourth is growing on nitrate as a sole nitrogen source. Sequences for the 16S-23S rDNA internal transcribed spacer (ITS) region determine that all four isolates fall within the LL-IV clade. Having ODZ *Prochlorococcus* in culture lays the foundation for genomic and physiological experiments to provide a greater understanding of the ecological role of ODZ *Prochlorococcus* as their habitat continues to grow.

S2P43: Light-harvesting efficiency of natural populations of the marine cyanobacteria *Synechococcus* increases with depth.

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Cyanobacteria of the genus *Synechococcus* play a key role as primary producers and drivers of the global carbon cycle in temperate and tropical oceans. *Synechococcus* use phycobilisomes as photosynthetic light-harvesting antennas. These contain phycoerythrin, a pigment-protein complex specialized for absorption of blue light, which penetrates deep into open ocean water. As light declines with depth, *Synechococcus* photo-acclimate by increasing both the density of photosynthetic membranes and the size of the phycobilisomes. This is achieved with the addition of phycoerythrin units, as demonstrated in laboratory studies. In this study, we probed the *Synechococcus* population in an oligotrophic water column habitat at increasing depths. We observed morphological changes and indications for an increase in phycobilin content with increasing depth, in summer stratified *Synechococcus* populations. Such an increase in antenna size is expected to come at the expense of decreased energy transfer efficiency through the antenna, since energy has a longer distance to travel. However, using a novel fluorescence lifetime depth profile measurement approach, we found that light-harvesting quantum efficiency increased with depth in stratified water column. Calculated phycobilisome fluorescence quantum yields were 3.5% at 70m and 18% at 130m. During winter-mixing conditions, *Synechococcus* presents an intermediate state of light harvesting, suggesting an acclimation of cells to the average light regime through the mixing depth (quantum yield of ~10%). Given this new photo-acclimation strategy, we suggest that primary productivity attributed to *Synechococcus* is likely higher than typically estimated.

S2P44: Proteome mapping of a cyanobacterium reveals distinct compartment organisation and cell dispersed metabolism

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Cyanobacteria are complex prokaryotes, incorporating a Gram-negative cell wall and internal thylakoid membranes (TMs). However, localisation of proteins within cyanobacterial cells is poorly understood. In this study we adapted the hyperLOPIT approach to map the proteins of the entire *Synechocystis* sp. PCC 6803 cell using spatial proteomics applied to cellular fractions enriched with various subcellular membranes. Via this approach we produced an extensive subcellular proteome map of an entire

cyanobacterial cell, identifying ~67% of proteins in *Synechocystis* sp. PCC 6803, ~1000 more than previous mapping studies. 1,712 proteins were assigned to six specific subcellular regions. Proteins involved in energy conversion localised to TMs. The majority of transporters, with the exception of a TM-localised copper importer, resided in the plasma membrane (PM). Most metabolic enzymes were soluble although numerous pathways terminated in the TM (notably those involved in peptidoglycan monomer, NADP+, heme, lipid and carotenoid biosynthesis), or PM (specifically, those catalysing lipopolysaccharide, molybdopterin, FAD and phylloquinol biosynthesis). We also identified the proteins involved in the TM and PM electron transport chains. The majority of ribosomal proteins and enzymes synthesising the storage compound polyhydroxybuyrate formed distinct clusters within the data, suggesting similar subcellular distributions to one another, as expected for proteins operating within multi-component structures. Moreover, heterogeneity within membrane regions was observed, indicating further cellular complexity. Cyanobacterial TM protein localisation was conserved in *Arabidopsis thaliana* chloroplasts, suggesting similar proteome organisation in more developed photosynthetic organisms. Successful application of this technique in *Synechocystis* suggests it could be applied to mapping the proteomes of other cyanobacteria, including marine *Synechococcus* and *Prochlorococcus* species. The organisation of the cyanobacterial cell revealed here substantially aids our understanding of these environmentally and biotechnologically important organisms. Future studies will focus on applying this technique to other cyanobacterial species to determine whether proteome organisation is conserved across the phylum.

S2P45: Urea as a nitrogen source for *Prochlorococcus* and *Synechococcus*

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Prochlorococcus and *Synechococcus* have adopted alternative strategies to occupy the oceanic ecosystem, which encompasses a range of water bodies from turbid, estuarine waters to transparent oligotrophic waters, and delineates a complex environment. Known physiological differences among these cyanobacterial strains include their tolerance to low temperatures, the response to nitrogen depletion and the preference for nitrate or urea for growth. Therefore, a number of observations suggest that the diversity observed in the strains and genomes of both marine cyanobacteria could also be reflected in the mechanisms of control of their C/N metabolism.

Urea is ubiquitous in nature, ranging from 1 nM to 50 µM in oceanic-estuarine waters, where it can contribute to 50% or more of the total nitrogen used by phytoplanktonic communities. Many, but not all *Prochlorococcus* and *Synechococcus* strains harbor the genes encoding the urea degradative enzyme urease (*ureABCDEFG*) and the urea transporter (*urtABCDE*) in their genomes. In this study, an initial characterization of the urea assimilation process in two marine cyanobacterial strains, *Prochlorococcus* sp. EQPAC1 and *Synechococcus* sp. BL107, has been performed. The promoter regions of the genes encoding the urease and the urea transporter have been analysed, showing at least one NtcA-binding site (a transcription factor involved in the regulation of C/N metabolism), which suggests their expression could be regulated by nitrogen availability. The co-transcription of the five *urt* genes was confirmed by RT-PCR performed with RNA

isolated from *Synechococcus* sp. strain BL107 cells grown under nitrogen starvation for 6 hours, and the expression of this operon was studied by qRT-PCR at different times under nitrogen starvation, and adding urea as the sole nitrogen source in the cultures. While the absence of nitrogen in the medium induced a strong increase of the expression of these genes in the cultures of *Synechococcus* sp. BL107, the addition of urea to the cultures had no significant effect in the expression of the *urt* operon. Finally, the characterization of the urease activity in extracts from *Prochlorococcus* and *Synechococcus* strains has been started, optimizing the conditions of the enzyme assay to detect this activity in those cell extracts.

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S2P46: Regulation of cyanobacterial biofilm development: Lessons from *Synechococcus* elongatus PCC 7942

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Information on the molecular mechanisms involved in cyanobacterial biofilm development started emerging only in recent years in spite of the environmental prevalence and the economic loss associated with these microbial assemblages. We revealed a biofilm self-suppression mechanism that operates in the model cyanobacterium *Synechococcus* elongatus that requires the type IV pilus (T4P) assembly complex. Recent studies uncovered two components required for the biofilm self-suppression mechanism: The RNA-chaperone Hfq and a protein annotated hypothetical which we denote EbsA (essential for biofilm self-suppression A). EbsA homologs are conserved and widespread in diverse cyanobacteria but are not found outside this clade. We revealed a tripartite complex of EbsA, Hfq and the ATPase homolog PilB, and demonstrated that either one of these components is required for pilus-assembly and for DNA competence, in addition to their role in biofilm self-suppression. Comparative analyses of extracellular proteins in wild type and mutants impaired in these components suggest that the T4P complex substantially affects the exo-proteome and imply its role in protein secretion. Additionally, we demonstrated that the self-suppression mechanism depends on the deposition of a factor to the extracellular milieu. This inhibitory substance governs transcription of an operon encoding small secreted proteins that enable biofilm formation and mutations in genes encoding components of the T4P complex result in transcription upregulation of this operon. Moreover, employment of a reporter strain allowing analysis of expression of this operon in individual cells suggested cell speciation during biofilm development namely, high expression is limited to a subpopulation in the culture. Further analysis of one of the small secreted proteins indicated its localization in the extracellular matrix. These data suggest a beneficial ‘division of labour’ upon biofilm formation where only some of the cells allocate resources to produce matrix proteins – ‘public goods’ that support robust biofilm development. We have also analysed reporter expression in a biofilm-forming mutant in response to conditioned medium harvested from a wild type culture at different time points following inoculation. Data indicated accumulation of the biofilm inhibitor with culture age, supporting involvement of intercellular communication similar to quorum sensing mechanism in S. *elongatus* biofilm regulation. Together, our

studies suggest unique characteristics of cyanobacterial T4P systems and assign them roles beyond the commonly known functions of pilus assembly and DNA competence.

S2P47: Exploring the diversity of the thioredoxin system in marine cyanobacteria

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(1) Balsera, M. and Buchanan, B.B. (2019). Free Radic. Biol. Med., 140, 28-35.

