Seasonality and dynamics of microbial consortia in the Bay of Banyuls

Stefan Lambert

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Sorbonne Université

École doctorale des Sciences de l'environnement d'Ile-de-France
Laboratoire d'Océanographie Microbienne - UMR 7621
Observatoire Océanologique de Banyuls

Seasonality and dynamics of microbial consortia in the Bay of Banyuls

Par : **Stefan Lambert**

These de doctorat d'oceanographie microbienne

Sous la direction de François-Yves Bouget
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Devant un jury composé de :

Rapporteur : Urania Christaki, Professeur, Université du Littoral Côte d'Opale, Wimereux

Rapporteur : Raffaele Siano, Ifremer, Laboratoire d'Ecologie Pélagique, Plouzané

Examinateur : Ingrid Obernosterer, CNRS, Banyuls-sur-mer

Examinateur : Behzad Mostajir, CNRS, MARBEC, Montpellier

Examinateur : Pierre Galand, CNRS, LECOB, Banyuls-sur-mer

Directeur de these : François-Yves Bouget, CNRS, LOMIC, Banyuls-sur-mer

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Abstract

The timing of major life events such as migration or mating in animals or flowering in plants has evolved to follow the Earth's revolution around the Sun. In temperate oceans, yearly transitions from winter to spring are accompanied by an increase in abundance of phytoplankton. Marine phytoplankton is at the basis of the food chain in the oceans, and plays an essential role in biogeochemical cycles, as it generates roughly 50% of the global primary production.

A time series was established at the coastal sampling station SOLA (Bay of Banyuls, North Western Mediterranean Sea) in 2007. Since then, environmental and biological parameters have been recorded at least twice a month. Using a metabarcoding approach, it was demonstrated, in the first chapter, that several photosynthetic eukaryotes, bacteria and archaea amplicon sequence variants (ASVs) displayed reoccurring seasonal patterns, despite stochastic environmental perturbations, inherent to coastal ecosystems. Day length and temperature were determined to be the main drivers of this rhythmicity.

The sampling frequency was increased to twice a week for three years (2015-2017) during the seasonal blooms of eukaryotic picophytoplankton (January to March). Network analyses, described in the second chapter, revealed that salinity and temperature deeply impacted the microbial community structure. In depth analysis of subnetworks highlighted that persistent ASVs during the 3 years, including rhythmic ones, switched their first neighbors depending on the environmental perturbations they faced. These observations suggest the existence of functional redundancy in marine microbial communities.

The third chapter reported on microcosm experiments conducted on natural microbial communities. These experiments confirmed that a +/-2°C temperature increment strongly affected the community structure. *Bathycoccus* and *Micromonas* dominated the incubated communities at low temperature, whereas diatoms, namely *Skeletonema*, prevailed at higher temperatures. These results help explain why *Bathycoccus prasinos* peak of abundance occurs every year at the temperature minimum at SOLA.

In conclusion, the breadth of data stemming from long term time series, such as the one in the Bay of Banyuls, not only offer global insight into the microbial diversity at these stations, but also give environmental context to data acquired *in vitro*. Furthermore, by integrating results from a time series and microcosms experiments, this manuscript helps unravel the impact of anthropologically driven climate change on marine microbial communities.

Key words: Mediterranean Sea | Time series | Metabarcoding | Microbial communities | Rhythmicity | Microbial networks | Microcosms

Résumé

Certains évènements majeurs de la vie, tels que la migration ou l'accouplement chez les animaux, ainsi que la floraison chez les plantes ont évolué afin de suivre la révolution de la Terre autour du soleil. Dans les océans tempérés, les transitions annuelles de l'hiver au printemps sont accompagnées d'une augmentation de l'abondance de phytoplancton. Le phytoplancton marin est à la base de la chaine alimentaire dans les océans et joue un rôle essentiel dans les cycles biogéochimiques, il produit notamment la moitié de la production primaire global.

Une série temporelle a été établie à une station d'échantillonnage côtière, SOLA (Baie de Banyuls, Nord-Ouest méditerranéen) en 2007. Depuis, les paramètres environnementaux et biologiques ont été mesurés deux fois par mois. En utilisant la technique du « metabarcoding », il a été démontré dans le premier chapitre de cette thèse, que plusieurs « amplicon sequence variants » (ASVs) assignés aux eucaryotes photosynthétiques, bactéries et archées avaient des motifs annuels récurrents, malgré les perturbations environnementales aléatoires, caractéristiques des zones côtières. La photopériode et la température étaient les principaux drivers de cette rythmicité.

La fréquence d'échantillonnage a été augmenté a deux fois par semaines pendant trois ans (2015-2017) lors des efflorescences saisonnières d'eucaryotes picophytoplanctoniques (Janvier-Mars). L'analyse de réseaux, décrit dans le deuxième chapitre, a révélé que la salinité et la température impactaient profondément la structure des communautés microbiennes. Puis, l'analyse des sous-réseaux a montré que des ASVs persistant lors des trois années, dont certains étaient rythmiques, changeaient de voisins les plus proches en fonction des contraintes environnementales auxquelles ils étaient exposés. Ces observations suggèrent l'existence de redondance fonctionnelle dans les communautés microbienne marine.

Le troisième chapitre rend compte d'expériences de microcosmes menées sur les communautés microbiennes naturelles qui ont confirmé qu'une variation de température de +/- 2°C affectait fortement la structure de la communauté. *Bathycoccus* et *Micromonas* dominaient les communautés incubées à basse température, tandis que les diatomées, principalement *Skeletonema* prévalait aux températures plus fortes. Ces résultats tendent à expliquer pourquoi le maximum d'abondance de *Bathycoccus prasinos* se produit tous les ans au minimum de température à SOLA.

En conclusion, la richesse des données issues de séries temporelles de longue durée, comme celle de la Baie de Banyuls, offre à la fois un aperçu global de la diversité microbienne à ces stations, mais permet aussi de remettre des données acquis *in vitro* dans leur contexte environnemental saisonnier. De plus, ce manuscrit intégrant à la fois les résultats d'une série temporelle et de microcosmes a permis d'éclaircir certain impact anthropologique sur les communautés microbiennes marines.

Mots clefs: Mer méditerranée | Séries temporelle | Metabarcoding | Communautés microbienne | Rythmicité | Réseaux microbien | Microcosmes

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2. **Lambert, S.**, Lozano, J.-C., Bouget, F.-Y., Galand, P. E., Switching neighbors in environmentally challenged coastal marine microbes. *In prep.*

3. Guyon, J.-B., **Lambert, S.**, Quentel, M., Aucouturier, J.-M., Groc, M., Vergé, V., Lozano, J.-C., Bouget, F.-Y., Contribution of light and temperature niches to seasonal patterns of photosynthetic picoeukaryotes. *In prep.*

Chapter 1

State of the art

1.1 Importance of marine microbes in the ocean

Marine microbes, comprised of eukaryotic phytoplankton, bacteria, archaea and viruses, dominate the biomass in the oceans and have vitally important contributions to marine ecosystes and global biogeochemical cycles (*1*). The ecological and metabolic diversity of these microorganisms explains how they can be involved in a wide range of functions (Fig.1.1). For example, photosynthesis, the process that transforms inorganic carbon into organic matter via light energy is undertaken by phytoplankton in the ocean (*2*). It is a fundamental event, and coupled with the grazing of phytoplankton by zooplankton, it transfers carbon compounds to higher trophic levels (*3*). Matching the output of land plants, whilst only representing a fraction of their biomass, marine phytoplankton are responsible for half of the global primary production (*4*). On the other hand, bacterioplankton consume the organic matter released by primary producers and either channel these compounds up the food chain or continuously recycle it (*5*). This process is known as the microbial loop, and it allows previously unusable organic matter to become available for higher trophic levels. Leftover organic matter is either transported as sinking particles to the deep ocean *via* the biological pump (*6*) or, if it is too recalcitrant, the microbial carbon pump keeps it stored in the ocean (*7*). Marine microbial communities also have primordial contributions to the biogeochemical cycles of important elements such as nitrogen and phosphorus (*8*).

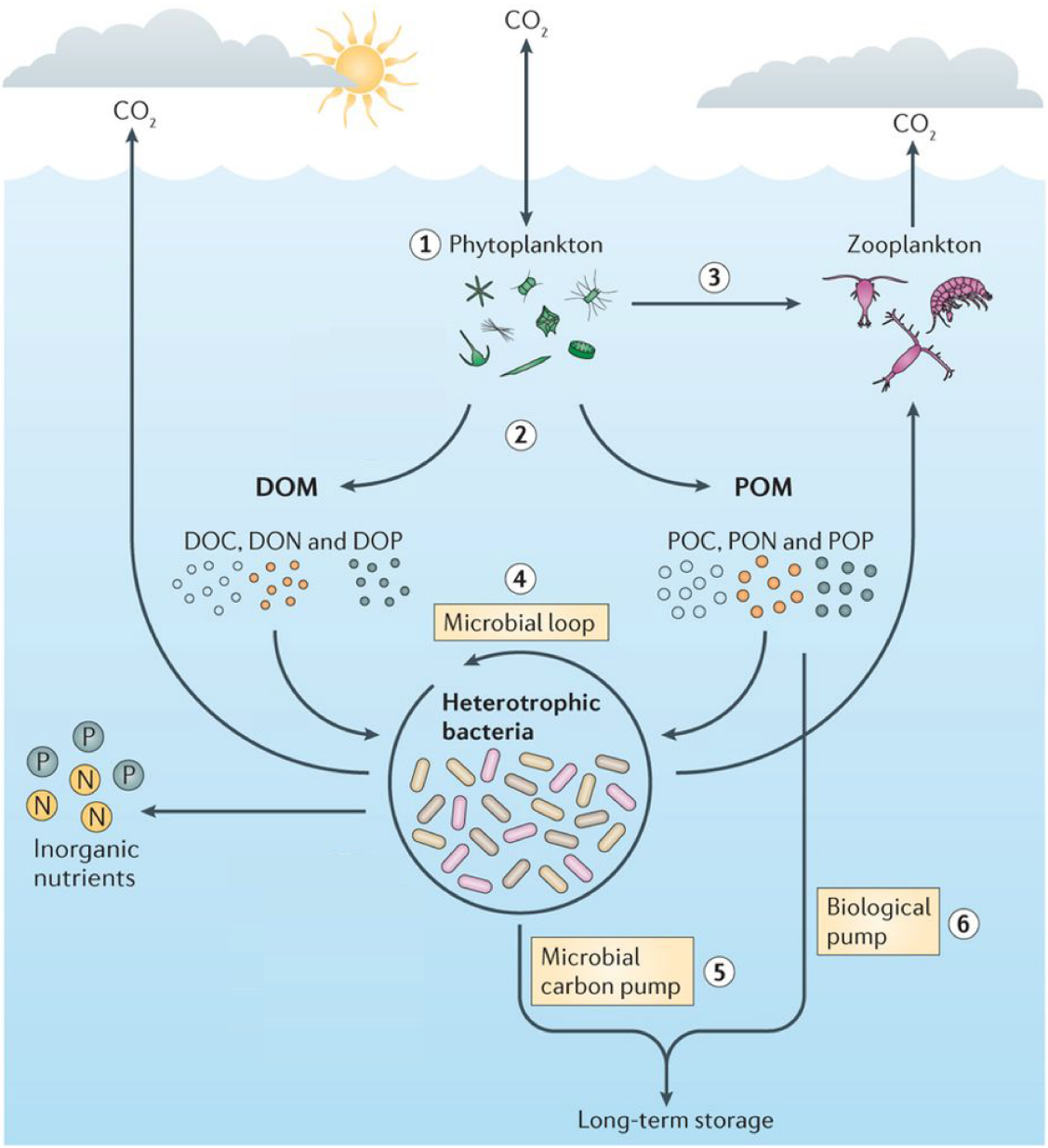

Figure 1.1: **Role of marine microbes in the fate of organic matter in the ocean.** (1) Phytoplankton, *via* photosynthesis, transforms inorganic carbon (CO2) into organic matter. (2) This organic matter is then released into the seawater either as dissolved organic matter (DOM; which includes dissolved organic carbon (DOC), dissolved organic nitrogen (DON) and dissolved organic phosphorous (DOP)) or particulate organic matter (POM; which includes particulate organic carbon (POC), particulate organic nitrogen (PON) and particulate organic phosphorous (POP)). (3) Zooplankton graze on the phytoplankton. (4) Heterotrophic bacteria recycle organic matter in the microbial loop. (5) Leftover organic matter, when it becomes too recalcitrant after successive microbial transformations, becomes sequestered in the ocean *via* the microbial carbon pump. (6) The sinking of POM is known as the biological pump. (Adapted from (*9*)).

1.2 Diversity of marine microbes

1.2.1 Eukaryotes

Microbial single celled eukaryotes, also known as protist, are involved in a wide range of ecological functions in the ocean. Protists are composed of different classes that are the result of multiple evolutionary processes. Their trophic characteristics can be complicated as some of them do not have a clear cut feeding process. Indeed, some protists are phototrophs and are capable of photosynthesis, such as mamiellophyceaes. Others are considered hetetrophic, such as cilliates, and some groups are mixotrophs, such as dinoflagellates.

Photoautotrophs are made up of numerous species with unique features and requirements. These features can be different pigments that react to specific wavelengths of light to carry out photosynthesis, or it can be the fact that they display different physiological structures. These precise structures were initially used to distinguish species, but now molecular tools offer a better resolution. Photoautotrophs not only exhibit preferential temperature and photoperiod niches but also specific nutrient and vitamin requirements. The denomination photoautotrophs actually represents multiple groups of microbes. Within this group are ecologically important divisions, such as Chlorophyta, Heterokonta, Haptophyta, Cryptophyta.

Chlorophyta, which are at the origin of the green lineage of plants (*10*), include some of the smallest known eukaryotes, such as *Ostreococcus* (*11*). Heterokonta, also referred as Stramenopile, are a complex group as they contain phototrophs, heterotrophs and mixotrophs. They have a large geographical distribution. Among the phototrophic Heterokonta, diatoms are abundant in the fossil records and play major roles in biogeochemical cycles (*12*). The Haptophyta division also contains phototrophs, heterotrophs and mixotrophs. Within phototrophic Haptophyta are coccolitophores, of which the most well known representative is *Emiliania huxleyi*. This species can form massive blooms and since it has a calcium carbonate structure, it impacts biogeochemical cycles (*13*). Additionally, it is highly susceptible to ocean acidification. Cryptophyta is a less well

known division, but display a specific photosynthetic pigment, phycobiliproteins, that allow them to convert light energy at low light intensities (*14*).