Thioredoxins (Trxs) are disulfide oxidoreductase proteins that control multiple cellular processes. In cyanobacteria, four different Trxs have been found, three of them corresponding to m (TrxA), x (TrxB) and y (TrxQ) types also present in plant chloroplast, while the fourth thioredoxin, TrxC, is unique to cyanobacteria. Trxs are reduced by thioredoxin reductases (TRs). Photosynthetic organisms have developed a light-dependent regulation system that connects reducing equivalents from photosynthetic electron transfer to the metabolism and core cellular processes via ferredoxin and ferredoxin-thioredoxin reductase (FTR). FTR is a heterodimeric enzyme composed of a catalytic subunit (FTRC) and a variable subunit (FTRV). FTR system is almost universal in photosynthetic organisms, however, in certain cyanobacteria it is missing. Using phylogenomic analysis and the availability of a wide variety of cyanobacterial genomes, four large clades of Trxs (TrxA, TrxB, TrxQ and TrxC) have been revealed. TrxA is present in all cyanobacteria in the phylogenetic subclade TrxA1. Analysis of the degree of conservation on the molecular surface reveals a patch of high conservation around the active site in this subclade. *Prochlorococcus* species contains only TrxA1. This subclade has a high degree of conservation on the molecular surface around the active site. The genome reduction that occurs in *Prochlorococcus* and marine *Synechococcus* can explain the loss of TrxB, TRxQ and TrxC types. However, the presence of Trxs in *Melainabacteria* and early cyanobacteria indicated that they were likely present in the common ancestor of all extant cyanobacteria. Our aim was to explore the presence of TRs in cyanobacteria. In all cyanobacterial genomes analyzed, the FTR system is present except for *Prochlorococcus* and *Gloeobacter* that contain another TR named DTR1 (1) and non-canonical TRs. The finding that all *Prochlorococcus* and *Gloeobacter* have DTR homologs suggests that the function of the FTR is done with this TR. Loss of iron-containing proteins, such as FTRC, could help reduce iron dependence, an element clearly limited in the oceans. In contrast, all marine *Synechococcus*, including those closely related to *Prochlorococcus*, have an intact FTR. Finally, most *Prochlorococcus* and other cyanobacteria also contain NTRC, a protein that contains a Trx fused to a NADPH dependent TR related to the reduction of 2-Cys peroxiredoxin.

(1) Balsera, M. and Buchanan, B.B. (2019). Evolution of the thioredoxin system as a step enabling adaptation to oxidative stress. Free Radic. Biol. Med., 140, 28–35.

S2P48: Nitrate assimilation in marine *Synechococcus* strains: Proteomic characterization and expression of the most relevant genes

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The marine picocyanobacteria *Prochorococcus* and *Synechococcus* are model microbes for marine ecology studies because of their abundance and significant contribution to global primary production. Since they proliferate in marine areas with a high diversity in the availability of nutrients, light and temperature, they show a number of adaptations to different environmental conditions.

Synechococcus and *Prochlorococcus* strains have demonstrated the ability to proliferate in oligotrophic environments, nitrogen being an important limiting factor1,2. Preliminary results from our group suggest the existence of a system that could allow *Synechococcus* sp. WH7803, representative of *Synechococcus* clades inhabiting mesotrophic areas of the ocean, to sense nanomolar concentrations of nitrate. In this work we describe changes in the proteome and the expression of the most relevant genes involved in nitrate assimilation, in response to nitrogen availability.

Three *Synechococcus* strains (WH7803, WH8102 and BL107) were grown under different conditions: no nitrogen, ammonium, or several nitrate concentrations as sole nitrogen source. Our results show significant changes in the abundance of crucial proteins in the metabolic pathway, such as the nitrate transporter or PII regulatory protein, which increase in the absence of nitrogen or in the presence of nitrate. This increase also occurs in proteins of interest such as cyanase, which participate in nitrogen metabolism and stress responses. In qRT-PCR expression studies, significant changes have been shown in central genes such as *narB* (encoding for nitrate reductase) or *nirA* (encoding for nitrite reductase).

1 Domínguez-Martín, M., Gómez-Baena, G., Díez, J., López-Grueso, M., Beynon, R., & García-Fernández, J. (2017). Quantitative proteomics shows extensive remodeling induced by nitrogen limitation in *Prochlorococcus marinus* SS120. mSystems,2:e00087
2 Berube, P., Rasmussen, A., Braakman, R., Stepanauskas, R., & Chisholm, S. (2019). Emergence of trait variability through the lens of nitrogen assimilation in *Prochlorococcus*. eLife, 8:e41043

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S2P49: Proteomics and metabolomics analysis of glucose utilization in *Prochlorococcus* and *Synechococcus*.

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Prochlorococcus and *Synechococcus* are the most abundant cyanobacteria in the ocean and important players in the global carbon cycle. Previous results from our group showed that *Prochlorococcus* can take up glucose, increasing the expression of genes involved in its metabolization upon glucose addition1. Besides, glucose uptake was detected in natural populations of *Prochlorococcus*2. Our current working hypothesis is that mixotrophy confers an evolutionary advantage to *Prochlorococcus* against other microorganisms

sharing the same ecological niche.

Using metabolomic and proteomic approaches, we compared the effect of the addition of 100 nM and 5 mM glucose on the metabolism of *Prochlorococcus* and *Synechococcus*, under light and dark conditions. Our results showed that 5 mM glucose addition led to a strong metabolic shift toward overall anabolic patterns in all the studied strains. After 5 mM glucose addition 469 metabolites increased their concentration. We will focus on the study of carbohydrates, especially those more related to glucose metabolism in cyanobacteria; glycolysis, TCA cycle, Calvin cycle and pentose phosphate pathway. Proteomic results showed that the relative abundance of proteins related with photosynthesis, ribosomes and ATPase decreased under darkness in the presence of glucose. Our metabolomics and proteomics results demonstrate that *Prochlorococcus* and *Synechococcus* use glucose and adapt their metabolisms to the availability of this sugar.

1 Gómez-Baena, G. et al. (2008) PLOS ONE 3, e3416
2 Muñoz-Marín, M. C. et al. (2013) PNAS 110, 8597-8602

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S2P50: An uncultured marine cyanophage encodes an active phycobilisome proteolysis adaptor protein NblA.

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Under nutrient limitation, cyanobacteria degrade their phycobilisomes (PBSs), a major light-harvesting complex, allowing the cell to control light energy capture. PBSs are water-soluble membrane-associated complexes in cyanobacteria and red algae that serve as a light-harvesting antenna for the photosynthetic apparatus. NblA, a small protein (ca. 6 kDa), is essential for degradation of PBS and causes a color change from blue-green to yellowish. Cyanobacteria, red algae and some freshwater cyanophages are known to contain *nblA* gene. A recent study, using assemblies from oceanic metagenomes revealed genomes from a novel uncultured marine cyanophage, which contain genes coding for PBS degradation protein, *NblA*. Here we examine the functionality of *nblA*-like genes from this marine cyanophage family by using a *Synechococcus* elongatus PCC7942 mutant lacking nblA, which does not bleach under nitrogen starvation. We complemented this mutant with a marine cyanophage nblA-like gene and removed nitrogen from the cyanobacterial growth medium in order to examine whether the complemented strain with the *nblA* gene mutant would restore the wild type phenotype under starvation. Based on

previous data and these studies, our findings reveal a functional NblA from a novel marine cyanophage lineage. Additionally, by analyzing genomes of cultured and uncultured marine cyanobacteria and cyanophages, we identified *nblA* genes that were absent from databases and found that multiplicity of nblA genes per genome is a widespread phenomenon among cyanobacteria. Furthermore, by applying low-temperature fluorescence spectroscopy we demonstrate the existence of a separate PBS disassembly step as part of the NblA-assisted PBS degradation.

S2P51: Impact of salinity on the physiology of a marine *Synechococcus* strain (*Synechococcus* sp. RS9907)

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Marine cyanobacteria of the genus *Synechococcus* are among the most abundant phototrophs in the ocean. Among the environmental factors which can significantly impact their growth, light and temperature have been the most widely studied. The impact of salinity has been studied mostly on freshwater cyanobacteria, but its effects on marine strains have been largely unexplored. As changes in seawater salinity connected to global warming are expected due to evaporation and ice melting, it is important to understand how this factor can impact the physiology of marine *Synechococcus*. To address this question, we performed long- and short-term salinity acclimation experiments with the strain *Synechococcus sp. RS9907*, with a focus on their PSII activity. During the long-term acclimation, we maintained RS9907 cultures in exponential growth phase over several months. Growth rates of RS9907 changed along the salinity gradient (from 18 to 50 PSU), with an important decrease towards low salinities. PSII quantum yield (Fv/Fm) and others photosynthetic parameters were measured using a Pulse Amplitude Modulation fluorometer (Phyto-PAM-II compact). Under optimal temperature (28ºC), the strain exhibited the highest PSII quantum yields from 36 to 50 PSU. In a series of short-term salinity shock experiments, we exposed cultures acclimated at 36PSU to low (18PSU) and high (50PSU) salinities. When samples were exposed to the low salinity shock, a decline in the Fv/Fm value and changes in other photochemical parameters were detected, indicating a salinity impact on PSII activity. However, the exposure to high salinity did not affect their photochemical performance. Our results show that salinity variations impact the physiology of marine *Synechococcus* and together with other environmental parameters, could determine their performance in future oceanic conditions.

S2P52: The marine picocyanobacterial PtoX functions as a photosystem II safety valve

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Plastoquinol terminal oxidase (PtoX) is widespread in oxygenic phototrophs. It has been suggested that it acts as a ‘safety valve’ during high light. Specifically that it uses photosystem II (PSII) derived electrons to reduce oxygen to water when the plastoquinone

(PQ) pool is at risk of overreduction. Effectively, it functions as a form of cyclic electron transfer around PSII. This is particularly novel since no other photosynthetic terminal oxidise is independent of cytochrome b6f. Yet convincing evidence for this 'safety valve' role has been lacking. Moreover, recent PtoX null mutants in model algae and plants suggests an altogether different role for PtoX: the maintenance of PQ pool redox poise during the transition to darkness.

Here we show that the marine cyanobacteria *Synechococcus* and *Prochlorococcus* (and their viruses!) encode a particularly divergent form of PtoX. Through comparative inhibitor studies and heterologous expression, we conclude that marine cyanobacterial PtoX does in fact function as a PSII 'safety valve' and appears to be essential for growth of some *Synechococcus*. Moreover, we show that viruses exploit this pathway during infection and that inhibition of this pathway dramatically reduces viral productivity. These data suggests a 'plastic' role for this family of proteins, which may be dependent on other components of the photosynthetic electron transport chain.

S2P53: Functional exploration of genes involved in marine *Synechococcus* type IV-B chromatic acclimation by mutagenesis

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Synechococcus is the second most abundant phytoplanktonic organism of the world ocean and contributes significantly to global primary production. This cyanobacterium displays a wide diversity of photosynthetic pigments in their light-harvesting antennae (phycobilisomes), reflecting the variety of light niches colonized by this ubiquitous microorganism. Some strains are specialized in harvesting either green or blue light, while others can dynamically modify their light absorption spectrum to match the dominant color. This process called 'Type IV chromatic acclimation' (CA4) has been linked to the occurrence of a small genomic island existing in two distinct configurations (CA4-A and -B). The analysis of metagenomic samples from the *Tara* Oceans circumnavigation revealed that these CA4-capable populations globally constitute the most abundant pigment type and that CA4-A and -B occupy complementary ecological niches [1]. Recently, we characterized two phycobilin lyases, MpeZ and MpeY, i.e. enzymes covalently attaching chromophores on phycobiliproteins that are specifically involved in the CA4-A process [2]. Here, we use a combination of approaches including mutagenesis to characterize two additional lyases, MpeW and MpeQ, and demonstrate their critical role in the CA4-B process. While MpeW attaches the green-light absorbing phycoerythrobilin to cysteine-83 of the -subunit of PE-II, MpeQ binds phycoerythrobilin to the same site in blue light and isomerizes it into the blue light-absorbing phycourobilin, in a similar but inverse way as do MpeZ and MpeY in CA4-A strains. Our results further elucidate the key molecular differences between the two types of *Synechococcus* chromatic acclimaters.

[1] Grébert et al. (2018) PNAS 115:E2010-9
[2] Sanfilippo et al. (2019) PNAS 116:6457-2

S2P54: A New Cyanophage Playground: Viral-encoded Fatty Acid Desaturases

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The interaction between cyanophages and their host is largely mediated by phage-encoded genes that redirect and modulate the virocell metabolism, termed Auxiliary Metabolic Genes (AMGs). The emergence of large viral metagenomic datasets allow the discovery of new AMGs originating from uncultured phages, revealing that cultured phages display only a small fraction of the genomic variability of these entities. However, inferring the encoded enzymes activity and role during infection based solely in sequence homology could lead to mistaken conclusions, for the selective forces are different in the virus and the host. In this work we found that uncultured cyanomyophages carry a fatty acid desaturase (FAD), capable of modulating the host's membrane fluidity and affect the activity of membrane-bound enzymes. We performed bioinformatic analysis and found two distinct families of cyanophage-encoded FAD, with distinct geographical distribution and abundance. Heterologous expression of the viral FADs (vFADs) in fresh water *Synechococcus* showed that both enzymes are delta-9 desaturases, catalyzing the desaturation of carbon number 9 in fatty acid chains of sixteen carbons long. Moreover, our search showed that vFADs are a widespread phenomenon in the virosphere, being present in *Phycodnaviridae*, *Ascoviridae*, and *Myoviridae* families, where most of the vFADs encode for putative '9 desaturases. This work adds a new layer to the interaction between cyanophages and their hosts, possibly affecting the way the virocell reacts to stress and the infection effect on the ecosystem, as it could change the composition of the debris left after burst.

S2P55: The dual role of a cyanophage thioredoxin

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Marine cyanophages are extremely abundant and have a major effect on cyanobacterial populations, diversity and evolution. One abundant family of cyanophages, the T7-like cyanopodoviruses, can be divided into two major clades, clade A and clade B. These clades differ significantly in their infection physiology and also in their abundance. Furthermore, clade A and B are similar in their genomes but have a set of clade specific genes. While differences in infection physiology are known, the genetic origins of these differences remain unclear. In this study, we set out to test whether the thioredoxin gene, found only in clade A cyanophages, contributes to the physiological differences between the clades. We hypothesized that it is involved in phage DNA replication similar to the role of E. coli thioredoxin in T7 replication. To test our hypothesis, we inactivated the gene in

the phage to study its function. We found that the deletion of this gene caused a decline in phage DNA replication and progeny production. These results suggest that the gene increases the burst size of the phage by increasing DNA synthesis. To our surprise, we also found that trxA expression is toxic to the cyanobacterial host. We hypothesized that this toxicity results from the inhibition of host growth by the protein product of the gene. To test this hypothesis, we adapted and used an inducible gene expression system based on the theophylline dependent translational riboswitch. We found that the thioredoxin gene inhibits host growth, and that this function is independent of its putative catalytic site. Our findings indicate that the cyanophage thioredoxin is responsible for some of the physiological differences between clade A and clade B T7-like cyanopodoviruses.

S2P56: Characterising the role of cyanophage plastocyanin during cyanobacteria infection.

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Cyanophage infecting *Prochlorococcus* and *Synechococcus* are ubiquitous in marine systems. How cyanophage infections play out, particularly in terms of subverting host metabolism, is however poorly understood. Genome sequencing and infection studies have demonstrated a great diversity in cyanophage genome size and infection strategies. Moreover, within these genomes are a number of host-like metabolic genes, named Auxiliary Metabolic Genes (AMGs). Cyanophage AMGs are diverse, encoding genes related to photosynthesis, nutrient depletion and central carbon metabolism. Intriguingly, some cyanophages are capable of inhibiting CO2 fixation while maintaining photosynthetic electron transport, supposedly to ensure consistent ATP production for phage replication. However, there is relatively little work focused upon characterising the function of specific cyanophage AMGs during host infection. Here, we set out to characterise the role of the cyanophage-encoded plastocyanin gene, *petE*. Plastocyanin is a key electron transport protein in cyanobacterial respiration and photosynthetic electron transport chains and the high abundance of the *petE* gene in cyanophage genomes suggests an important role during infection. Using the freshwater strain *Synechococcus elongatus* spp. PCC7942 as a heterologous host, we generated knock-in mutants where the PCC7942 gene was replaced with the *petE* gene from the marine *Synechococcus* sp. WH7803 or cyanophage S-RSM4. We compared growth and photophysiology measurements under different light intensities to assess if the cyanophage plastocyanin facilitates greater tolerance to light. Alongside this we are using proteomics to investigate where the cyanophage PetE protein, as well as other cyanophage AMGs, localise during infection of a marine *Synechococcus* host, to give further clues of the role of such AMGs in the infection process.