Heterotrophs are characterized by their capacity to graze on picophytoplankton and feed off bacterioplankton. Without being exhaustive, the main contributors to this group are Ciliates, Rhizaria and Flagellates. Ciliates have multiple cilia (small hairs) on their cell body for motility. Due to their large size, they can feed off bacteria as well as microalgae and other protists as well (*15*). However, many remain mixotrophs since they can scavenge and use the chloroplast of ingested prey (kleptoplastidy) (*16*). The Rhizaria supergroup displays a large diversity of organisms with multiple structures. For example, Cercozoa can have flagellates (*17*), Foraminifera display calcareous tests (*18*) and Radiolaria have intricate mineral skeletons (*19*). Flagellates are a highly diverse group as well of which some representatives are Heterokonta (also known as Stramenopiles) and alveolates. Heterotrophic Heterokonta tend to be small, round or oval organisms that can have two flagella, one hairy flagellum and one hairless flagellum that are of unequal size (*20*). Heterokonta encompass different lineages and display a wide trophic range as they can be heterophic, mixotrophic or phototrophic (as seen previously with diatoms). Belonging to the alveolates is an ecologically important group, the dinoflagellates. They can have two flagella and a theca (cellulose plates) that protects them. Furthermore, they cover a large range of functions as not only are some phototrophic but they can also be grazers and predators on other protists and bacteria (*21*). All things considered, heterotrophic protists comprise a large diversity of organisms with multiple shapes, sizes and appearances. They also display multiple trophic functions since they consume algae, other protists or bacteria. But depending on the state of the ecosystem, some can show periods of mixotrophy or even phototrophy. Consequently, their physiology and taxonomical classification remains complex particularly given the lack of data concerning cultivated individuals.

1.2.2 Prokaryotes

Marine prokaryotes are composed of bacteria and archaea. These organisms are usually taxonomically classified based on the phylogeny of their 16S ribosomal RNA. However,

it is not because two prokaryotes are phylogentically related that they are functionally similar. Even though microorganisms in the same division share core genes (16s rRNA, "house keeping" etc.) it should be noted that thanks to several processes, such as horizontal gene transfer or gene loss, bacteria can gain, or loose, functions relatively rapidly, making them functionally diverse, even for closely related prokaryotes.

Alphaproteobacteria are among the most abundant bacteria found in culture independent studies. They are usually found in surface samples and can occur in a wide range of ecosystems, such as the water column, sediments, fresh water, ice etc.

SAR 11 is a branch of the *Alphaproteobacteria* that was discovered thanks to novel sequencing methods (*22*). It is one of the most abundant bacteria in the oceans and is composed of multiple subclades that have preferences for different depth and ecosystems (*23*).

Gammaproteobacteria is a large group that contains many ecologically and medically (i.e pathogens) important organisms. Among the marine *Gammaproteobacteria*, the vibrio bacteria is probably the most well studied, since it is relatively easily maintained in culture. Vibrio are curved rod shaped bacteria that have been isolated from the water column, sediment and from other organisms such as squid or oysters. They are susceptible to communicate between themselves *via* quorum sensing (*24*). Other examples of well known *Gammaprotobactetia* are *Alteromonas*, *Pseudoalteromonas* and *Shewanella*, but are less well known due to the lack of cultured representatives.

Bacteroidetes is a highly diverse group that encompasses, for example, *Flavobacteria*, *Bacteroides* as well as *Cytophaga*. Due to the lack of *Bacteroidetes* in culture, information concerning their physiology is rather limited. However, they are often found in conjunction with phytoplankton blooms and are speculated to take part in the degradation of organic matter (*25*).

Cyanobacteria, with a majority of their members cultured and their complete genome sequenced, are presumably the most well understood group of marine bacteria. Cyanobacteria are capable of photosynthesis, which is not found in other marine bacterial groups. They have been observed in most marine ecosystems except for the cold polar seas (*26*).

Cyanobacteria are divided into two main genera, *Procholorococcus* and *Synechococcus*. *Procholorococcus* is the most abundant genus and was identified with the use of flow cytometry (*27*). On the other hand, *Synechococcus* is less abundant but was observed earlier because its specific pigment, phycoerythrin, is more easily detected (*28*).

Marine *Archaea* were first discovered by Carl Woese and collegues' in the late 70's (*29*). This discovery lead to a new branch in the tree of life. At fisrt all *Archaea* were thought to be extremophiles, since they were generally found in extreme habitats. But by applying universal primers, *Archaea* were discovered in the seawater (*30*) and were then shown to be quite abundant. *Archaea* are divided into two main groups, *Crenarchaeota* and *Euryarchaeota*.

1.2.3 Microbial ecosystem stability: the plankton paradox

Clearly, there is a tremendous diversity in marine microbes. But one might ask, how is this diversity maintained? Presumably, some species must grow better than others and should have dominated the microbial biomass by now. This notion is expressed in Hutchinson's "paradox of plankton" that discusses competitive exclusion (*31*). In the early 60's Hutchinson asked how was it possible for multiple species of phytoplankton to coexist in the same environment when they are all competing for the same nutrients. This question is based on the competitive exclusion theory that stats that when a group of species are competing for the same resources, eventually the most efficient species will outcompete the others, dominate the limited resources and lead the other species towards extinction. Despite this Hutchinson observed multiple phytoplankton species in the lake he was sampling. Hutchinson then offered potential solutions to this paradox.

Firstly, he dismissed the idea that different species could take advantage of microhabitats resulting from varying physical conditions (for example depth). Indeed, he considered that the lakes he was sampling were too homogeneous to support multiple different species. He then suggested that, since it was reasonably possible that phytoplankton existed in symbiosis, one less efficient organism could provide essential vitamins to a more efficient organism, which would lead to an equilibrium in the population. He also briefly suggested

that predators could impact competition between species enough to allow for coexistenceof multiple species. Additionally, he pointed out that, under changing environmental conditions, no single organisms could continually outcompete the others for sufficient enough time to exclude them, which would prevent reaching an equilibrium.

Since Hutchinson's paper, ecologists have offered several solution to the paradox. One explanation is the fact that homogeneous well-mixed conditions (which was part of Hutchinson's assumptions) do not really exist. In reality, there are constant variations in environmental conditions, that give rise to numerous microhabitats that allow for the coexistence between competitive species. Moreover, competition and predation models suggest that plankton will not arrange into a steady state but instead bring about oscillations and chaos, with continual variations in the microbial community (*32*).

Furthermore, predation and viruses promote the coexistence of species. Indeed, the "Killing the winner" theory explains how predators and viruses affect community composition. By being shape and size specific, grazers affect differently organisms in the system, which prevents a dominant species from becoming too abundant (*33*). Additionally, since viral lysis is linked to host abundance, and the fact that viruses tend to be species specific, they are remarkably efficient in restraining dominant species in marine habitats (*33*).

1.3 Regulation of microbial community composition

1.3.1 Abiotic factors

Marine community compositions are subject to multiple environmental parameters. Physical factors can structure the water column, which inherently influence microbial community dynamics. One of the first studies to investigate bloom initiation was in 1953, when Sverdrup offered the critical depth hypothesis (*34*). This hypothesis states that at any moment and location in the ocean, phytoplankton growth is equal to the loss of phytoplankton biomass (by sinking, grazing or respiration). However, when the mixed layer becomes less deep than the critical depth (*35*), then the growth rate can surpass the loss rate, which leads to a phytoplankton bloom. Sverdrup's critical depth hypothesis

resulted in the assumption that improved growth rates, and thus the bloom, were not just correlated but were actually induced by improved temperature, light and stratification conditions. Opposing this view, Behrenfeld offered his dilution-recoupling hypothesis that states that bloom initiation is not necessarily due to the depth of the mixed layer, but rather it is the seasonal mixing that tips the balance between growth and loss, which then leads to a bloom (*36*). Subsequently, other physical impacts were hypothesized to impact community composition, such as turbulences in the water column (*37*) or eddies (*38*).

Other abiotic factors have been shown to affect community composition. For example, at the time series station ALOHA in the North Pacific Subtropical Gyre, which is considered a stable habitat, it has been shown that wind speed and solar irradiance affected community composition (*39*). In the Western English Channel, temperature and nutrients, mainly phosphate, drove bacterial community structure (*40*). Furthermore, in high latitude sampling sites, light had a drastic effect on eukaryotic community dynamics. A size fractionation of photosynthetic cells was observed, as large cells were found in spring and summer, whereas small cells dominated the biomass the rest of the year (*41*). Similarly, seasonal haptophytes were driven by light and temperature during a two year time series in Norway (*42*). Nutrient levels can impact community composition by limiting or promoting the growth of microbes at specific moments of the year. This is particularly visible at coastal sites, where heavy rains and river runoffs can lift nutrient limitations and enhance the growth of microbial species, as observed in the Bay of Bengal (*43*) and in the Gulf of Trieste (*44*).

1.3.2 Biotic factors

Simultaneously, community composition is also shaped by an array of biotic factors. Microbial community members form a complex system with intricate interactions between its members, which can be sorted into two main types.

Positive interactions. For example, microalgae generate dissolved organic matter, either *via* exudates, zooplankton grazing or following viral lysis, which is utilized and

remineralized by heterotrophic bacteria (*45*). This event could be considered allelopathy. But, on the other hand, several microalgae have been shown to be auxotrophic for vitamin B_1 and B_{12} (*46*, *47*), and could possibly interact with bacteria to obtain these required micronutrients *in situ*. Both these interactions show that phytoplankton and bacteria possibly have a mutualistic relationship (Fig.1.2).

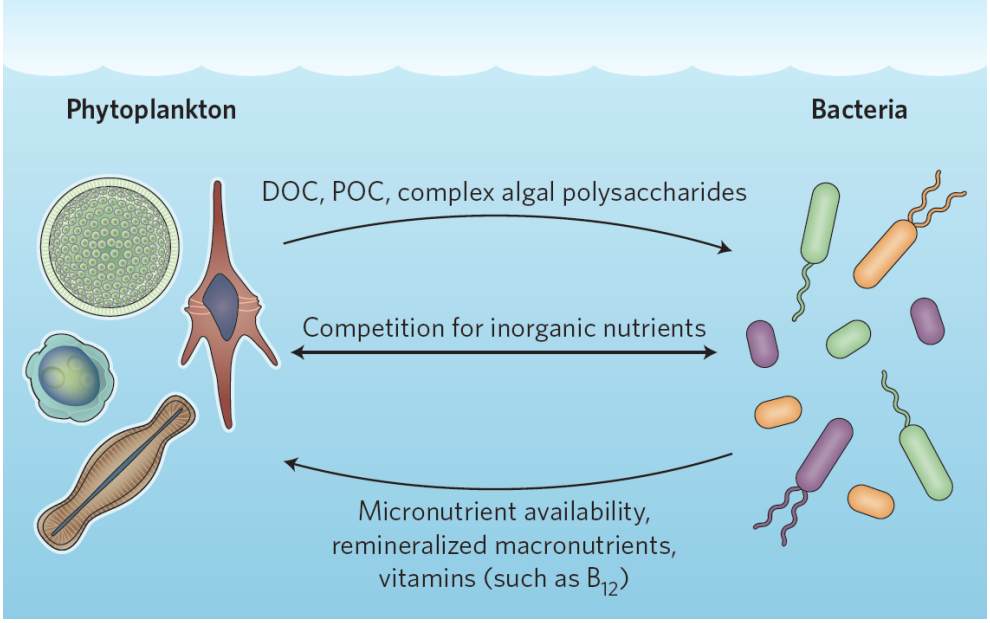

Figure 1.2: **Interactions between phytoplankton and bacteria.** Phytoplankton and bacteria can exchange compounds, such as carbon or vitamins, but they can also be in competition for inorganic nutrients. As these compounds are required for their respective growth, this leads to complex interaction networks (*48*).

Negative interactions. As inorganic nutrients are limited in the ocean, phytoplankton and bacteria can also be in competition since they both need those resources. In a fresh water lake, parasitic groups in the small eukaryote fraction were found to have an impact on microbial community composition, suggesting the ecological relevance of parasitism, a usually ignored biotic interaction (*49*). Within the marine food web, one of the main negative interaction is predation, which has been shown to induce bloom termination (*50*). Predation, which is also a form of competition as zooplankton and protists are rivals when it comes to phytoplankton grazing, serves other roles such as the remineralization of nutrients and trace metals (*51*).

1.3.3 Microbial interactions as networks

To help researchers investigate increasingly larger datasets, novel methods are being implemented that help visualize possible interactions within microbial communities. Correlation networks (Fig.1.3) depict individual microbes as nodes and the edge connecting two nodes can be computed by several methods, some of which have been studied and compared recently (*52*). Intuitively, if these connections are positive it could suggest a mutualistic interaction between nodes, and if it is negative, this could mean that two nodes are mutually exclusive, for example if they have a predator-prey interaction. Furthermore, with the aim of investigating time lagged interactions between individuals, the local similarity analysis has been developed (*53*). This time dependent analysis is particularly useful for time series studies as it can demonstrate a progression in microbial interactions. For example, it has helped researchers suggest possible symbioses or parasitism between dinoflagellates and specific eukaryotes (*54*).

In practice, interpreting network results is not that straight forward, as nodes could be co-occurring or mutually exclusive for multiple reasons. Furthermore, network analyses are powerful tools to visualize data and emit new theories, however, drawing conclusions directly from networks is not recommended (*55*).

In reality, abiotic and biotic factors conjointly affect community dynamics. It has recently been presented that temperature and salinity along with cyanobacteria could influence bacterial composition in the Baltic Sea (*56*). Similar results were found where temperature and phosphate drove microbial dynamics, which demonstrated a covariance with cyanobacteria as well (*57*). Furthermore, in the North Sea, bacterial community composition was primarily influenced by phytoplankton blooms, but also, and on a longer time scale, by temperature (*58*). Clearly it remains a complicated endeavor to study separately abiotic and biotic factors *in situ*.

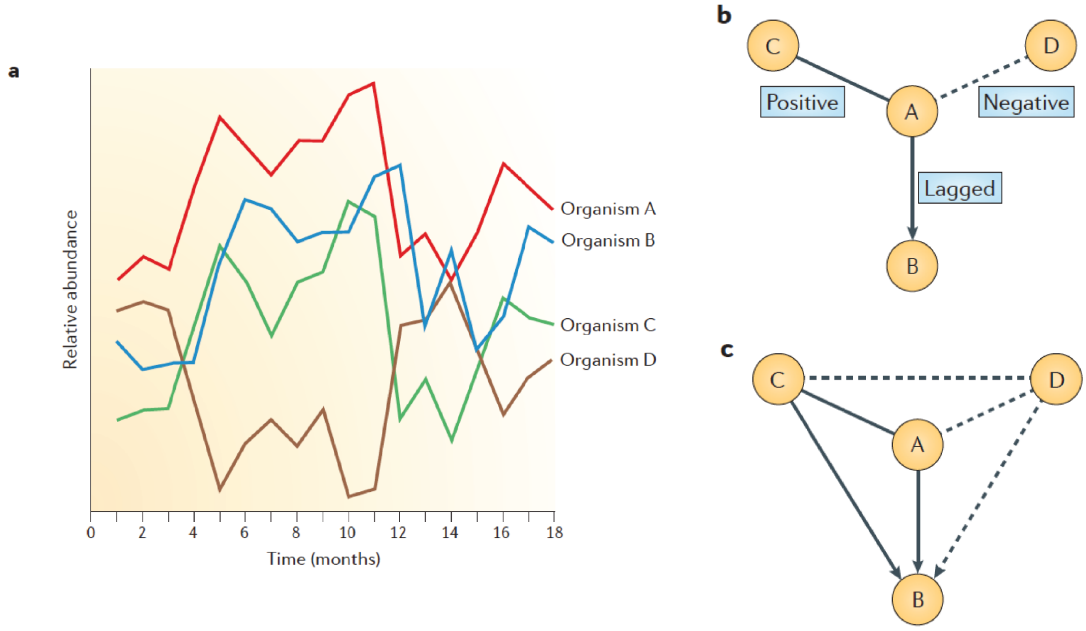

Figure 1.3: **Representation of microbial interactions as a network.** (a) Hypothetical abundances of four organisms over time. Organisms A and C are positively correlated, A and B as well, but with a time lag of one month and A and D are negatively correlated. (b) The previously described correlations are then translated into a network. (c) Using the previous nomenclature (from (b)), this network describes all the correlations between the four hypothetical organisms (*59*).