S2P57: Picocyanobacteria: Growth and physiological responses to light and photoperiod.

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We are studying the growth, physiological and transcriptomic responses of strains of picocyanobacteria originating from different ocean depths, latitudes and oxygen concentrations [O_2], including offshore, oligotrophic strains *Prochlorococcus* marinus MIT9313, SS120 and MED4, and onshore *Synechococcus* and *Cyanobium* strains. We simulate a matrix of eco-physiological niches approximating current and hypothetical future

ocean zones. Using PSI MC1000-OD Multicultivators, we track real-time cell density and chlorophyll while controlling temperature, photoregime, [O_2], spectral color and light level. MIT9313, known as a low light (LL) strain, is actually tolerant of high light, under low [O_2]. In contrast the LL strain SS120 and the high light (HL) MED4 grow poorly under low [O_2]. We suggest that some niche partitioning is driven by [O_2] rather than by light level, per se. In parallel we are testing a regime of photoperiods to simulate depth-dependent light and spectral attenuation, and range expansion polewards into regions with wider annual fluctuations in photoperiod. Real-time monitoring reveals strong diel oscillations which we are analyzing using time-series modelling to extract the influence of the environment upon the amplitudes and phases of diel cycling.

S2P58: Novel anti-phage resistance mechanisms channelled through transcriptional regulation.

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Marine picocyanobacteria of the genera *Prochlorococcus* and *Synechococcus* are the most abundant photosynthetic organisms in the oceans and substantially contribute to marine primary production. The coexistence with highly abundant cyanophages, viruses that infect cyanobacteria, is impacting the abundance, diversity and evolution of the cyanobacterial hosts. This is likely possible due to effective mechanisms of resistance. Broad host-range T4-like cyanophages have identical transcriptional programs in multiple sensitive host strains and recruit their host RNA polymerase. However, the regulation of the transcriptional program remains largely unknown. Generalist cyanophages are able to attach to and enter resistant cyanobacterial cells but cannot complete the infection cycle whereas resistant cyanobacteria commonly show reduced transcription of phage genes. The vast majority of these marine cyanobacteria lacks known resistance mechanisms suggesting that a currently unknown intracellular defence system is at play. The objective of this study is to elucidate how the phage transcriptional program of marine T4-like cyanophages is regulated in sensitive cyanobacteria and to unveil the mechanisms of defence at the transcriptional level in resistant cyanobacteria. As model organisms we will use the T4-like cyanophage Syn9 with the sensitive *Synechococcus* strain WH8109 and the resistant strain CC9311, all of which can be genetically modified. I will present first results on phage and host proteins that might be involved in the regulation of phage promoter activity.

S2P59: From single cell growth to productive ecosystems: Insights from mathematical modelling.

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The evolution of oxygenic photosynthesis in the ancestors of modern-day cyanobacteria gave rise to perhaps the most important biological process within our biosphere. While many properties of growth in axenic cultures are reasonably well understood, cyanobacteria and other microorganisms have evolved as parts of interconnected and dynamic ecosystems. Phototrophic microorganisms, in particular *Prochlorococcus*, are primary engines of biogeochemical cycles and dominate primary production in marine ecosystems.

The purpose of this contribution is to summarize how mathematical modelling can help us to understand how marine microbes interact and collaborate: what are the pre-requisites and energetic trade-offs for cooperation and division of labor? How do metabolic diversity and mutualistic relationships emerge? To tackle these challenging questions, we can build upon high quality quantitative models of microbial growth and resource allocation developed over the past decade. Our premise is that the perspective of cellular resource allocation offers a unique opportunity to understand the constraints and energetic trade-offs that govern the emergence of dependencies between photo- and heterotrophic microorganisms in marine environments.

In particular, within the contribution I seek to outline how computational resource allocation models of cyanobacterial growth, based on quantitative insight into microbial growth physiology, offer the potential to advance ecosystem simulations, and hence our understanding of microbial interactions.

S2P60: Photosynthesis-associated methane production by Cyanobacteria.

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A substantial amount of the potent greenhouse gas CH4 is produced and emitted from oxygenated aquatic systems. This phenomenon that contrasts textbook knowledge where CH4 is produced under strictly anoxic conditions was termed “The Methane Paradox”. Phytoplankton, and *Cyanobacteria*, in particular, are often spatially and temporally associated with high CH4 concentrations in oxygen supersaturated waters. We showed that *Cyanobacteria* release CH4 as what appears to be a byproduct of photosynthesis. This comes in addition to our knowledge that cyanobacteria produce CH4 by demethylation of methylphosphonates, DMSP, and likely methylamines. Interestingly, two abundant marine *Cyanobacteria* showed opposing trends, with *Prochlorococcus* having the highest CH4 production rate per biomass among *Cyanobacteria* and *Synechococcus* the lowest. First investigations show that different inhibitors of photosynthesis have different effects on CH4 production, however, so far, the exact mechanism of photosynthesis-associated CH4 production has not been identified. Nevertheless, our results, together with additional CH4 production pathways, suggest that cyanobacteria are widespread, omnipresent methanogens.

S2P61: Guilty by association in cyanobacterial genomes: the ribosome-assembly GTPase EngA and the multitask regulator PipX.

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The Cyanobacterial Linked Genome (CLG) is a web tool that generates flexible gene networks based on synteny at custom threshold (https://dfgm.ua.es/genetica/investigacion/cyanobacterial_genetics/dCLG/). It connects PipX with the ribosome-assembly GTPase EngA. PipX is a small protein exclusive of cyanobacteria that binds to the nitrogen regulators NtcA and PII according to the C/N ratio and energy status. On the other hand, EngA is a eubacterial protein essential in all systems studied so far. The physical interaction between PipX and EngA proteins, already demonstrated by a variety of approaches, provides the first proof of concept for the

generated CLG. EngA plays essential roles in ribosome biogenesis in both bacteria and chloroplasts and has been found membrane associated in E. coli as well as in Arabidopsis thaliana thylakoids, where it has been connected with the photosystem II repair cycle. EngA shows a unique domain structure (G1-G2-KH-like) in which two G domains are tandemly repeated. GD1 contains the main determinants for interactions with PipX.
In previous works we established that pipX overexpression or gain-of-function mutations decreases growth in *Synechococcus* elongatus PCC7942, a “PipX toxicity” phenomenon observed whenever the PipX/PII ratio is increased. For a long time, we have been speculating that PipX toxicity could be caused by its binding to an unknown binding partner, and we now wondered whether EngA could be that enigmatic protein.
To integrate previous information on PipX with our latest results concerning PipX-EngA interactions we propose that PipX binds to EngA to slow down growth under conditions in which the levels of PII and EngA effectors (together signalling energy status and C/N ratios) allow formation of a significant number of PipX-EngA complexes that interfere with ribosome assembly. This scenario, that could be achieved by genetic manipulation resulting in increases of the intracellular PipX/PII ratio, would occur under non-optimal growth conditions, including the environmentally relevant temperature of 20ºC. The current model for PipX-EngA regulatory interactions and future research will be discussed.

S2P62: Bacterial growth phases in *Synechococcus elongatus* PCC7942.
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Cyanobacteria as photosynthetic bacteria belongs to the microalgae and they are in the focus of several biotechnological applications. Settlement of ideal growing conditions are important for the effective biomass production. In the natural environment constant bacterial growth is seldom found, in contrast to laboratory conditions, when bacteria are cultured in rich media at an optimal temperature for a while. In a nutrition rich medium bacteria can sustain a continuous, relatively fast, balanced growth for a given time that called exponential growth phase. This is followed by stationary phase, where the cell number ceases to increase. In the late stationary phase the cells commencing to change their genetical regulations and enter a so called viable but not culturable (VBNC) state that we defined as bacterial G0 state. Recently we want to shed light on the differences between the cells of exponential and stationary phases and characterize the two states and transition between them in *Synechococcus elongatus* PCC7942. Part of this project has received funding from the Hungarian National Research, Development and Innovation Office grant GINOP-2.3.2-15-2016-00058 and the European Union‘s Horizon 2020 Research and Innovation Programme under grant agreement N° 101000501.

S2P63: A novel auxiliary metabolic gene cassette found in viral metagenomic data.
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Prochlorococcus and *Synechococcus* utilise phycobiliproteins to expand the range of light energy harvested for photosynthesis. Phycobilins, the pigments responsible for absorbing

light in the green gap of chlorophyll, are linear tetrapyrroles, whose biosynthesis relies on heme as a precursor. Recently, marine bacteriophage encoded genes have been discovered that have the potential to complement bacterial heme synthesis and augment cyanobacterial photosynthesis, the so-called auxiliary metabolic genes(AMGs). The synthesis of heme requires 5-aminolevulinic acid(ALA), which is synthesised via one of two different pathways. In α-proteobacteria, ALA is formed via the Shemin(C4)-pathway as a condensation reaction of glycine and succinyl-Coenzyme A by the 5-aminolevulinic acid synthase(ALAS;*hemA*), whereas other bacteria use the C5-pathway utilising glutamyl-tRNA for ALA formation. AMGs for pigment synthesis, heme oxygenase(*ho1*) and ferredoxin-dependent bilin reductases(*pebS, pcyX*) have already been identified encoded on cyanophage genomes. Bioinformatic analysis of viral metagenomic data from multiple marine and freshwater samples identified a conserved AMG cassette. This cassette is present in both habitats and consists of a putative *hemA, ho1* and *pcyX* genes. The flanking viral genes of the cluster suggest a bacteriophage, possibly capable of infecting an α-proteobacterium. An identical gene cluster arrangement was furthermore identified in a giant-phage from freshwater, which probably infects methanotrophic bacteria. The putative ALAS from marine phages shows high similarity regarding its amino acid sequence compared to well-characterised ALASs, like the one from *Rhodobacter capsulatus* with ~ 50 % (E-value 1e-145) identity. In addition, structural modelling using Phyre2 confirmed this on a three-dimensional level. To verify the biochemical activity of the phage ALAS, heterologous expression of *hemA* in *E. coli* was conducted and the protein was purified via affinity chromatography. Currently, the enzyme assay is set up to allow quantitative measurements of product formation via the Ehrlich reagent. Additionally, in a functional complementation study, plasmid-encoded phage ALAS will be used to recover auxotrophic *E. coli* and *R. capsulatus* Δ*hemA* mutants. This is the first report of a phage-encoded *hemA*, and its occurrence in a cassette with cyanophage-associated AMGs suggests a potential role in linear tetrapyrrole synthesis. Its novelty is based on the finding that it encodes for an ALAS, which is normally only present in α-proteobacteria. The results of this study will provide insights into the role of AMGs involved in pigment-biosynthesis outside the cyanobacteria, but might also answer questions of host specificity and mechanisms of horizontal gene transfer.

S2P64: Recurring cyanophage infection results in increased fitness rather than changes in host range.

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As obligate parasites, bacteriophages depend on their host's intracellular mechanisms in order to propagate, evolve and persist. Hence, phage host range is a characteristic of major importance, which interplays with the availability of susceptible hosts in the environment to determine the fate of phage particles. Host range, and host compatibility in general, is an evolvable trait: adaptation may result in host range expansion or constriction, host switching and changes in fitness on a specific host. Evidence suggests that the evolutionary trajectory of a phage depends in part on host availability. Here, we compare the means of adaptation between a narrow and a broad host range cyanophage in different host availability conditions. This will allow us to understand which adaptive trajectory is more likely to occur: (1) changes in host range and acquiring the ability to infect a novel, previously resistant host, or (2) changes in fitness on the original host and selection of

variants that are better adapted to it (or both). We repeatedly infected a specific susceptible host with and without the presence of a resistant non-host belonging to different *Prochlorococcus* ecotypes, and tested several susceptible-to-resistant host ratios. When plating phages on the original susceptible host at the end of the experimental procedure, we observed a major increase in plaque size. This increase was evident when cultures were composed of susceptible host alone, but also in some conditions where the resistant non-host was present. The increase in plaque size was found to be gradual in the three replicates propagated on the susceptible host alone. This suggests that several mutations accumulated during the process of adaptation, each contributing to the eventual phenotype in an additive manner. We also observed different timelines and plaque size distributions between the three lineages, suggesting different population composition in each of them. No host range expansion or switching was observed for either of the two phages, even though the resistant non-host constituted the majority of the culture in some conditions. The increase in plaque size indicates that cyanophages adapted increased fitness on their original susceptible host rather than acquire the ability to infect a novel host in the interactions examined, even when a resistant non-host was present. We are currently looking into the genotypic changes associated with the observed adaptations.

S2P65: A toolkit for engineering the highly productive cyanobacterium *Synechococcus* sp. PCC 11901.

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Synechococcus sp. PCC 11901 (PCC 11901) is a recently discovered fast-growing marine cyanobacterial strain. It has a capacity for sustained biomass accumulation to very high cell densities, even outperforming the marine model strain *Synechococcus* sp. PCC 7002. Nevertheless, very few genetic tools have been characterised in this strain. Here, we outline our progress towards the development of a synthetic biology toolkit based on the CyanoGate MoClo system to unlock the biotechnological potential of PCC 11901. We have characterised several neutral sites suitable for stable genomic integration that do not affect growth even at high densities. Furthermore, we have found that PCC 11901 is amenable to transconjugation and have characterised a suite of known and new genetic parts, including constitutive promoters , terminators, inducible systems, and CRISPR interference tools. We envision that this toolkit will lay the foundations towards the adoption of *Synechococcus* sp. PCC 11901 as a robust model strain for engineering biology and green biotechnology.

S2P66: Redox regulation of protein activity by the thioredoxin system in *Prochlorococcus*.

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In most cyanobacteria and plants, the iron-sulfur ferredoxin:thioredoxin reductase (FTR) enzyme catalyzes the transfer of photosynthetically-derived electrons to proteins for disulfide reduction, resulting in the modulation of protein activity. Of the most primitive targets in evolution, selected proteins of the Calvin-Benson cycle and associated processes, such as the oxidative phosphate pentose pathways, are regulated by this system. In contrast to other oxygenic photosynthetic organisms, the ancient cyanobacterium *Gloeobacter* and the ocean-dwelling green oxyphotobacteria *Prochlorococcus* lack an FTR gene, raising the question of how these photosynthetic organisms link carbon fixation and related metabolic processes to light and other changing environmental conditions. During the last few years, we have explored this issue and have found that an evolutionary-unrelated enzyme has substituted FTR in its function likely for adaptation of the redox regulatory mechanisms to the prevailing environment. This would explain how marine phytoplankton *Prochlorococcu*s living in Fe-deficient environment substitutes a Fe-S enzyme by a flavoenzyme. In this Workshop, we will present our latest findings on the thioredoxin system in *Gloeobacte*r and *Prochlorococcu*s, and promote a discussion including evolutionary, biochemical and structural aspects of the regulatory pathway.

S2P67: Effect of temperature on the macromolecular composition and resource allocation of bacterioplankton in the lab and in the ocean.

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Microorganisms are strongly affected by the surrounding temperature, yet how exactly temperature affects cell structure and function is still unclear. Two different hypotheses have been raised as to how temperature affects resource allocation in microorganisms, resulting in opposite cellular and biochemical responses, as well as cellular elemental ratios. According to the translation-compensation hypothesis (TCH), high temperatures results in an increase in enzymatic activity (including protein translation), resulting in a decrease in the amount of ribosomes the cell needs for the same amount of protein production (low RNA:protein). Alternatively, the growth rate hypothesis (GRH) states that high temperature increases the growth rate of microorganisms, which then require increased ribosome content to produce the required protein for growth (high RNA:protein). In order to test these two hypotheses, two research approaches were undertaken: 1) In-vitro laboratory experiments, where different *Prochlorococcus* and *Alteromonas* strains were grown across multiple temperatures; 2) In-situ field measurements in the Eastern Mediterranean Sea (EMS), where the temperature changes naturally over the year. We found support for both the TCH and the GRH, depending on the organism and conditions. Broadly speaking, a tendency was observed for the RNA:protein ratio and RNA/cell of *Alteromonas*, especially HOT1A3, to decrease with temperature, in accordance with the TCH. Contrary, that of *Prochlorococcus* mostly increased with temperature and growth rate, thus supporting the GRH. The results of the natural population also supported the GRH, as the changes in MM composition in the deep chlorophyll maximum (DCM) were corelated with growth rates/viability estimates such as BP/cell and the high nucleic acid

(HNA)/low nucleic acids (LNA) ratio. Interestingly, a considerable part of the cellular P was not allocated to ribosomes (between 61.5±22.4 in laboratory *Alteromonas* to >90% in the high-light *Prochlorococcus* and natural populations), raising the question which cellular molecules contain these P reserves. The results of this study highlight the complexity of cellular response to changing temperatures, suggesting that the TCH and GRH may each be applicable to different organisms or environmental conditions.