1.4 Seasonality of marine microbes

1.4.1 Initial observations

Marine macroscopic events such as red tides or coastal bioluminescence, have been observed during history and were hypothesized to be seasonal (*60*, *61*). More recently, with the development of satellite imagery, phytoplanktonic blooms have been observed around the globe (Fig.1.4).

However, knowledge concerning marine microbial community composition and function is lacking, as it mainly derives from isolated 16S and 18S rRNA gene analysis studies. The development of *"-omics"* is helping improve the understanding of community function, but it is not sufficient to predict interactions, nutrient limitations or responses to environmental factors. Therefore, details concerning marine microbial community compositions, dynamics or functions when facing naturally or anthropologically evolving ecosystems are unobtainable with standard, and often segregated studies. Long term

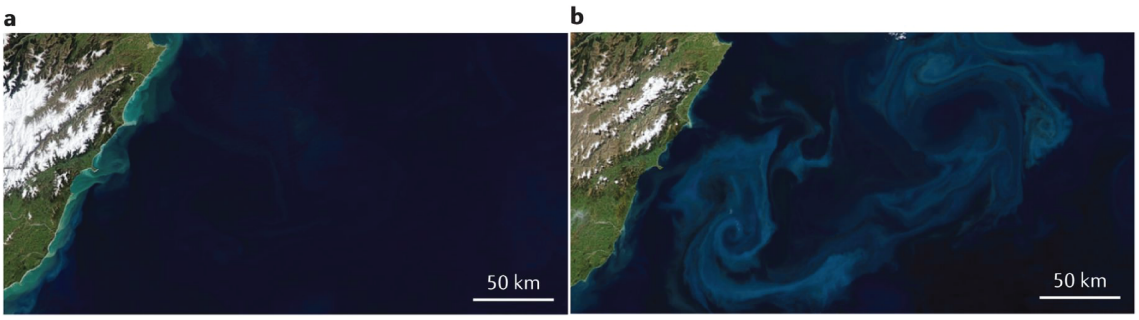

Figure 1.4: **Macroscopic event.** Satellite image of the eastern coast of New Zealand before (a) and during (b) a diatom bloom (adapted from (*9*)).

and frequent sampling at a same study site, insuring a temporal investigation, could help elucidate microbial community characteristics as well as improve the prediction of microbial community reoccurrences. In the context of climate change, and considering that phytoplankton is the basis of the marine food chain, there is a need for robust predictions of algal blooms. Knowing how susceptible phytoplankton can be to changing temperatures (*62*), the predicted changes in seawater temperature, that have been increasing for the past 30 years (Fig.1.5), could have a major impact on bloom dynamics and microbial community composition overall. With the aim of monitoring changes in community composition, long term sampling sites have been implemented around the globe (Fig.1.6).

1.4.2 The establishment of time series

Various studies covering diverse time scales and focalizing on different questions have been published in the last two decades. Without being exhaustive, some significant discoveries were found at different long term sampling sites, for example, at the Hawaiian Ocean Time-series (HOT) 25 years of data allowed to confirm that three, previously unknown, major players in the microbial marine community are *procholorococcus*, the alphaproteobacteria SAR11 and planktonic archaea (*63*). At the Bermuda Atlantic Time-series Study (BATS) site, several years of sampling allowed for a better understanding of the evolutionary diversification of SAR11 clades (*64*). Depicting the Bray-Curtis similarity between samples, according to the number of months between samples, Fuhrman and colleagues have shown that communities at the San Pedro Oceanic Time series (SPOT)

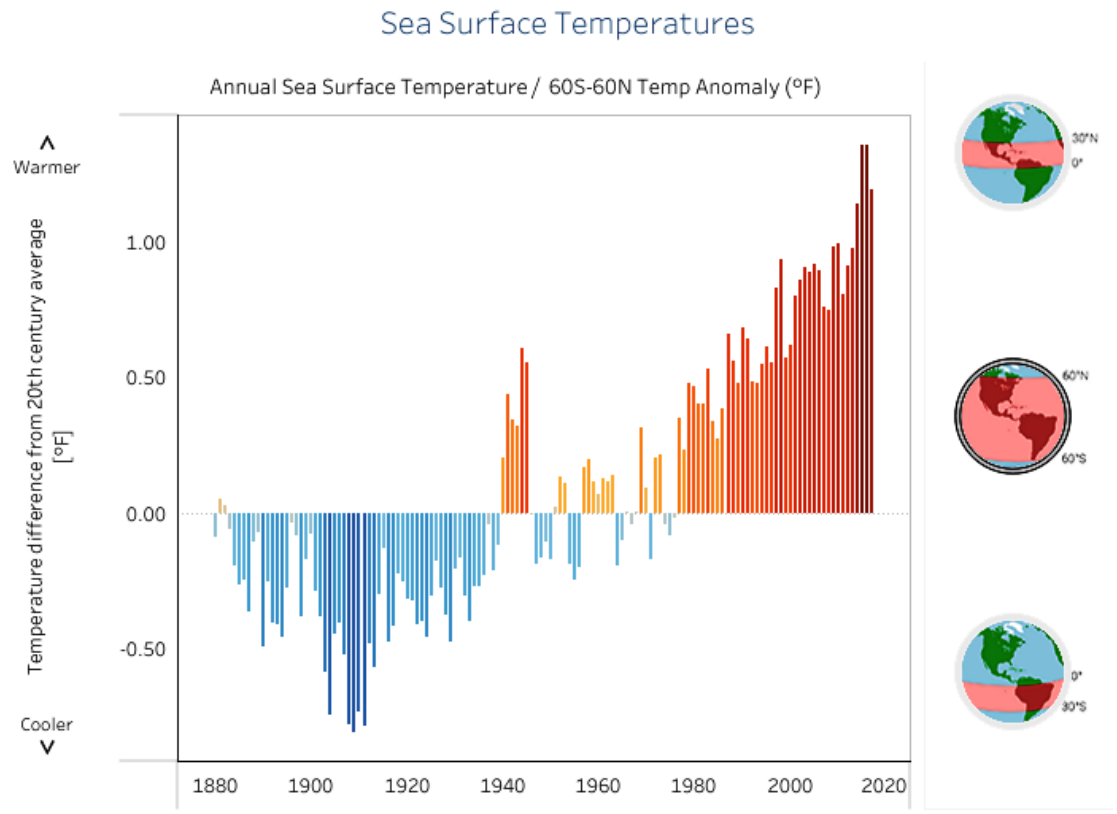

Figure 1.5: **Temperature anomaly.** Sea surface temperature differences from the average sea surface temperature of the 20th century since 1880 between the 60S and 60N latitudes in degrees Fahrenheit (from: `https://www.globalchange.gov/browse/indicators/indicator-sea-surface-temperatures`).

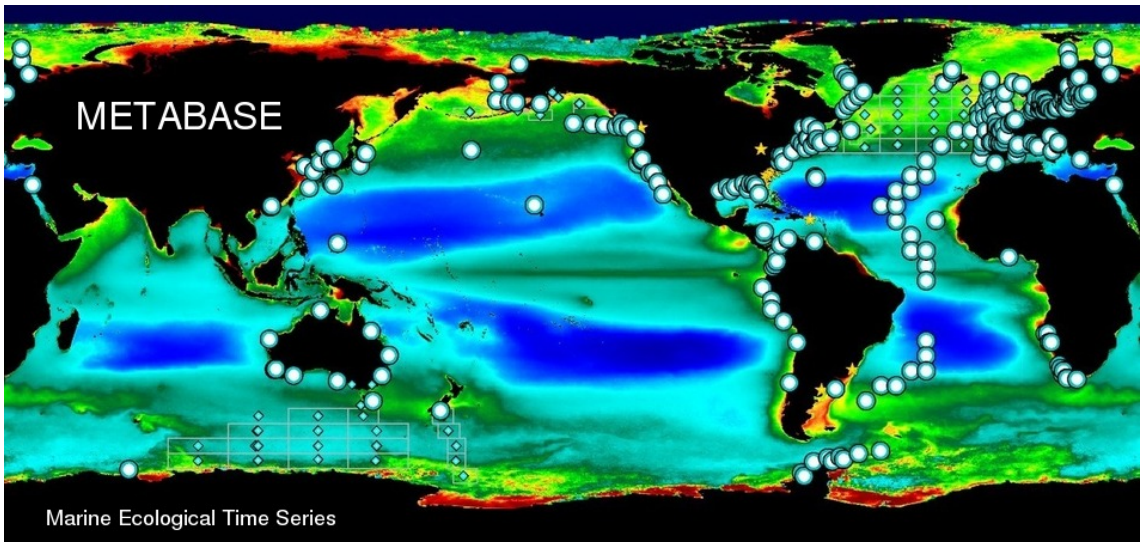

Figure 1.6: **Global investigation.** Map showing the location of active marine time series (from: `https://www.st.nmfs.noaa.gov/copepod/time-series/`.)

are more similar when they are 12 months apart and more dissimilar when they are 6 months apart (Fig.1.7). Moreover, this pattern is reoccurring yearly during 10 years (*59*). The Bray-Curtis similarity is an equation that quantifies the similarity of two sites, based on the species count of each site. The result of the equation is between 0 and 1, where two sites that have a Bray-Curtis similarity of 0 do not share any species, whereas two sites that have a Bray-Curtis of 1 share all the species (*65*). In the literature, it is also possible to read about the Bray-Curtis *dissimilarity*, with reversed values (0 meaning all species are shared and 1 implying that no species are shared). Both are commonly used, and there does not seem to be a consensus as to which one is favored.

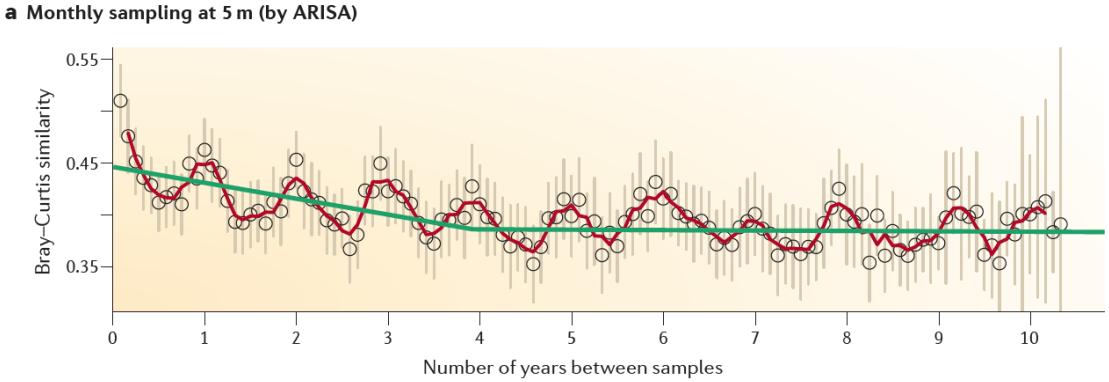

Figure 1.7: **Temporal investigation.** Bray-Curtis similarity between monthly samples at the San Pedro Ocean Time-series (SPOT) over 10 years (adapted from (*59*)).

Furthermore, coastal time series have been carried out in the Western channel near Plymouth and surface bacterial populations were shown to have a high seasonality (*66*). Marine archaea community structures have also been studied in a time series, and it was shown that both rare and abundant archaea were seasonally inclined and that different ecotypes of archaea had different patterns (*67*, *68*). More recently, a 21 day study of the dynamics of picoplankton communities revealed a daily succession of archaea, bacteria and eukaryotes highlighting the importance of microbial interactions during a bloom (*69*).

Although long term sampling stations are powerful tools to investigate marine microbial community dynamics, there is still room for improvement. The question of determining the right balance between sampling effort and the resolution of observations has been raised (*70*). Most time series started off with a monthly frequency as sampling open ocean sites is not that straightforward and, at the time, sequencing was still expen-

sive. Nowadays, most sampling sites have observed that a weekly or even daily sampling frequency is required to capture high resolution community dynamics (*71*, *72*).

At the same time, there have also been advancements in taxonomic analysis. Initial time series studies had no other choice than to use the tools that were available at that time, such as denaturing gradient gel electrophoresis or sequencing clone libraries (*22*, *73*), which only allowed for a coarse taxonomic resolution of community composition. With the increased prevalence of next generation sequencing, researchers are able to access taxonomy at a finer resolution. For examples, the assemblage of Operational Taxonomic Units (OTUs) (*74*) went from a similarity threshold of >97% to >99%, which greatly improved the taxonomical resolution. Besides, several research groups are transitioning from OTUs to Amplicon Sequence Variants (ASVs) (*75*) or even to oligotyping (*76*) or minimum entropy decomposition (*77*).

Previously, most long term sampling sites were confined to analyze bacterial genetic data, as it was the only domain to have a strong database at the time, and eukaryote data was mainly derived from microscopy observations (*45*, *78*). More recently, certain studies have started exploring multi-domain data (*69*, *71*), whereas other studies are now including viruses in their analysis (*79*, *80*). Moreover, the use of *"-omics"* data for time series studies is revealing not only the taxonomy of microorganisms, but also the microbial activity and functions found *in situ* (*81*, *82*). With modern sequence analysis tools (*75*, *76*), and cheaper sequencing prices, analyzing high resolution time series is becoming easier and more accessible to an increasing number of laboratories. By increasing the prevalence of temporal investigations, and coupling them with recent multi-omics possibilities would allow for a more in-depth examination of marine microbial communities around the globe.