S2P68: Nutrient concentration or nutrient ratio? The effect of media composition on *Prochlorococcus* growth and decline.

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Dissolved nutrients in the ocean, mainly Nitrogen (N) and Phosphorus (P), play major roles in stimulating growth and primary production, thus ensuring life in the sea. Common mathematical models of bacterioplankton growth predict that growth rate will only be affected by extracellular nutrient concentrations near the Km of the uptake mechanism, yet this has not been tested experimentally. We asked whether *Prochlorococcus*, a globally abundant model cyanobacterium, can respond to high media nutrient concentrations or changes in media N:P ratio in batch culture through changes in growth rate. We examine two alternative hypotheses; i) Growth yield (maximum biomass) in lab cultures depends on media nutrients concentrations, but growth rate does not. This is because laboratory media concentrations are much higher than concentrations at sea and assumed the Km of the uptake mechanism. ii) Both yield and growth rate change as a function of media concentrations, and specifically the N:P ratio. Three *Prochlorococcus* strains were studied, isolated from two different niches: High-light-adapted MED4 and MIT9312 grow at the ocean surface, where nutrients are scarce, and low-light-adapted MIT9313 grows deeper in the water where nutrients are more available. All strains were grown in multiple nutrient conditions: Pro99 (nutrient-replete medium), low N, low P and low N/low P media. Culture growth was monitored non-invasively using fluorescence, and cell counts were obtained using flow cytometry. Strains MED4 and MIT9312 did not change their growth rate in response to nutrient concentrations, whereas the growth rate of MIT9313 was significantly lower in the lowN media, but this difference was quantitatively minor. In contrast, as expected, the maximal culture fluorescence of all cultures was affected by nutrient concentrations. Interestingly, differences were observed in the decline rate and in the shape of the decline-curve, where lowN media is characterized by a sharper and faster decline, whereas lowP media has slower decline. Furthermore, both MED4 and MIT9312 seem to survive longer under P-starvation compared to MIT9313, which seems to be more sensitive to starvation. To conclude, it seems that different *Prochlorococcus* strains respond and adapt uniquely to nutrient limitation, possibly as a result of niche-adaptation.

S2P69: Study of carotenoid-binding proteins in marine picocyanobacteria.

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In photosynthetic organisms, the excess of light energy needed for photosynthesis can produce highly reactive molecular species that damage the photosynthetic apparatus. Cyanobacteria have evolved a reversible photoprotective mechanism in which that energy is dissipated as heat. In most cyanobacteria, photoprotection involves dynamic interactions among the light-harvesting antenna (the phycobilisomes, PBS), the photoactive Orange Carotenoid Protein (OCP), and the Fluorescence Recovery Protein (FRP). The OCP is

composed by an all-helical carotenoid binding N-terminal domain (NTD) and a C-terminal domain (CTD) with a mixed alpha-beta fold. Moreover, a carotenoid spans both domains. Recently, two new families of carotenoid-binding proteins have been described, the Helical Carotenoid Proteins (HCPs) are homolog to the NTD and a family homolog to the CTD named C-terminal domain-like carotenoid proteins (CCPs)1. Various photoprotective mechanisms exist in different ecotypes of *Synechococcus*, including the non-photochemical quenching (NPQ) via the OCP and FRP. However, *Prochlorococcus* lacks OCP-related proteins. Our bioinformatics analysis revealed that OCP from marine *Synechococcus* is relatively poorly conserved. Only 65% sequence identity to the OCP1 of freshwater strains (typically 85-90% identical); therefore, they are the most common divergent OCP2. In addition, we are analyzing the carotenoid profile of marine *Synechococcus* under different conditions. A fair amount of biophysical, structural, and biochemical studies has contributed to understanding the NPQ process via OCP in cyanobacteria. However, there is a lack of knowledge in the regulation. Interestingly, preliminary results indicate putative binding sites for NtcA to regulate OCP that might exist in marine *Synechococcus*, connecting the nitrogen metabolism regulation and the photoprotective mechanisms. Experiments are underway to elucidate this possible association.

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Session 3: Predation and trophic interactions

S3P61: **Protist impacts on marine cyanovirocell metabolism.** Cristina Howard-Varona, Simon Roux, Benjamin Bowen, Leslie Silva, Rebecca Lau, Sarah Schwenck, Samuel Schwartz, Tanja Woyke, Trent Northern, Matthew Sullivan, Sheri Floge

S3P62: Influence of environmental parameters on cyanobacteria-phage interactions Laure Arsenieff, Debbie Lindell

S3P63: *Synechococcus* ecotypes exhibit distinct physiological responses to Fe stress corresponding to their biogeographic distributions. B. Shafer Belisle, Nathan A. Ahlgren.

S3P64: THE CURIOUS CASE OF *PROCHLOROCOCCUS* HEMOLYSINS. Waseem Bashir VK, Dikla Aharonovich, Daniel Sher

S3P65: Cyanophage dynamics at the San Pedro Ocean Time Series: Generalists, specialists, and one-shot-wonders. Emily Dart, Jed Fuhrman, Nathan Ahlgren.

S3P66: Effect of different *Synechococcus* prey on nanoflagellate ingestion rate Bryan Hamilton, Kristen Hunter-Cevera**.**

S3P67: Vesicles deliver products of leaking function in the Black Queen Hypothesis. Zhiying Lu, Jeffrey Morris

S3P68: Genetic determinants of cyanophage resistance differ among isolates of marine *Synechococcus* spp. Marcia Marston, Shawn Polson, Jennifer Martiny.

S3P69: Host recognition in generalist and specialist cyanophages. Lea Reuveni, Debbie Lindell.

S3P70: Abundance patterns of cyanophages in the North Pacific Ocean. Sigitas Šulčius, Debbie Lindell

S3P71: Variable uptake of *Prochlorococcus*-derived metabolites by the gamma-proteobacterium Alteromonas macleodii. Kathryn H. Halloran, Krista Longnecker, Rogier Braakman, Allison Coe, Sallie W. Chisholm, and Elizabeth B. Kujawinski

S3P72: Nutrient cycling in picocyanobacteria-heterotroph interactions. Maria del Mar Aguilo-Ferretjans, Despoina Sousoni, James Kerr, David J. Scanlan, Joseph A. Christie-Oleza

S3P61: Protist impacts on marine cyanovirocell metabolism

Cristina Howard-Varona[1], Simon Roux[2], Benjamin Bowen[3], Leslie Silva[6], Rebecca Lau[3], Sarah Schwenck[5], Samuel Schwartz[4], Tanja Woyke[2], Trent Northern[3], Matthew Sullivan[1], Sheri Floge[4]

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The fate of oceanic carbon and nutrients depends on interactions between viruses, prokaryotes, and unicellular eukaryotes (protists) in a highly interconnected planktonic food web. To date, few controlled mechanistic studies of these interactions exist, and where they do, they are largely pairwise, focusing either on viral infection (i.e. virocells) or protist predation. Here we studied population-level responses of *Synechococcus* cyanobacterial virocells (i.e. cyanovirocells) to the protist *Oxyrrhis* marina using a systems biology toolkit combining transcriptomics, endo- and exo-metabolomics, photosynthetic efficiency measurements, and microscopy. The cyanovirocells had a small intracellular response, displaying known patterns of virus-mediated metabolic reprogramming while releasing diverse exometabolites during infection. When protists were added, many exometabolites disappeared, suggesting microbial consumption. Additionally, the intracellular cyanovirocell impact was largest, with 4.5-fold and11-fold more host transcripts and endometabolites, respectively, responding to protists, especially those involved in resource and energy production. Physiologically, photosynthetic efficiency also increased, and together with the transcriptomics and metabolomics findings suggest that cyanovirocell metabolic demand is highest when protists are present. These data illustrate unique responses of cyanovirocells to protist predators that are not yet considered when linking microbial physiology to global scale biogeochemical processes.