1.5 Laboratory studies

1.5.1 Microsoms

To have a better grasp on naturally occurring processes, it is sometimes required to reduce the complexity of the system. By allowing researchers to change one variable at a time, microcosms are the perfect tool to investigate the impact of individual parameters. For example, a study (*56*) wanted to investigate the possible impact of climate change on their study site. Given the context of the Baltic Sea, they assumed that temperature would increase and salinity would decrease. With the use of microcosms, they were capable of replicating these assumptions and examined their impact on community composition. They observed specific responses of communities to the expected changes in environmental parameters. Higher temperatures lead to earlier cyanobacterial blooms, whereas low salinity had a strong impact on the bacterial community. Combined, the effects of global warming in the Baltic Sea could reduce the availability of carbon for marine microbes and thus impact the bacterial community dynamics. Furthermore, the effect of three UV conditions (PAR+UV-A+UV-B, PAR+UV-A and PAR only) were tested on marine bacterioplankton communities from the South Atlantic in 25 L microcosms during eight days. Taxonomical analysis showed that PAR+UV-A and PAR displayed more similarity than PAR+UV-A+UV-B. Different radiation conditions thus have different impacts on marine microbial community composition (*83*). However, researchers must remain cautious when generalizing results inferred from microcosms studies as transcriptional changes can be drastic when dealing with complex marine communities that are incubated (*84*).

1.5.2 Strains physiological characterization

At an even finer scale, culture work allows to ask very specific questions and investigate processes at the molecular level. A culture study done on six strains of *synechococcus* that were isolated from different latitudes, maintained the strains at 22°C, then exposed them to a cold stress (13°C). The strains demonstrated different molecular capabilities that reflected adaptations to their preferred thermic niches. Furthermore, these molecular

adaptations could suggest a correlation between clade radiation and temperature tolerance in *synechococcus* (*85*). Another study demonstrated a similar result but with the *micromonas* genus. Thermal responses of eleven strains from four species of *micromonas* were studied by culture work and modeling. As these strains have specific responses to changing temperatures, it has been suggested that the *micromonas* genus could be used as a sentinel species to anticipate the impact of climate change (*62*). The complexity of marine microbial interactions and behavior makes clear conclusions almost impossible to achieve with *in situ* studies alone. Thus, even though time series studies allow to develop novel hypotheses, there is a need to confirm these ideas specifically and precisely in culture studies. Despite the recent improvements in technology, allowing for better sequence analysis and complex modeling, understanding marine microbe interactions and functions remains challenging.

1.6 Study site

The sampling station, Service d'Observation Laboratoire Arago (SOLA), presented in this manuscript is situated in the Bay of Banyuls, a coastal, generally oligotrophic site, located in the North Western Mediterranean Sea (Fig.1.8). The Service d'Observation en Milieu LITtoral (SOMLIT) program, which SOLA is part of, aims to monitor the changes in coastal ecosystems on the long term, and to quantify the influence of environmental and anthropologic variability on these systems (`http://somlit.epoc.u-bordeaux1.fr/`). This program has allowed for a coordinated effort on the entire French coastline, with a homogeneous sampling of physical, chemical and biological variables.

SOLA being a coastal site (Fig.1.9), fresh water such as rainfall, storms and runoffs from multiple rivers in the vicinity impact nutrient and salinity levels at the sampling station. These sporadic events have been shown to have an impact on the phytoplankton community (*87*).

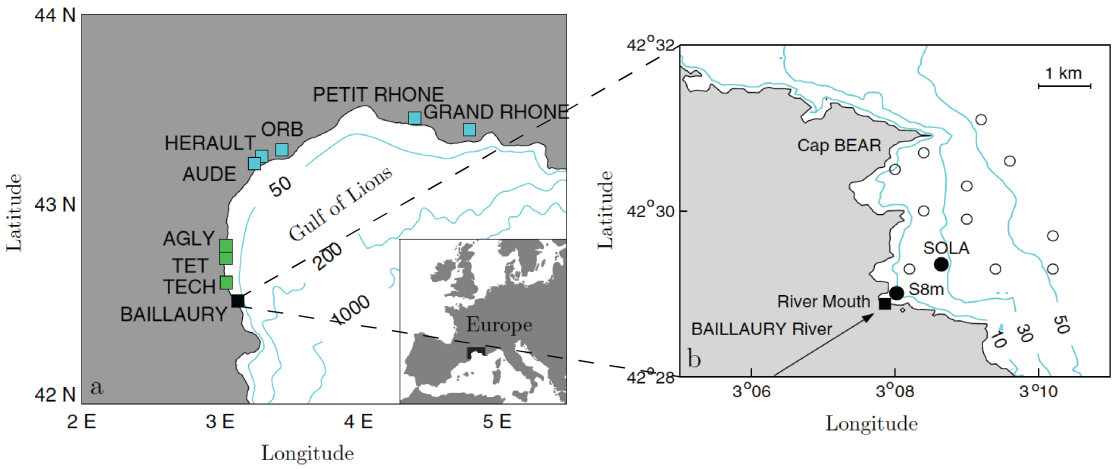

Figure 1.8: **Geographical context.** (a) Map showing the location of local rivers, with the black square being the intermittent river near the sampling site. Green and blue squares are the nearby and farther rivers, respectively. (b) Close up of the sampling site, showing the sampling site SOLA (adapted from (*86*)).

Figure 1.9: **Study site.** Picture of the buoy at SOLA and the coastline of the bay of Banyuls (©*Stefan Lambert)*.

1.7 Hypothesis

In order to study the seasonality of microbial communities, it is fundamental to acquire data from a long-term sampling site. This data will help elucidate the influence of environmental factors on marine microbial community composition. This manuscript was structured around three main questions:

- Are marine microbes capable of conserving a yearly rhythm despite sporadic environmental factors?
- Are microbial co-occurrences affected by challenging environmental events?

- Can microcosms help elucidate the dynamics of microbial communities when exposed to varying environmental factors?

First, a seven-year time series was used to elucidate the yearly rhythmicity of microbial taxa (Chapter II). Then we investigated, at a higher resolution, the co-occurrences that happened during 3 years at SOLA when faced with different environmental challenges (Chapter III). And finally, to bridge the gap between *in situ* observations and *in vitro* experiments, microcosms experiments were carried out to verify the effect of multiple environmental factors independently (Chapter IV).

Chapter 2

Rhythmicity of coastal marine picoeukaryotes, bacteria and archaea despite irregular environmental perturbations

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Prologue

Seasonality is the result of the rotation of the Earth on its axis and around the Sun. Major life events have evolved to follow this seasonality and result in drastic macroscopic events such as migration or mating in animals or flowering in plants. In temperate oceans however, yearly transitions from winter to spring are accompanied by an increase in abundance of phytoplankton. Marine phytoplankton is at the basis of the food chain in the oceans and plays an essential role in biogeochemical cycles, as it generates roughly 50% of the global primary production. Yearly variations in bacterial and archaeal populations have also been observed. Environmental and biological parameters have been recorded twice a month at SOLA Station (Bay of Banyuls, North Western Mediterranean Sea) since 2008. We have investigated this time series, using a metabarcoding approach targeted towards the three domains of life. Several photosynthetic eukaryotes, bacteria and archaea amplicon sequence variants (ASVs) showed reoccurring seasonal patterns. Day length and temperature were determined to be the main structuring factors. Furthermore, though a minority of ASVs were truly rhythmic over the entire time series, they represented more than 31.3%, 31.6% and 75.6% of photosynthetic eukaryotes, bacterial and archaea total ASV sequences, respectively. Determining the rhythmicity of seasonal ASVs could provide a better insight into their ecological roles in coastal environments. Heatmaps showed co-occurrences between ASVs of different domains hinting that, even in a continuously changing environment, a strong influence of biological co-occurrences controlling the population dynamics throughout the time series exists. Moreover, rhythmic autotrophs could be leading the re-occurrences of heterotrophs but shared environmental niches may be driving seasonality as well. Determining the rhythmicity of seasonal ASVs could provide a better insight into their ecological roles in coastal environments.

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ARTICLE

Rhythmicity of coastal marine picoeukaryotes, bacteria and archaea despite irregular environmental perturbations

Stefan Lambert[1] · Margot Tragin[2] · Jean-Claude Lozano[1] · Jean-François Ghiglione[1] · Daniel Vaulot[2] · François-Yves Bouget[1] · Pierre E. Galand[3]

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Abstract

Seasonality in marine microorganisms has been classically observed in phytoplankton blooms, and more recently studied at the community level in prokaryotes, but rarely investigated at the scale of individual microbial taxa. Here we test if specific marine eukaryotic phytoplankton, bacterial and archaeal taxa display yearly rhythms at a coastal site impacted by irregular environmental perturbations. Our seven-year study in the Bay of Banyuls (North Western Mediterranean Sea) shows that despite some fluctuating environmental conditions, many microbial taxa displayed significant yearly rhythms. The robust rhythmicity was found in both autotrophs (picoeukaryotes and cyanobacteria) and heterotrophic prokaryotes. Sporadic meteorological events and irregular nutrient supplies did, however, trigger the appearance of less common non-rhythmic taxa. Among the environmental parameters that were measured, the main drivers of rhythmicity were temperature and day length. Seasonal autotrophs may thus be setting the pace for rhythmic heterotrophs. Similar environmental niches may be driving seasonality as well. The observed strong association between Micromonas and SAR11, which both need thiamine precursors for growth, could be a first indication that shared nutritional niches may explain some rhythmic patterns of co-occurrence.

Introduction

Regular and predictable fluctuations of environmental parameters have a great impact on life. Seasonality sets the pace for many reoccurring life events, such as mating or migrations in animals, flowering in plants and blooms in plankton communities [1–3]. Phytoplanktonic blooms in temperate oceanic areas are a typical example of seasonal events. Several classical theories, from Sverdrup's "Critical Depths Hypothesis" [4] to Behrenfeld's "Dilution-Recoupling Hypothesis" [5], have attempted to explain the mechanisms triggering bloom formation. However, these theories do not aim to explain the reoccurrence and seasonality of specific microbial taxa. In macroscopic organisms, seasonality results from a fine interplay between external environmental factors and the internal circadian clock, which is an endogenous timekeeper [6]. In marine microorganisms, circadian rhythms are less well known and they have been reported only in cyanobacteria and in some eukaryotic microalgae [7–10]. However, the effect of environmental forcing on the seasonality of entire bacterial communities has been studied more extensively and reoccurring microbial communities are often observed responding to environmental changes [11–15].

Oceans are fluctuating habitats that are often marked by a strong seasonality. These regular environmental changes allow for an overall high microbial community diversity, since the environment can accommodate different species in

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✉ François-Yves Bouget
francois-yves.bouget@obs-banyuls.fr

✉ Pierre E. Galand
pierre.galand@obs-banyuls.fr

[1] CNRS, Laboratoire d'Océanographie Microbienne (LOMIC), Observatoire Océanologique de Banyuls, Sorbonne Université, Banyuls sur Mer, Paris, France

[2] CNRS, UMR7144, Station Biologique de Roscoff, Sorbonne Université, Roscoff, Paris, France

[3] CNRS, Laboratoire d'Ecogéochimie des Environnements Benthiques (LECOB), Observatoire Océanologique de Banyuls, Sorbonne Université, Banyuls sur Mer, Paris, France

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the same space, but at different times of the year [16]. Within a year, diversity also varies locally with peaks observed in winter at high latitudes [15, 17] and community composition changes with seasons. Seasonal cycles in abiotic and/or biotic factors drive these community changes [18, 19]. To understand the seasonality of marine microbial communities, several long term sampling sites have been established within the last couple decades leading to some important findings on the seasonality of major microbial groups in the surface of the ocean [14, 20–23] and the reoccurring patterns of microbial community composition [12, 24].

Most of earlier studies focused on bacteria and there are only few reports on the seasonality of the other domains of life. For marine archaea, it has been shown that both rare and abundant members of the community were re-occurring seasonally and that different ecotypes of archaea had different seasonal patterns [20, 25]. For phytoplankton, evidence for global patterns of temporal dynamics were obtained by compiling seasonal data of chlorophyll *a* concentrations [26]. Molecular techniques also revealed that microbial eukaryote assemblages displayed seasonality patterns in surface marine waters [27, 28], but interestingly not always in the deeper ocean [28]. Reports on the seasonality of archaea and eukaryotes are scarce, but there are even fewer time series studies covering simultaneously the three domains of life. Steele et al. [29] identified the microorganisms that co-occurred during a 3-year study at the SPOT station (Southern California, USA). At the same site, a 21-day study of the dynamics of phytoplankton, archaea and bacteria revealed a rapid succession of microbial species during a bloom [30], which highlighted the importance of taking into account microbial interactions when studying the seasonality of marine microbial communities. However, long-term surveys of the annual dynamics and succession of photosynthetic picoeukaryotes, bacteria and archaea are currently lacking. Moreover, most time series have covered open ocean sampling sites and there are very few studies dealing with the long term monitoring of microbial communities at coastal sites. In the Mediterranean Sea, coastal environments are characterized by quite variable conditions caused by land to sea transfer of nutrients, organic matter and pollutants through seasonal river discharge during periods of strong precipitations. In such fluctuating environments, predictable patterns of reoccurring microbial communities would be less likely.

The main objective of this study was to test if the eukaryotic phytoplankton, bacteria and archaea communities demonstrated significant patterns of rhythmicity at a coastal site. We conducted a 7-year survey of the taxonomic diversity of microbial plankton community at the Banyuls Bay microbial observatory (SOLA) in the North Western Mediterranean Sea, and investigated the environmental factors that could contribute to microbial seasonality. We also used statistical tools to quantify the rhythmicity of the picoplankton and to detect patterns of co-occurrence between eukaryotic picophytoplankton (less than 3 µm), bacteria and archaea.

Materials and methods

Environmental sampling

Surface seawater (3 m depth) was collected roughly every 2 weeks from October 2007 to January 2015 at the Service d'Observation du Laboratoire Arago (SOLA) sampling station (42°31′N, 03°11′E) in the Bay of Banyuls-sur-Mer, North Western Mediterranean Sea, France. Seawater was collected in 10 l Niskin bottles and then kept in 10 l carboys until arrival to the laboratory within one hour. A subsample of 5 l was prefiltered through 3 µm pore-size polycarbonate filters (Merck-Millipore, Darmstadt, Germany), and the microbial biomass was collected on 0.22 µm pore-size GV Sterivex cartridges (Merck-Millipore) and stored at –80 °C until nucleic acid extraction.

For cytometry, unfiltered seawater samples were fixed at a final concentration of 1% glutaraldehyde, incubated for 15 min at ambient temperature in the dark, frozen in liquid nitrogen and stored at −80 °C. Cytometry analyses were performed on a Becton Dickinson FacsCalibur. Cells were excited at 488 nm and discriminated by SSC and red fluorescence (measured at 670 nm; chlorophyll content). Orange fluorescence (measured at 585 ± 21 nm), produced by phycoerythrin, was used to discriminate *Synechococcus* from *Prochloroccocus* populations [15].

The physicochemical (temperature, salinity, nitrite, nitrate, ammonium, phosphate and silicate) and biological (chlorophyll *a*) parameters were provided by the Service d'Observation en Milieu Littoral (SOMLIT).

DNA extraction, amplification and sequencing

The nucleic acid extraction followed protocols published earlier [25]. Briefly, the Sterivex filters were thawed on ice, followed by addition of lysis buffer (40 nM EDTA, 50 nM Tris, 0.75 M sucrose) and 25 µl of lysozyme (20 mg ml^{-1}). The filters were then incubated on a rotary mixer at 37 °C for 45 min. The 8 µl of Proteinase K (20 mg ml^{-1}) and 26 µl of sodium dodecyl sulfate (20% v/v) were added before incubating at 55 °C for 1 h. Total DNA was extracted and purified with the Qiagen AllPrep kit (Qiagen, Hilden, Germany) following the kit's protocol.

Specific primer pairs were used to target different domains of life. We used primers 515 F (5'-GTGY CAGCMGCCGCGGTA) [31] and NSR951 (5'-TTG

GYRAATGCTTTCGC) [32] to amplify the V4 region of 18S rRNA eukaryote gene. Primers 27 F (5'-AGRGTTY GATYMTGGCTCAG) [33] and 519 R (5'-GTNTTAC NGCGGCKGCTG) [34] were used for regions V1-V3 of the bacterial 16S rRNA gene, and finally primers 519 F (5'-CAGCMGCCGCGGTAA) [35] and 1041 R (5'-GG CCATGCACCWCCTCTC) [36] to amplify regions V4-V6 of the archaeal 16S rRNA gene.

As with all primers, there can biases introduced during the amplification steps, either because some taxa can be preferentially amplified, or because of the uneven number of rRNA gene copies between taxa. A known example is the absence of haptophytes when classical 18S rRNA V4 primers are used [37]. Our eukaryote primers do amplify haptophytes, but no primers are perfect, we hope to have reduced primer biases in this study.

Sequencing was carried out with Illumina MiSeq 2 × 300 bp kits by Research and Testing Laboratory (Lubbock, Texas). We noticed that the R2 reads were of lower quality and therefore chose to conduct our analysis with R1 reads only (300 bp). Having a good quality R2 reads would have been more informative. It could have improved taxa differentiation, taxonomic assignation and overall sequence quality. However, we remain confident, considering the length of the R1, that our data are robust. All the sequences were deposited in NCBI under accession number SRP139203.

Sequence analysis

The analysis of the raw sequences was done by following the standard pipeline of the DADA2 package (https://benjjneb.github.io/dada2/index.html, version 1.6) in "R" (https://cran.r-project.org) with the following parameters: trimLeft = 21, maxN = 0, maxEE = c(5,5), truncQ = 2. Briefly, the package includes the following steps: filtering, dereplication, sample inference, chimera identification, and merging of paired-end reads [38]. DADA2 infers exact amplicon sequence variants (ASVs) from sequencing data, instead of building operational taxonomic units from sequence similarity. In total, we had 159, 160 and 158 samples for the eukaryotic phytoplankton, bacteria and archaea datasets respectively, and an average of ca. 27,000, 29,000 and 16,000 reads per sample respectively. The sequence data were normalized by dividing counts by sample size. This could influence our seasonality analyses, but considering our raw data, we found that the most appropriate transformation was to use proportional abundances [39]. The taxonomy assignments were done with the SILVA v.128 database (https://www.arb-silva.de/documentation/release-128/) and the "*assignTaxonomy*" function in DADA2 that implements the RDP naive Bayesian classifier method described in Wang et al. [40]. For some ASVs, in order to obtain a finer taxonomical resolution, we did an additional BLAST [41] search (blastn, 95% minimum similarity), which results can be found in the column "Blast" of the supplementary table 1. We also did a PR2 [42] assignation for the rhythmic eukaryotic phytoplankton (supplementary table 1). In this study, we aimed to focus more specifically on autotrophic picoeukaryotes in order to highlight the co-occurrence patterns and rhythmicity of phototrophs versus heterotrophs. We have therefore selected a subset of the eukaryotic datasets by retaining sequences belonging to the divisions: Chlorophyta, Dinoflagellata (without including Syndiniales, which are parasitic), Ochrophyta and Haptophyta. Here we considered all non-parasitic Dinoflagellata to be photosynthetic, but it should be noted that organisms from this group display a range of metabolisms: phototrophic, mixotrophic and heterotrophic [43].

Statistics

The Lomb Scargle periodogram (LSP) was used to determine if periodic patterns were present in microbial ASVs. The LSP, based on the Fourier transform, was originally adapted by astrophysicists to detect periodic signals in time series that were unevenly sampled due to limited access to telescopes and varying weather conditions [44, 45]. The LSP was then successfully used in biological studies to determine the periodicity of an unevenly sampled signal [46]. Owing to the robustness of the method and the fact that the sampling effort at SOLA was unevenly spaced, the LSP appeared as the best tool for our study. Computing the peak normalized power (PNmax) of the LSP was accomplished via the "Lomb" package (https://cran.r-project.org/web/packages/lomb/) in the "R" software. ASVs were considered rhythmic when they had a PNmax > 10. The threshold for PNmax is automatically calculated by the package. In summary, the LSP gives both the significance of the rhythmicity and the period of the rhythm. The LSP looks for all possible rhythmic patterns in a signal, regardless of their period. To estimate the time of the year of maximal abundance, we determined for each year and each rhythmic ASV the week of the year with the highest number of sequences. Then we selected, over the entire time series, the week that most often showed highest number of sequences.

The Shannon index, to estimate community diversity, was calculated for each sample and for eukaryotic phytoplankton, bacteria and archaea, respectively, with the function "diversity" from the "Vegan" package in "R" (https://cran.r-project.org/web/packages/vegan/).

Distances between samples were calculated for eukaryotic phytoplankton, bacteria and archaea based on community composition with a canonical correspondence

analyses (CCA). Contribution of environmental factors were added as arrows, and their significance was tested with an analysis of variance (ANOVA) from the "Vegan" package in "R".

Patterns of co-occurrences between taxa were measured with the sparse partial least squares (sPLS) regression [47]. The sPLS was used to relate the abundance matrices of eukaryotic phytoplankton against bacteria and archaea with these parameters: ncomp = 3, mode = 'regression', in the "mixOmics" package (https://cran.r-project.org/web/packages/mixOmics/) in "R". Relationships between taxa were then visualized by a heatmap with the "CIM" function, from the same package.

Eukaryotic phytoplankton, bacteria and archaea ASV tables containing reference sequences, taxonomy and proportional abundance in the different samples are available as supplementary table 1.

Results

Environmental conditions

Chlorophyll *a* concentrations showed yearly reoccurring patterns with maxima reaching up to 2.50 µg l^{-1} during the winter to spring transitions, and minima at 0.04 µg l^{-1} during summer months (Fig. 1). Similarly, temperature levels showed yearly patterns but with much less pronounced inter-annual variations. Water temperature at SOLA were warmest during the months of August and September usually, reaching 22 °C, and coldest between February and March, with values as low as 10 °C. Salinity fluctuated from 38.49 to 34.27 psu, with an average of 37.63 psu. Nitrate levels extended from undetectable to 9.52 µmol l^{-1} with an average of 0.90 µmol l^{-1}. Phosphate concentrations varied from 0.01 µmol l^{-1} to 0.36 µmol l^{-1} with an average of 0.04 µmol l^{-1}. Nitrate, phosphate and chlorophyll *a* concentrations had highest values at the winter/spring transition and lowest in summer. However, salinity, nitrate and phosphate concentrations varied more than average in November 2011, March 2013 and January 2014 when decreases in salinity levels co-occurred with increases in nitrate and phosphate levels (Fig. 1).

Eukaryotic phytoplankton, bacteria and archaea community composition

Overall, the datasets yielded 6398, 6242 and 918 ASVs for the eukaryotic phytoplankton, bacterial and archaeal communities respectively. Within the eukaryotes, 1801 ASVs corresponded to autotrophs (eukaryotic phytoplankton). The Shannon index showed similar patterns of diversity for autotrophic eukaryotes, bacteria and archaea, with higher values at the beginning and the end of winter, and lower values during late summer (Supplementary Fig. 1). Bacterial communities had, on average, the highest diversity, followed by eukaryotic phytoplankton and then archaeal communities.

Canonical correspondence analyses (CCA) were performed on the eukaryotic phytoplankton, bacteria and archaea datasets to investigate the relationships between community composition and measured environmental variables (Fig. 2a–c). The communities showed a strong seasonal pattern but the environmental parameters that we measured explained only 7, 12 and 14% of the variance for the eukaryotic phytoplankton, bacteria and archaea communities respectively (Supplementary Table 2). The main explaining factors were temperature (T), day length (DL) for the three datasets, and also Nitrate (NO_3) and Salinity (S) for bacteria (ANOVA, $p = 0.001$). Temperature and day length explained close to half of the total variance for eukaryotic phytoplankton, bacteria and archaea (Supplementary Table 2). The eukaryotic phytoplankton communities grouped together according to the month of sampling. The communities showed more divergence on the CCA plots during the months of April and May, whereas they were grouped during the other months (Fig. 2a). Bacterial communities showed a similar seasonal structure with higher separation between samples from March to June (Fig. 2b). Finally, the archaea had a comparable structure of monthly successions, but with highest variability between samples from July to October (Fig. 2c).

From 2007 to 2015, at the division level, the photosynthetic picoeukaryote community was composed of Dinoflagellata, Chlorophyta, Ochrophyta and Haptophyta (44.01% of the sequences, 29.45, 13.23, 13.31% respectively) (Supplementary Fig. 2). Dinoflagellata were dominated by Dinophyceae (99.19% of the sequences) and Chlorophyta by Mamiellophyceae (94.36%). Within Mamiellophyceae, three main genera were found, *Micromonas*, *Bathycoccus* and *Ostreococcus* (64.59, 31.89 and 3.49%, respectively) (Supplementary Fig. 2). Bacteria (Supplementary Fig. 3) were dominated by the phyla *Proteobacteria* (76.74%) and *Cyanobacteria* (12.12%). The main contributors of the *Proteobacteria* were *Alphaproteobacteria* (89.79%, mainly SAR11) and *Gammaproteobacteria* (9.93%). *Synechococcus* ASVs represented 95.8% of *Cyanobacteria* sequences. Finally, archaea were divided between the *Thaumarchaeota* (64.36%) and the *Euryarchaeota* (35.07%) (Supplementary Fig. 4).

Rhythmicity of the environmental and biological compartments

In order to test if environmental factors and microbial taxa had significant rhythmic patterns during the 7-year time

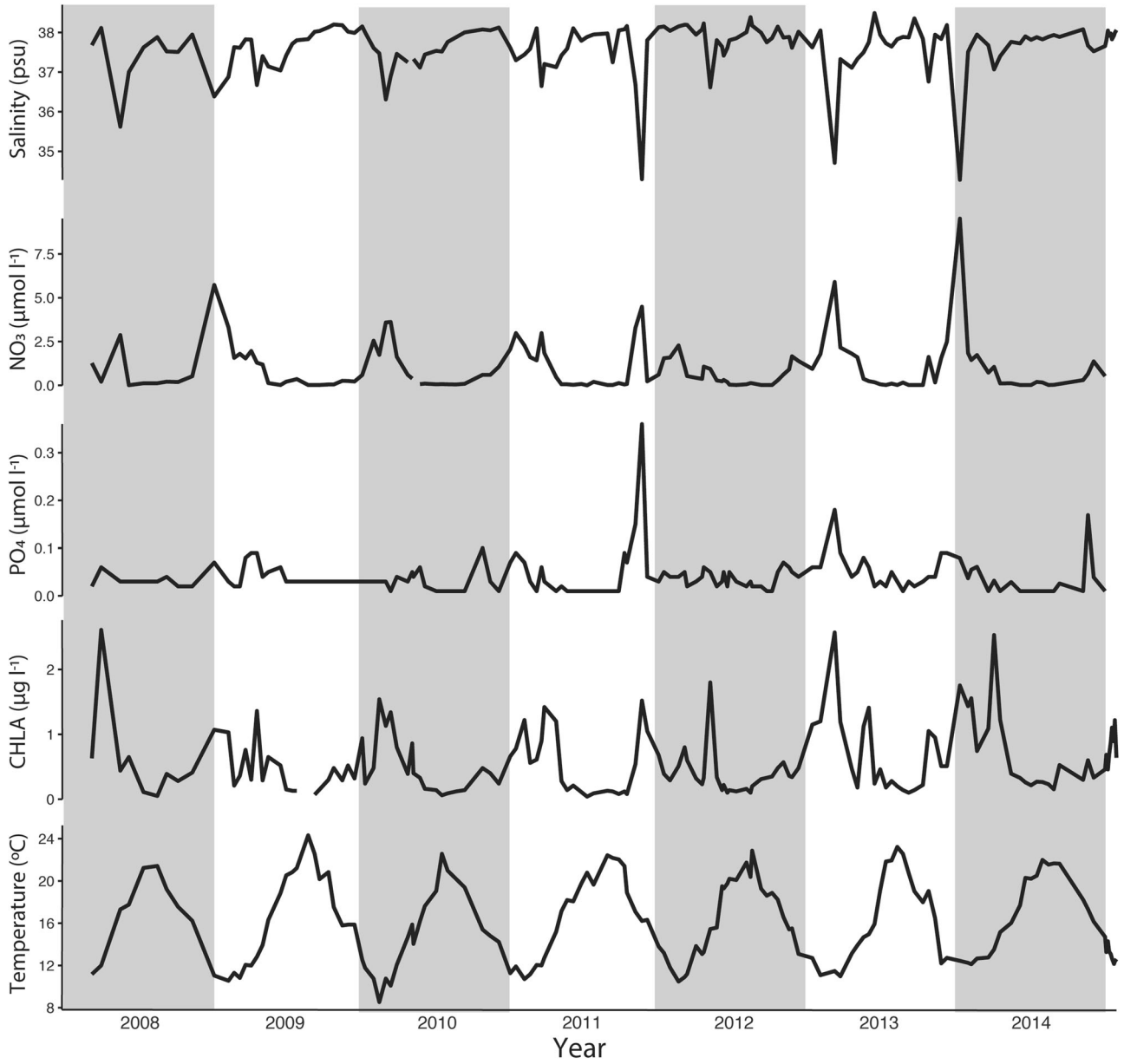

Fig. 1 Salinity, nitrates (NO_3), phosphates (PO_4), chlorophyll *a* (CHLA) and temperature from 2008 to 2015 at the SOLA station in the Banyuls Bay

series, the Lomb Scargle periodogram (LSP) algorithm was applied to the eukaryotic phytoplankton, bacteria, archaea and environmental datasets. The most rhythmic environmental parameters were day length and temperature with a PNmax score of 60.00 and 55.67 respectively. Other rhythmic factors were NO_2, NO_3, chlorophyll *a* and NH_4 but with lower PNmax scores of 37.17, 24.27, 21.37 and 13.44, respectively. $SIOH_4$, PO_4 and salinity had PNmax scores that did not cross the statistical threshold to be considered rhythmic (PNmax scores of 9.95, 7.03 and 5.45, respectively). A total of 15 picoeukaryote, 89 bacteria and 31 archaea ASVs had significant patterns of rhythmicity. The rhythmic ASVs and environmental factors all had a period of one year. Theses rhythmic microbial ASVs were selected for further detailed analysis.