S3P62: Influence of environmental parameters on cyanobacteria-phage interactions

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In marine environments viral infection is a major biological factor involved in the regulation of microbial communities. Nevertheless, both the viral and bacterial protagonists also interact with their environment which affects cellular activities and processes. Over the past few decades, studies have demonstrated the effects of abiotic factors (such as temperature, irradiance and nutrients) on virus-algae interactions. These parameters play a considerable role in infection kinetics, affecting both host and viral properties, which can dramatically alter the outcome of their interactions. Elucidating how environmental parameters regulate host-virus interactions is thus of prime importance to unveil how they coexist and how these interactions change along environmental gradients in the oceans. In marine communities, cyanobacteria of the genera *Prochlorococcus* and *Synechococcus* are highly abundant and contribute greatly to primary production. The viruses that infect them, the cyanophages, are considered to be main drivers in the mortality, diversity and evolution of their hosts. To date, responses to factors such as light, pH or nutrient availability have been investigated for only a few phage-cyanobacteria systems, but not temperature. In this context, the main objective of my research is to investigate the interplay between cyanobacteria and their phages under different abiotic pressures. Here I will present the influence of temperature on cyanophage decay and infection properties, with future work focusing on the effect of temperature and nutrients on cyanobacteria-phage interactions. Results obtained in this study will provide insights into the drivers governing cyanobacterial-cyanophage interactions across gradients of environmental change in the oceans.

S3P63: *Synechococcus* ecotypes exhibit distinct physiological responses to Fe stress corresponding to their biogeographic distributions

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Primary production in aquatic systems is often limited by nutrient availability, which can dramatically impact microbial community composition and selects for specific adaptations to these limited conditions. Iron (Fe) limits growth in roughly 40% of the world's oceans, particularly in high-nutrient low-chlorophyll (HNLC) regions, but phytoplankton in coastal waters can experience Fe-limitation as well. Biogeographic distributions suggest that *Synechococcus* ecotypes are adapted to several distinct Fe niches. Coastal ecotypes I and IV and HNLC ecotypes CRD1 and CRD2 appear to be adapted to waters with Fe-limiting conditions. Oligotrophic ecotype II is abundant in regions with midrange levels of Fe availability, while ecotype III appears to thrive in oligotrophic but high-Fe habitats. However, physiological work on representative cultures to confirm these distinct niches is lagging'of the above major ecotypes, Fe physiology experiments have only been conducted on ecotypes I and III. To fill this gap in knowledge of Fe-adaptation in *Synechococcus*, we evaluated growth dynamics under various Fe concentrations for isolates from ecotypes II, IV, and CRD1. Our results demonstrate a higher tolerance of HNLC CRD1 strains to Fe-limiting conditions as expected. Likewise we will present comparative analysis of Fe-stress responses for all of the above ecotypes. This work provides an important expanded view of the range of adaptive responses to Fe availability across *Synechococcus* ecotypes and corresponding to the diverse niches they occupy across the global oceans.

S3P64: THE CURIOUS CASE OF *PROCHLOROCOCCUS* HEMOLYSINS

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Hemolysins are toxic membrane rupturing molecules secreted as a defence or predatory mechanism by many species of bacteria. Their presence in marine organisms has always been a point of interest that has raised multiple hypotheses addressing their intended function. We report the presence of a set of genes that are annotated to have hemolysin-like features in the genomes of *Prochlorococcus* marinus strains. In-silico studies into available P. marinus genomes showed the presence of these genes across these genomes in varied frequencies. A detailed analysis of the retrieved list of hemolysin like genes suggests that we can classify the genes into three broad categories based on the reason they are annotated as a hemolysin like gene, which is the presence of Calcium Binding Sites, RTX_N terminal repeats or FTS_J RNA binding domains. One of these genes (annotated as Hemolysin A/ FTS_J RNA Methyl Transferase) closely resembles the hemolysin genes reported in known hemolytic pathogens such as Brachyspira hyodysenteriae and Helicobacter pylori. However, this same gene also resembles RNA methyltransferases. Our preliminary analysis found that the encoded protein could be of dual annotation because of sequence similarity to both groups of genes and the presence of key features such as a characteristic amino acid tetrad (K-D-K-E) for the FTS_J Group. Studies had shown that the same gene from Mycobacterium tuberculosis has a dual function when expressed, but the role the gene serves in the cell remains unclear. To begin

with, we are working on recombinantly expressing the MIT9313 protein, in order to test its biochemical activity (hemolysis/RNA methyltransferase) and in the long term we hope to infer its role in the biology of *Prochlorococcus.*

S3P65: Cyanophage dynamics at the San Pedro Ocean Time Series: Generalists, specialists, and one-shot-wonders

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Cyanophage exert important controls on the abundance and productivity of cyanobacteria communities as well as impact strain-level diversity within these communities. Long term time series show that natural phage communities exhibit predictable seasonal patterns while models, such as Kill-the-Winner, suggest a continual overturn of virus and host strains. However, important, basic knowledge is still lacking on the dynamics and population structure of the phage strains that are most successful at controlling host populations. Here we analyze multi-year stain level dynamics of the cyanobacteria genera *Prochlorococcus* and *Synechococcus* and the T4 cyanophage that infect them at the San Pedro Ocean Time Series. By utilizing 5 years worth of cellular and viral metagenomes along with cyanobacterial ITS marker gene data, we demonstrate that closely related phage strains exhibit different long term dynamics. The cyanophage community consists broadly of three groups. 1) Generalist phage remain at low, constant relative abundance throughout the time series and exhibit no discernible seasonal pattern or significant correlation with host ecotypes as is consistent with them having a broad host range. 2) Seasonal specialist phage occur repeatedly at high relative abundance and are strongly correlated with host ecotypes that exhibit similar seasonal patterns. 3) One-shot-wonder' phage occur sporadically at high relative abundance and correlate both with host strains that also occur briefly within the time series and with high abundance seasonal host strains. Additionally, phylogenetic analyses indicate that phage relatedness is not a predictor of their long term dynamics; phage within the same monophyletic group fall into the above three categories. These data suggest that cyanobacterial lysis and productivity are impacted by a combination of both stable, seasonally re-occurring and sporadically successful phage strains. These results indicate that the complex co-evolutionary arms race between cyanobacteria and cyanophage at this location is influenced by both recurrent and stochastic processes. This in turn has important implications for informing future models of phage-host dynamics.

S3P66: Effect of different *Synechococcus* prey on nanoflagellate ingestion rate

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At the Martha's Vineyard Coastal Observatory (MVCO) located on the US Northeast Shelf, *Synechococcus* is the most numerically abundant phytoplankter. *Synechococcus* dynamics are governed by both environmental ('bottom-up') and biological ('top-down') variables. Loss processes in particular shape abundance features (i.e. spring bloom) and can determine the maximum concentrations of cells reached. Grazing, viral lysis, and sedimentation are a few of the known removal processes for photosynthetic picoplankton. Grazing is especially important to understand as this process directly transfers nutrients and energy to higher trophic levels. At MVCO, however, the identity of protist grazers that consume *Synechococcus* is unknown as these organisms are notoriously difficult to isolate

and concentrate. We have performed a time series of enrichments for protist grazers and have isolated four marine protists belonging to the genera Pseudobodo, Goniomonas, and Paraphysomonas. We have also designed a microfluidic device to aid in grazer isolation and concentration attempts. This device has enabled us to significantly lower background bacteria levels during grazer ingestion rate experiments, allowing for better control of protist-to-*Synechococcus* encounter rate. From ingestion experiments between Paraphysomonas bandaiensis and eight different clade representatives of *Synechococcus*, we find that this protist shows distinct ingestion preferences for certain strains over others. These experiments not only help us understand how grazing may shape *Synechococcus* population diversity patterns, but also provides key rates that can be utilized to understand top-down control of cell abundance.

S3P67: Vesicles deliver products of leaking function in the Black Queen Hypothesis

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Background:
The Black Queen Hypothesis (BHQ) is a sweeping hypothesis that explains how free-living microorganisms rely on each other to live more efficiently and the heart of BHQ is that leaking function become public goods and is used by beneficiaries. However, how the products of leaking function from one organism approach to another is still unknown.

Method:
Using *Prochlorococcus* MIT9312 and its heterotrophic bacterium Alteromonas EZ55 as model organisms, we separated and concentrated the extracellular products of Alteromonas EZ55 in the media and evaluated its effects on *Prochlorococcus* growth. Proteomic analysis, cryo-electron microscopy observation and detection of *Prochlorococcus*-vesicle interaction by flow cytometry were also conducted.