Timing of yearly reoccurrences and relative abundance of rhythmic ASVs

Among the 135 ASVs (Fig. 3, Supplementary Table 3) that showed significant reoccurrences throughout the year, different domains displayed different patterns. Bacterial rhythmic ASVs showed phases of maximal abundance that spread throughout the year, whereas eukaryotic

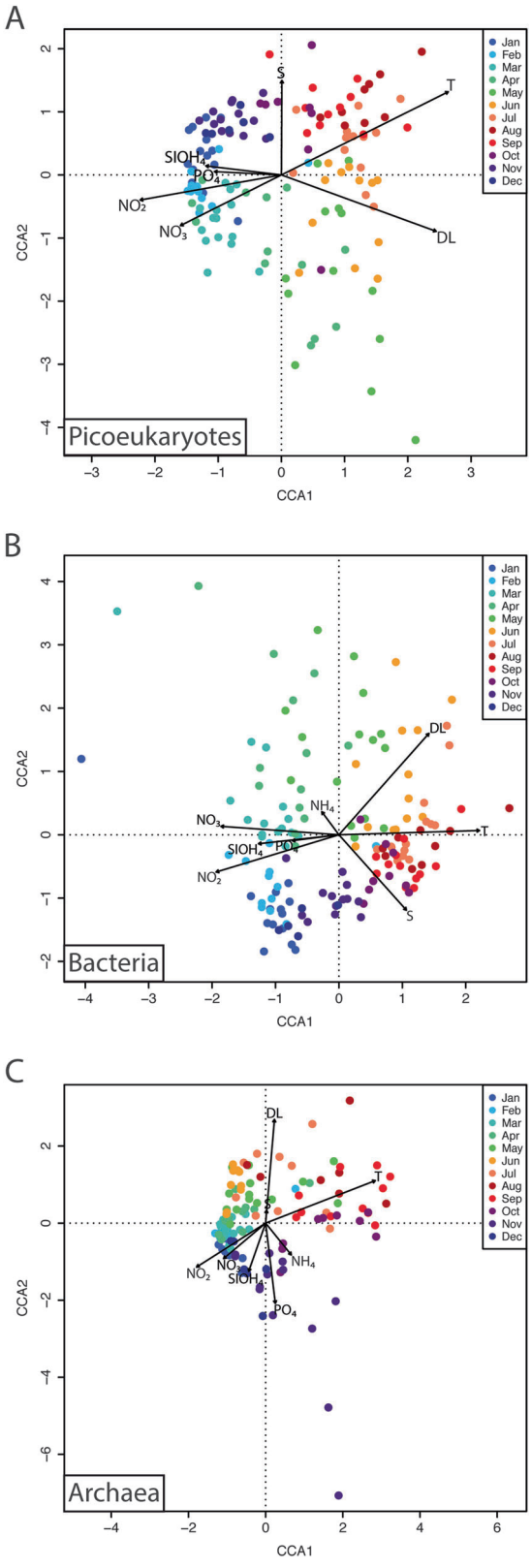

Fig. 2 Canonical correspondence analyses (CCA) of the eukaryotic phytoplankton (**a**), bacteria (**b**), and archaea (**c**) community composition in relation to environmental factors. The communities are color coded according to the month of sampling. The arrows represent the different environmental factors (T: temperature, DL: day length, NH_4: ammonium, NO_3: nitrates, NO_2: nitrites, PO_4: phosphates, $SIOH_4$: silicates, S: salinity)

phytoplankton and archaeal rhythmic ASVs phases were confined to certain moments of the year. Eukaryotic phytoplankton rhythmic ASVs had maximal abundance from November to April, while archaeal rhythmic ASVs had maximal abundance from September to March.

On average 30.5% of the eukaryotic phytoplankton sequences were rhythmic but the proportion varied throughout the year. Rhythmic ASVs represented up to 96% of the sequences in January and as low as 2.5% of the sequences in July (Fig. 4b). All classes followed a similar pattern with high levels (50 to 60% of total sequences) from mid-Autumn to mid-Spring (October to April) and lower levels (less than 15% of total sequences) during the rest of the year. The lowest number of rhythmic sequences were seen during the summer months (Fig. 4b). Flow cytometry showed that picoeukaryotes had low abundances during the summer months and high abundances during winter months (Fig. 5).

At the eukaryotic class level, (Fig. 4a), the Mamiellophyceae rhythmic ASVs were found mostly from the end of November to the end of March. The Dinophyceae rhythmic ASVs had peaks of abundances year-round. The Dictyochophyceae rhythmic ASV was only abundant at the beginning of February. Within Mamiellophyceae, the *Bathycoccus prasinos* ASV peaked around the middle of February (7th week of the year) (Fig. 4c) with a distribution going from January to April (Fig. 4d). *Micromonas commoda* was recurrent from December to the end of March (Fig. 4c) and distributed from February to April (Fig. 4d). *Micromonas sp.1* ASV was more present at the end of November (Fig. 4c) with a distribution from November to February (Fig. 4d). *Micromonas bravo*, however, had ASVs peaks from December to February (Fig. 4c) and was present from October to April (Fig. 4d).

Rhythmic bacterial ASVs were present throughout the year (Fig. 6a), and represented in average 31.3% of the sequences, with variations from 18 to 45.7% of the sequences (Fig. 6b). The contributors to the rhythmic ASVs were *Acidimicrobiia*, *Alphaproteobacteria*, *Betaproteobacteria*, *Cyanobacteria*, *Flavobacteria*, *Gammaproteobacteria*, SAR202 and candidate *Proteobacteria* SPOTSOCT00m83 (Fig. 6a, b).

The different rhythmic bacterial classes showed different types of patterns. The *Acidimicrobiia, Gammaproteobacteria*, SAR202 and candidate *Proteobacteria* SPOTSOCT00m83 showed high numbers from October to April and were almost absent during the summer months (Fig. 6b). They displayed similar reoccurrence patterns as well, mainly from December to February (Fig. 6a).

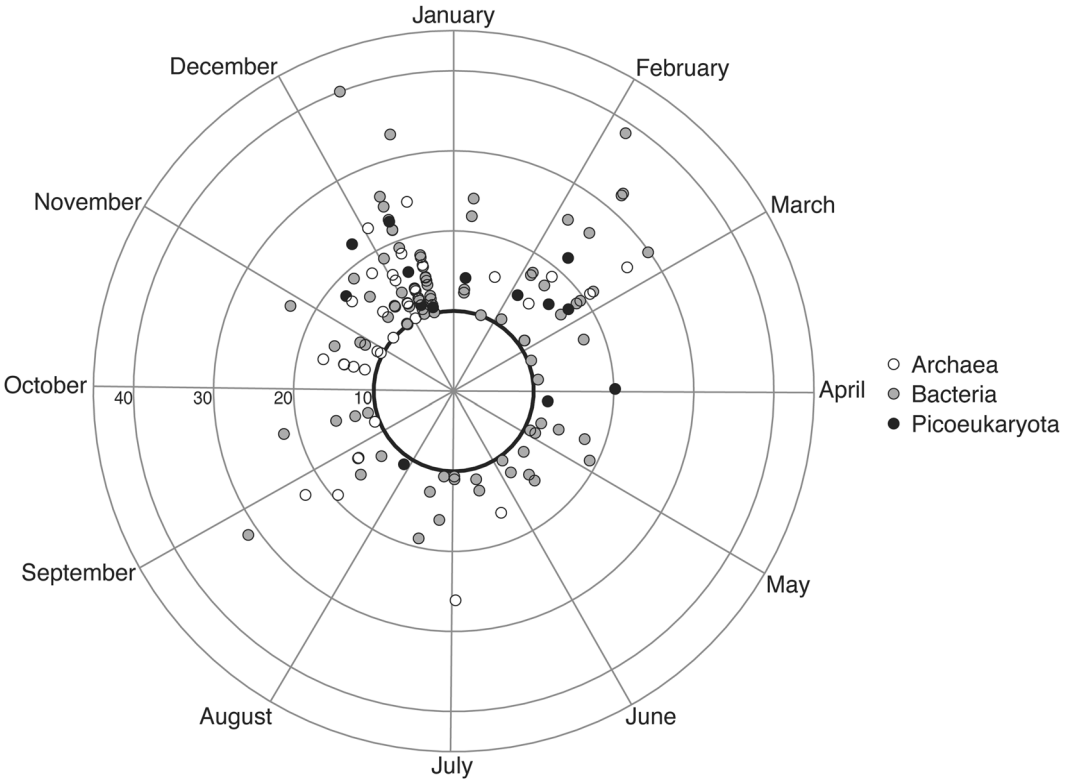

Fig. 3 Polar plot showing when during the year the rhythmic ASVs reoccur and the strength of reoccurrence (PNmax, calculated *via* the LSP). The black circle shows the statistical threshold for significant rhythmicity (PNmax = 10). The ASVs are color coded according to which domain of life they belong to

Cyanobacteria rhythmic ASVs demonstrated an opposite pattern, with high levels during the warm summer and autumn months (March to October) and low levels the rest of the year (Fig. 6b). Cytometry data showed the same seasonal pattern in terms of cell abundance (Fig. 5). *Flavobacteria* had similar patterns as cyanobacteria. However, their reoccurrence patterns were different. Rhythmic *Cyanobacteria* ASVs reoccurred from the end of March to October, whereas *Flavobacteria* ASVs had two periods of maximal reoccurrence, one from March to July and another during December (Fig. 6a). *Betaproteobacteria* ASVs were more abundant from January to May and were absent the rest of the year (Fig. 6b), and were only recurrent at the end of February (Fig. 6a). *Alphaproteobacteria* rhythmic ASVs displayed similar sequence numbers throughout the year, accounting for half of the rhythmic ASVs sequence numbers (15%) (Fig. 6b). Similarly, the *Alphaproteobacteria* ASVs reoccurrences covered the whole year except for March (Fig. 6a).

Amongst the rhythmic *Alphaproteobacteria*, a majority of ASVs belonged to SAR11. All sub-groups of SAR11 (SAR11Ia, SAR11Ib, SAR11Ic, SAR11IIa, SAR11IIIa and SAR11IV) had high numbers of rhythmic ASVs from September to the end of February (Supplementary Fig. 5A). These groups also showed higher number of sequences during winter months (Supplementary Fig. 5B), except for SAR11IIIa which had higher sequence abundance from June to November (Supplementary Fig. 5B).

Finally, archaeal rhythmic ASVs had maximum occurrences from the end of August to March, both for *Euryarchaeota* and *Thaumarchaeota* (Fig. 6c). Rhythmic ASVs dominated the dataset as they represented an average of 74.6% of total sequence numbers, ranging from 47.3 to 89.2% (Fig. 6d). Within the *Euryarchaeota* phylum, rhythmic ASVs of Marine group II (MGII) and Marine group III (MGIII) were found. Rhythmic MGII ASVs showed reoccurrence patterns from September to March (Fig. 6c) and highest relative sequence numbers from July to October (Fig. 6d). MGIII rhythmic ASVs had a more restrained occurrence, from end of November to beginning of December (Fig. 6c) and were less present in relative abundance (Fig. 6d). The *Thaumarchaeota* rhythmic ASVs displayed high levels of presence throughout the year with the exception of the months of September. The months preceding and succeeding September showed a steady decrease and increase of relative sequence number, respectively (Fig. 6d). *Thaumarchaeota* had high occurrences all year, except from March to May (Fig. 6c).

We also observed a large number of ASVs that were not rhythmic and thus had peaks of abundance at different

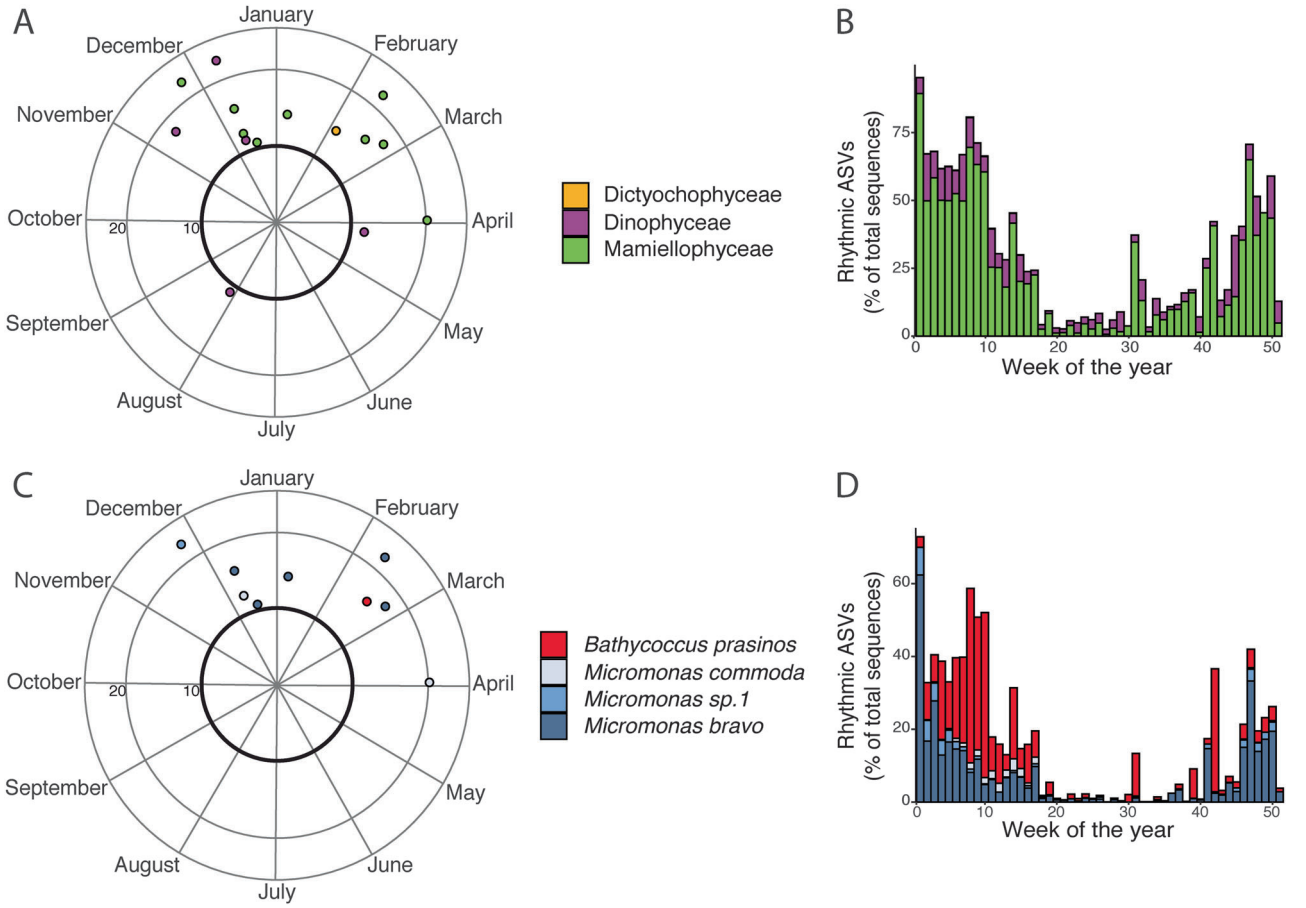

Fig. 4 Polar plots representing the rhythmic eukaryotic phytoplankton classes (**a**) and the rhythmic Mamiellophyceae ASVs (**c**). The bar plots show the proportion of sequences belonging to rhythmic ASVs averaged per week of the year for eukaryotic phytoplankton classes (**b**) and Mamiellophyceae ASVs (**d**)

moments from year to year. Non-rhythmic ASVs had different patterns of seasonal dynamics. Some ASVs, like the Gymnodiniphycidae ASV00020, were absent from most of the samples but shows sudden and irregular peaks of abundance (Supplementary Fig. 6). Other, like the Gymnodiniphycidae ASV00036, were more frequent and had irregular peaks of sequence abundance that co-occurred with irregular environmental events such as freshening sea surface waters and increased nitrate concentrations (Supplementary Fig. 6).

Co-occurrence at the ASV level

To determine co-occurrences, heatmaps were created with the rhythmic ASVs after calculating Sparse Partial Least Squares (sPLS) regressions for one dataset against the other (bacteria vs. picoeukaryote, bacteria vs. archaea and archaea vs. picoeukaryote). For bacteria vs. picoeukaryotes (Fig. 7a), the highest correlation scores (>0.6) were between *Micromonas sp.1* (ASV 00013) and a SAR11 sequence (ASV 00054) as well as 3 *Rhodospirillaceae* (ASV 00020, ASV 00112 and ASV 00266). A Dinophyceae (ASV 00011) also had a high correlation (0.55) with the same *Alphaproteobacteria* ASVs. Other high correlations were found between *Bathycoccus prasinos* and *Alpha-* and *Gammaproteobacteria*. *Micromonas bravo* (ASV 00002) also had high correlations with an *Alphaproetobacteria* (ASV 00112). A Dinophyceae ASV (ASV00053) displayed a specific high correlation with a group of bacteria that were not correlated to other eukaryotic phytoplankton. This is probably due to the fact that Dinophyceae is the only rhythmic picoeukaryote to peak in summer (Fig. 4a).

The archaea vs. picoeukaryote heatmap revealed high correlation (>0.5) between *Bathycoccus prasinos* and MGII ASVs (ASV 00050 and ASV 00008). *Micromonas bravo* (ASV 00040) showed a similar trend. On the other hand, *Micromonas commada* (ASV 00084) had high correlations (>0.5) with MGIII ASVs (ASV 00012 and ASV 00028). As with the bacteria dataset, the Dinophyceae, ASV 00053, displayed high correlations when all other eukaryotic phytoplankton ASVs had low correlations (Fig. 7b).

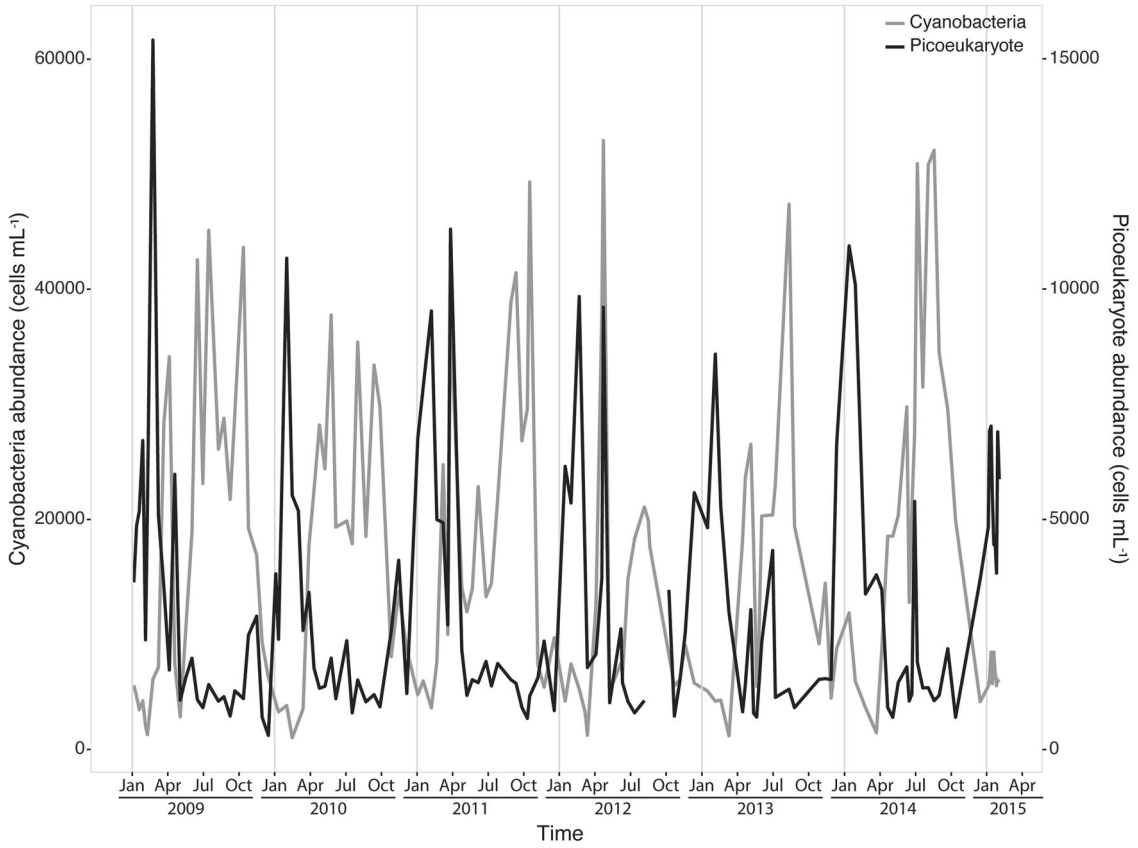

Fig. 5 Photosynthetic picoeukaryote and cyanobacteria abundance determined by flow cytometry from 2009 to 2015 at the SOLA station in the Banyuls Bay

In the bacteria vs. archaea heatmap the main co-occurrences were observed between a *Thaumarchaeota* ASV and a *Gammaproteobacteria* ASV, as well as between a MGII and *Alphaproteobacteria* ASV (Supplementary Fig. 7).

Discussion

Our 7-year survey in the NW Mediterranean Sea showed that within all domains of life some taxa showed significant patterns of rhythmicity with a one year period. The number of rhythmic taxa differed between domains. Phototrophic picoeukaryotes had 1% of rhythmic ASVs, bacteria 3.1 % and archaea 3.4%, but these ASVs represented a large proportion of the total number of sequences (31.3, 31.6 and 75.5%, respectively). The large proportion of rhythmic sequences supports the idea of microbial communities that come back year after year at the same season. The concept of re-occurring communities has been demonstrated in several long term studies [14, 20, 24] but coastal observations are quite scarce [48]. The Banyuls Bay is a coastal site with seasonal characteristics specific to the NW Mediterranean. It has a marked seasonality but interestingly it is also characterized by strong and ephemeral inputs of nutrients brought from sediment mixing during episodic winter storms and during flash floods from incoming rivers [49]. Nutrients are known to strongly structure communities by promoting planktonic blooms and by stimulating the growth of certain microbes [12, 50]. However, despite irregular nutrient supply from year to year, as illustrated by salinity and phosphate variations during winter and spring (Fig. 1), we could still observe a large number of rhythmic eukaryotic phytoplankton, bacteria and archaea sequences. The CCA analysis (Fig. 2) confirmed that day length and temperature were major factors structuring the communities and we can suppose that they directly or indirectly control the dynamics of the rhythmic taxa.

Day length has been shown to be a strong driver of community structure in temperate and polar marine environments such as the English Channel [14], or a high-Arctic fjord [50]. Temperature is another strong driver as it can affect gene expression and subsequently the structure and the function of the microbial communities [51]. The availability of nutrients has also been shown to be an important factor in community composition as demonstrated in the

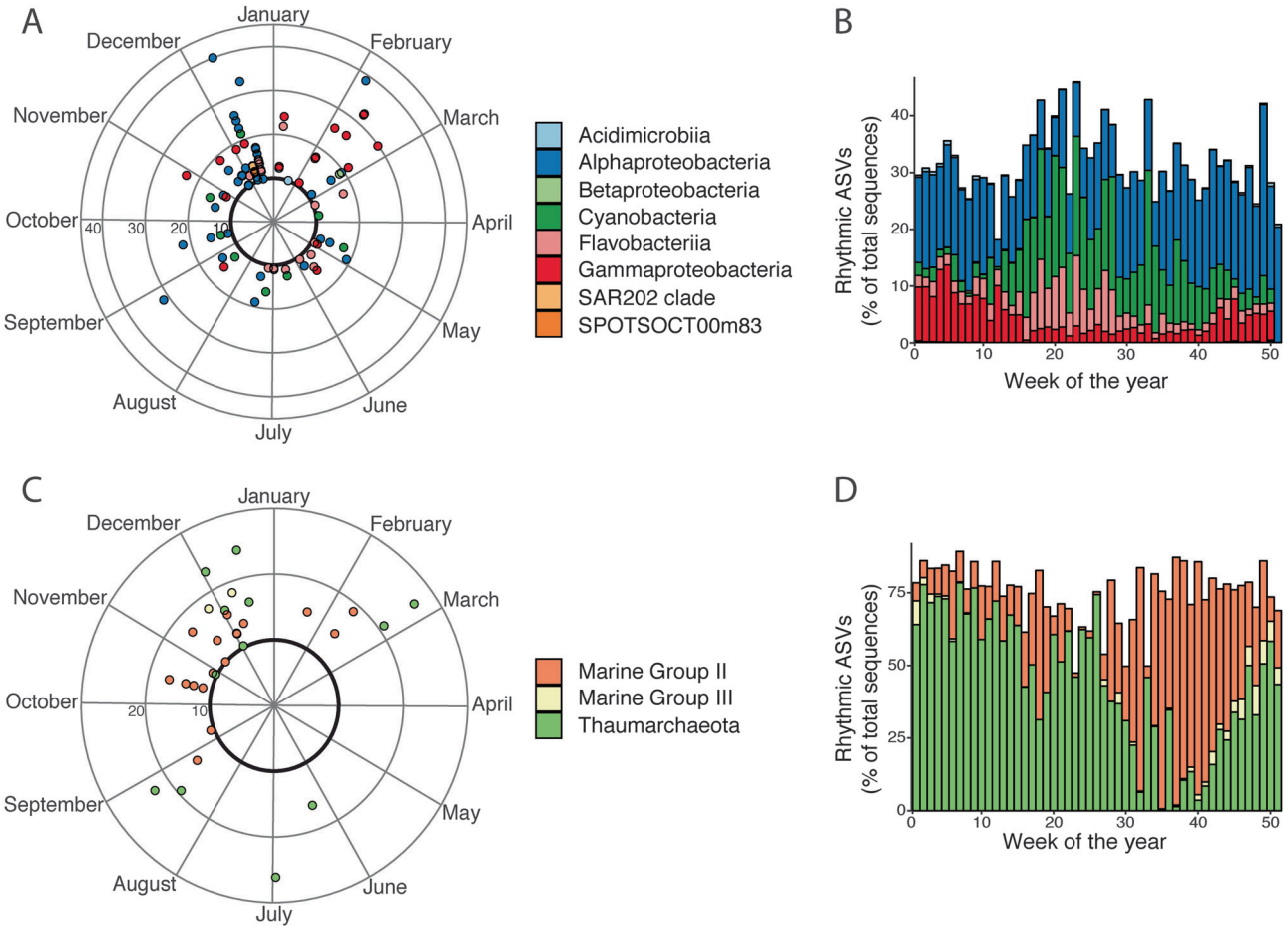

Fig. 6 Polar plots representing the rhythmic bacteria ASVs (**a**) and the rhythmic archaea ASVs (**c**). The bar plots show the proportion of sequences belonging to rhythmic ASVs averaged per week of the year, for bacteria (**b**) and archaea (**d**)

BATS time series in the Atlantic Ocean [3]. Our data from the Banyuls Bay shows that in a coastal ecosystem, environmental parameters like temperature and day length have such a structuring effect that sporadic meteorological events do not appear to impact the overall microbial rhythms of reoccurring dominant groups of eukaryotic phytoplankton, bacteria and archaea. However, even though rhythmic ASVs could be predominately influenced by day length and temperature, we observed non-rhythmic ASVs, which were influenced by irregular environmental factors. For example, the dynamics of the Gymnodiniphycidae ASV00036 was associated to the irregular peaks of nitrate concentration (Supplementary Fig. 6).

The importance of day length in driving the rhythm of individual microorganisms brings the question whether seasonality is driven by circadian clocks in marine microbes. The presence of a functional circadian clock governing day/night biological processes bas been demonstrated in the mamiellophyceae *Ostreococcus* [8, 52], however, the existence of a photoperiod dependent regulation of blooms remains to be established formally in this order. In diatoms from northern Norwegian coastal waters, it has been reported that the timing of the spring bloom varies little from year to year whether water stratification had occurred or not [53]. The authors hypothesized that the photoperiod was the major factor that relieved diatoms resting spores from dormancy, leading to seasonal blooms. However, the internal mechanisms triggering these rhythms remain unknown since the presence of circadian clocks remain to be shown in diatoms. Amongst the prokaryotes, cyanobacteria are the only known group to have a genuine circadian clock [7] and the occurrence of circadian clock remain to be established in heterotrophic bacteria and archaea. The rhythmicity of some heterotrophic microorganisms could thus be governed directly by day length or indirectly through interactions with the rhythmic autotrophs. Interestingly, altogether, eukaryotic and prokaryotic autotrophs were present during the entire year, but they showed clear differences in their seasonal dynamics. Picoeukaryotes had highest abundance from autumn to spring, and cyanobacteria during the summer.