Results:
The results showed that both the > 50 KDa and < 50 KDa fractions could promote the growth of *Prochlorococcus*, and > 50 KDa fraction have stronger growth promotion and its activity was high temperature-sensitive. By proteomics approach, a series of leaking function-related proteins were identified, such as catalase, superoxidase, membrane transporters and hydrolase from >50 KDa fraction. Subcellular localization analysis indicated that the targeted location of most of these identified proteins were not extracellular and their molecular weight were smaller than < 50 KDa. Cryo-electron microscopy observed vesicles in >50 KDa fraction. Fluorescence microscopy and flow cytometry further confirmed that the attachment of vesicles to *Prochlorococcus*.

Conclusion:
The above results suggested that, in the BHQ-involved microorganisms, helpers might package their public goods (products of leaking function) into vesicles, and delivered them to beneficiaries.

S3P68: Genetic determinants of cyanophage resistance differ among isolates of marine *Synechococcus* spp.

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Synechococcus and cyanophage communities in marine coastal environments are genetically diverse and temporally variable. Significant proportions of *Synechococcus* communities can be lysed daily by cyanophages, although estimates vary widely ranging from less than 1% to over 40%. To better predict rates of viral-induced mortality, an understanding of the genetic determinants responsible for host resistance and viral infectivity is needed. In this study, we conducted pairwise-coevolution experiments in continuous culture using two strains of marine *Synechococcus* (WH7803 and WH8101) and six cyanophage strains (four myoviruses and two podoviruses). We isolated *Synechococcus* cells from different time points in each experiment and then sequenced their genomes to examine the genetic basis of resistance. We also tested each isolates breadth of resistance using a panel of genetically distinct cyanophage strains including both myoviruses and podoviruses along with coevolved viral isolates. In general, phage-resistant *Synechococcus* isolates differed by one to eight nucleotides from the ancestral cells and there were multiple ways in which a *Synechococcus* strain could become resistant to the same virus. Interesting differences were observed among WH7803 and WH8101 phage-resistant isolates. In *Synechococcus* WH7803 isolates, many mutations were observed in genes located within a single genomic island. In addition, when WH7803 isolates became resistant to one virus, they often gained resistance to other strains of cyanophage (both myoviruses and podoviruses). The location of mutations within the genomic island influenced both the breadth of resistance to diverse cyanophages and also how frequently resistance-breaking phage mutants were observed. In phage-resistant *Synechococcus* WH8101 isolates, the majority of mutations (over 80%) were not in genomic islands and resistance was more specific, thus when WH8101 isolates became resistant to one virus, they rarely became resistant to other viral strains. An understanding of the genomic dynamics of viral-host interactions will provide insights into the processes that allow natural communities of *Synechococcus* and cyanophages to coexist and persist.

S3P69: Host recognition in generalist and specialist cyanophages

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In the oceans, cyanobacteria live alongside viruses that infect them (cyanophages). The two main families of cyanophages are T7-like podoviruses that have a narrow host-range and T4-like myoviruses with members that have either a narrow host-range and are termed specialists or have a broad host-range and are generalist phages. In previous research conducted in our lab, it was shown that extracellular resistance due to impaired attachment was common against specialist phages but rare against generalist phages. We hypothesized that generalist cyanophages recognize conserved cell-surface components that are essential to their hosts. Here we tested this hypothesis by selecting for resistance in two *Prochlorococcus* strains (MED4 and MIT9515) against two generalist and three specialist phages, in a total of 7 interactions, and comparing the frequency of resistant strains that appeared. The assumption is that if a gene is essential to its host in its current form, then the frequency of resistant mutants will be very low. Our results show that while there are mutants resistant to specialist phages, no resistant colonies appeared against generalist phages. Furthermore, the frequency of resistant cells to specialist phages varied greatly depending on the interaction, including a two-fold difference in occurrence of

resistance in a single *Prochlorococcus* strain towards two different phages. These results support our hypothesis that generalist phages attach to a receptor that is essential to their cyanobacterial host cyanobacteria, which is different to specialist phages.

S3P70: Abundance patterns of cyanophages in the North Pacific Ocean.

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Viruses are an important factor in cyanobacteria mortality and have major influence on fluxes of organic and inorganic matter in the oceans. However, their spatial patterns of diversity and abundance as well as the underlying drivers remain poorly known. In this study, we investigated the latitudinal changes and environmental predictors of two cyanophage families (T4-like myoviruses and T7-like podoviruses) across the North Pacific Subtropical Gyre and transitional zone towards the subpolar gyre using samples collected during three SCOPE Gradients research expeditions (2016, 2017 and 2019) and using a solid-phase single-molecule PCR (Polony) approach. Despite the variation in cyanophage abundances between different years, there was a consistent pattern in distribution of T4-like and T7-like cyanophages along the North Pacific Transitional Zone. We observed a latitudinal shift in cyanophage community composition, from the dominance of T4-like cyanophages at lower latitudes (23°N-31°N) to the dominance of T7-like cyanophages at higher latitudes (34°N-38°N) along the North Pacific Transitional Zone. Along each vertical profile cyanophage distribution was correlated with cyanobacteria abundance and chlorophyll *a* concentration. Regression analysis showed that main predictor for the variability in T4-like and T7-like cyanophage abundance over the latitudinal and vertical transects was *Prochlorococcus* abundance, explaining up to 70% of total variability.

S3P71: Variable uptake of *Prochlorococcus*-derived metabolites by the gamma-proteobacterium *Alteromonas* macleodii.

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Phytoplankton such as *Prochlorococcus* are often implicated in the release of dissolved organic carbon (DOC) to the ocean. Yet we have a limited understanding of the molecular composition of DOC, the sources and sinks of specific molecules that make up this carbon pool, and the biological interactions mediated by these compounds. Understanding these dynamics is critical to understanding the carbon cycle and microbial ecology of the ocean. Recent work in our laboratories has explored the complex suite of metabolites released by *Prochlorococcus* strains in culture, under varied growth conditions. Interestingly, some metabolites, such as the branched-chain amino acids and their precursors, are frequently excreted at high levels. In this study, we examined the growth of a copiotrophic gamma-proteobacterium, *Alteromonas macleodii* strain MIT1002, when cultured with these excreted metabolites as a carbon source. We found that *A. macleodii* is able to grow using certain metabolites but not others, pointing to unexpected substrate specificity for this bacterium. To follow up, we tested the ability of *A. macleodii* to remove a subset of these metabolites from solution by monitoring the change in dissolved metabolite concentration during growth. We found that it can remove the valine precursor, 3-methyl-2-oxobutanoic acid, from solution, but not the other branched chain amino acid precursors. *Alteromonas macleodii* has pathways for processing all of these compounds. Our observations therefore suggest a high level of transporter specificity and may reflect the importance of 3-methyl-

2-oxobutanoic acid in other biochemical pathways, or the importance of *Prochlorococcus* exudates to this strain of *A. macleodii*. These results help illuminate microbial interactions influencing *Prochlorococcus* ecology and provide additional constraints on the sources and sinks of compounds within labile DOC.

S3P72: Nutrient cycling in picocyanobacteria-heterotroph interactions

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Nutrients are scarce in the photic layer of the ocean, limiting growth and cell densities. Nevertheless, the rapidly cycling of the nutrients available within these oligotrophic ecosystems and high efficiency in their use, allow a higher productivity than previously anticipated and helps sustain the entire marine food web. The unicellular phototrophs that populate and feed these ecosystems have adapted to extreme oligotrophic conditions via various means. One of these is based on niche specialisation which facilitates collaborative interactions where nutrients are recycled between phototrophic and heterotrophic members of the planktonic community. We have performed long-term phototroph-heterotroph co-culture experiments under natural oligotrophic seawater conditions to underpin the physiological interactions that take place under these relevant conditions. Focussing here on model picocyanobacteria from the genus Prochlorococcus and Synechococcus, grown in combination with a diverse range of marine heterotrophic bacteria, we show that it is not the concentration of nutrients but rather their circulation that maintains a stable interaction and a dynamic system, although this was not achieved under all combinations. High-throughput proteomic and metabolite data generated from these co-cultures revealed mechanistic insights into the cross-feeding process that occurs in each one of these systems. Thus, I will present a comprehensive understanding of the networks of marine phototroph-heterotroph interactions at a broad scale and challenge the general belief that marine phototrophs and heterotrophs do not always compete for the same scarce nutrients and niche space, but instead suggest these organisms more likely benefit from each other because of their different levels of specialization and complementarity within long-term stable-state systems.

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