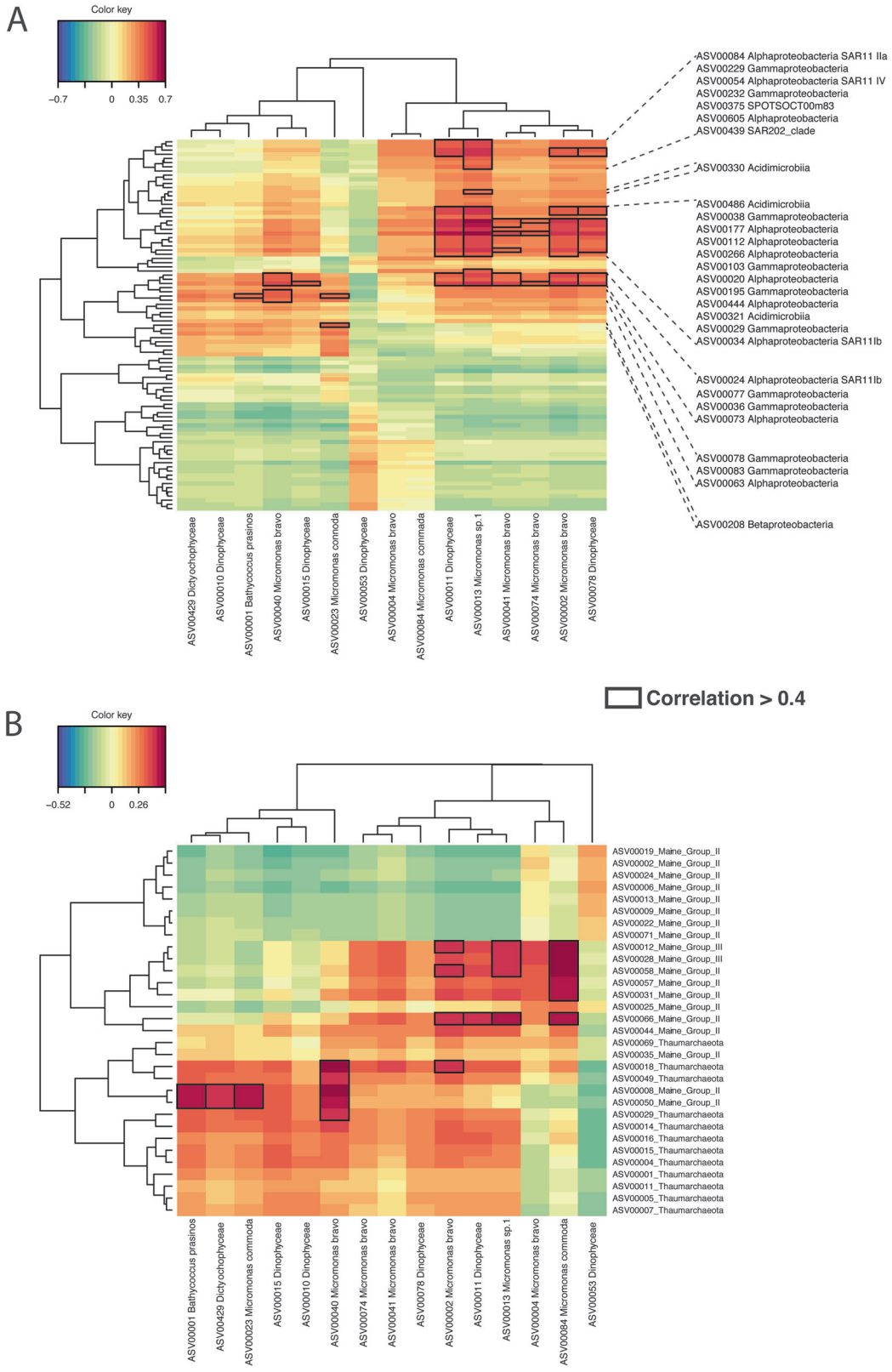

Fig. 7 Heatmap based on a sPLS regression showing co-occurrences between rhythmic eukaryotic phytoplankton ASVs and bacteria ASVs (**a**) and between rhythmic eukaryotic phytoplankton ASVs and archaea ASVs (**b**). Correlations > 0.4 are highlighted

We observed a large number of rhythmic ASV sequences, which were mainly seen within abundant members of the communities. The eukaryotic phytoplankton were represented primarily by Mamiellophyceae and Dinophyceae, which have important ecological roles as primary producers and as links in the predator/prey food chain [54]. Among prokaryotic rhythmic ASVs, there were many representative of the SAR11, known for being the most common group of marine bacteria [23]. Seasonality has been observed for SAR11 and Flavobacteria groups earlier [15, 55]. Rhythmic archaea were found in MGII, MGIII and Thaumarchaeota, which have already been shown to have reoccurring yearly patterns [20, 25]. The dominance of abundant groups within rhythmic ASVs raises the question as whether our analysis underestimated the rhythmicity of less abundant ASVs. It should be noted, however, that some rare ASVs, with occurrences of 0.034, 0.009 and 0.115% respectively for the eukaryotic phytoplankton, bacteria and archaea dataset, were also found to be rhythmic. In agreement with our observations, Alonso-Sáez et al. [55] recently showed that, also in a coastal system, both rare and abundant bacterial species had patterns of rhythmicity in the Atlantic Ocean [55] and that many species that remained rare all year long also showed significant patterns of rhythmicity. Rhythmicity of marine microbes, at the ASV level, remains to be verified in other sites as there have been only few studies conducted at this level of resolution. While the re-occurrence of entire communities is now well documented [13, 14, 24], the long-term monitoring of individual taxa is not common [12, 21] and the use of statistics to test patterns of ASV rhythmicity is even less frequent.

There have been very few studies looking at the temporal dynamics across the three domains of life in marine microbial ecosystems. One of the first long term study covering the three domains did not focus on the rhythm of the individual taxa but rather looked at co-occurrence networks [29]. They showed that correlations between microbes were more prevalent than correlations between microbes and environmental factors. This is probably due to the stability of the deep chlorophyll maximum at their study site [29]. More recently, Needham and Fuhrman looked at the succession of phytoplankton, archaea and bacteria, but only during 6 months [30]. Another study in the same ecosystem, relying on automated sampling, showed daily and highly dynamic population variations in the three domains of life, and extensively described the biological interactions that took place during the sampling period [56]. A study looking at bacterioplankton diversity and phytoplankton microscopy counts, has shown that despite interannual variations in phytoplankton blooms, bacterioplankton microdiversity patterns seem stable in both bloom and non-bloom conditions [57]. The present dataset showed high co-occurrence between some eukaryotic phytoplankton and prokaryotes ASVs. The most significant correlations were found between Mamiellophyceae and the alphaproteobacteria SAR11. This co-occurrence could be explained by the fact that *Micromonas* and SAR11 might interact by exchanging compounds such as vitamins, growth factors and organic carbon [58, 59]. However, SAR11 was recently shown to be auxotrophic to the thiamine precursor 4-amino-5-hydroxymethyl-2-methyl pyrimidine [60], thus resulting in similar needs as *Micromonas* for thiamin precursors [61]. The co-occurrence of these two microbes therefore may be explained by their requirement for similar nutritional niches rather than by a relationship of interdependency depending on environmental factors.

In conclusion, through the analysis of our time series we demonstrated that a large proportion of members of eukaryotic phytoplankton, bacteria and archaea datasets, showed rhythmicity with a one year period of reoccurrence over then entire time series. The main drivers of seasonality were photoperiod and temperature. Sporadic meteorological events and irregular nutrient supply characteristic of our coastal site did not affect significantly the seasonality, indicating that the yearly rhythms were robust. Rhythmicity was found in both autotrophs (picoeukaryotes and cyanobacteria) and heterotrophic prokaryotes. Seasonal autotrophs, which respond to light, may be setting the pace for rhythmic heterotrophs but similar environmental niches may be driving seasonality as well.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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Supplementary information
Type of files:
Figures: JPEG image files (.jpg)
Tables: MS Excel spreadsheet documents (.xlsx)

Supplementary table 1: Eukaryotic phytoplankton, bacteria and archaea ASV tables containing reference sequences, taxonomy and proportional abundance in the different samples.

Supplementary table 1 is available here: https://www.nature.com/articles/s41396-018-0281-z#Sec14

Supplementary table 2:
Explained variance of environmental factors relative to the CCAs of the three domains of life. The ration expresses the explained variance associated to temperature and day length divided by the explained variance of all environmental factors (Temperature, Day Length, Ammonium, Nitrate, Nitrite, Phosphate, Silicate, Salinity).

Supplementary table 2:

	Picoeukaryotes	**Bacteria**	**Archaea**
Total variance explanation (%)	0.07397	0.1229	0.1428
Temperature and day length variance explanation (%)	0.03581	0.05542	0.07612
Ratio	0.4841097	0.4508774	0.5328746

Supplementary table 3: Description of the rhythmic eukaryotic phytoplankton, bacteria and archaea ASV containing ASV number, Percentage of total sequences, Number of sequences, PNmax, Week of maximum occurrence, Taxonomy and Blast results.

Description of the rhythmic eukaryotic phytoplankton ASVs [SILVA assignment] (1/2)

Rhythmic eukaryotic phytoplankton (1/2)

ASV number	Percentage of total sequences	Number of sequences	PNmax	Week of maximum occurrence	Kingdom	Phylum/Division	Class	Order
ASV00001	9.294	328595	16.088	7	Eukaryota	Chlorophyta	Mamiellophyceae	Mamiellales
ASV00002	8.242	264486	21.908	6	Eukaryota	Chlorophyta	Mamiellophyceae	Mamiellales
ASV00004	3.845	93816	10.771	50	Eukaryota	Chlorophyta	Mamiellophyceae	Mamiellales
ASV00010	1.954	49054	11.815	14	Eukaryota	Dinoflagellata	Dinophyceae	Gymnodiniphycidae
ASV00011	1.816	48284	22.631	49	Eukaryota	Dinoflagellata	Dinophyceae	Peridiniphycidae
ASV00013	1.310	44418	22.292	47	Eukaryota	Chlorophyta	Mamiellophyceae	Mamiellales
ASV00015	1.174	38764	17.927	45	Eukaryota	Dinoflagellata	Dinophyceae	Peridiniphycidae
ASV00023	0.711	27345	20.168	13	Eukaryota	Chlorophyta	Mamiellophyceae	Mamiellales
ASV00040	0.368	16177	17.58	8	Eukaryota	Chlorophyta	Mamiellophyceae	Mamiellales
ASV00041	0.499	16149	14.189	1	Eukaryota	Chlorophyta	Mamiellophyceae	Mamiellales
ASV00053	1.061	12902	11.046	31	Eukaryota	Dinoflagellata	Dinophyceae	Gymnodiniphycidae
ASV00074	0.292	9601	15.893	49	Eukaryota	Chlorophyta	Mamiellophyceae	Mamiellales
ASV00078	0.342	9430	11.486	49	Eukaryota	Dinoflagellata	Dinophyceae	Gymnodiniphycidae
ASV00084	0.378	8556	12.413	49	Eukaryota	Chlorophyta	Mamiellophyceae	Mamiellales
ASV00429	0.034	1365	14.383	5	Eukaryota	Ochrophyta	Dictyochophyceae	Pedinellales

Description of the rhythmic eukaryotic phytoplankton ASVs [SILVA assignment] (2/2)

Rhythmic eukaryotic phytoplankton (2/2)

ASV number	Family	Genus	Blast	Accession	Homology
ASV00001	Mamiellales	Mamiella	Bathycoccus prasinos	XR_002608757.1	100%
ASV00002	Mamiellales	Micromonas	Micromonas bravo	KT860894.1	100%
ASV00004	Mamiellales	Micromonas	Micromonas bravo	KU244671.1	100%
ASV00010	Gymnodiniumade	NA	Warnowia sp. Isolate	KY980035.1	100%
ASV00011	Peridiniales	Heterocapsa	Heterocapsa rASVndata isolate	KY980285.1	100%
ASV00013	Mamiellales	Micromonas	Micromonas sp.1	**KU244667.1**	**95%**
ASV00015	Peridiniales	Heterocapsa	Heterocapsa rASVndata isolate	KY980397.1	100%
ASV00023	Mamiellales	Micromonas	**Micromonas commoda**	**KY980374.1**	**100%**
ASV00040	Mamiellales	Micromonas	Micromonas bravo	KX602138.1	100%
ASV00041	Mamiellales	Micromonas	Micromonas bravo	KT860894.1	99%
ASV00053	Gymnodiniphycidae	Gyrodinium	Gyrodinium cf. gutrula	FN669511.1	100%
ASV00074	Mamiellales	Micromonas	Micromonas bravo	KT860894.1	99%
ASV00078	Gymnodiniphycidae	Gyrodinium	**Uncultured Gyrodinium**	**KX602145.1**	**99%**
ASV00084	Mamiellales	Micromonas	Micromonas commoda	KU244669.1	100%
ASV00429	NA	NA	Pedinellales sp.	JF794054.1	95%

Description of the rhythmic eukaryotic phytoplankton ASVs [PR2 assignment] (1/2)

PR2 taxonomic affiliation for rhythmic eukaryote phytoplankton (1/2)

OTU number	kingdom	supergroup	division	class	order	family	genus
Otu00001	Eukaryota	Archaeplastida	Chlorophyta	Mamiellophyceae	Mamiellales	Bathycoccaceae	Bathycoccus
Otu00002	Eukaryota	Archaeplastida	Chlorophyta	Mamiellophyceae	Mamiellales	Mamiellaceae	Micromonas
Otu00004	Eukaryota	Archaeplastida	Chlorophyta	Mamiellophyceae	Mamiellales	Mamiellaceae	Micromonas
Otu00010	Eukaryota	Alveolata	Dinoflagellata	Dinophyceae	Gymnodiniales	Gymnodiniaceae	Gymnodinium
Otu00011	Eukaryota	Alveolata	Dinoflagellata	Dinophyceae	Peridiniales	Heterocapsaceae	Heterocapsa
Otu00013	Eukaryota	Archaeplastida	Chlorophyta	Mamiellophyceae	Mamiellales	Mamiellaceae	Micromonas
Otu00015	Eukaryota	Alveolata	Dinoflagellata	Dinophyceae	Peridiniales	Heterocapsaceae	Heterocapsa
Otu00023	Eukaryota	Archaeplastida	Chlorophyta	Mamiellophyceae	Mamiellales	Mamiellaceae	Micromonas
Otu00040	Eukaryota	Archaeplastida	Chlorophyta	Mamiellophyceae	Mamiellales	Mamiellaceae	Micromonas
Otu00041	Eukaryota	Archaeplastida	Chlorophyta	Mamiellophyceae	Mamiellales	Mamiellaceae	Micromonas
Otu00053	Eukaryota	Alveolata	Dinoflagellata	Dinophyceae	Gymnodiniales	Gymnodiniaceae	Gyrodinium
Otu00074	Eukaryota	Archaeplastida	Chlorophyta	Mamiellophyceae	Mamiellales	Mamiellaceae	Micromonas
Otu00078	Eukaryota	Alveolata	Dinoflagellata	Dinophyceae	Gymnodiniales	Gymnodiniaceae	Gyrodinium
Otu00084	Eukaryota	Archaeplastida	Chlorophyta	Mamiellophyceae	Mamiellales	Mamiellaceae	Micromonas
Otu00429	Eukaryota	Stramenopiles	Ochrophyta	Dictyochophyceae	Dictyochophyceae_X	Pedinellales	Pedinellales_X

Description of the rhythmic eukaryotic phytoplankton ASVs [PR2 assignment] (2/2)

PR2 taxonomic affiliation for rhythmic eukaryote phytoplankton (2/2)

OTU number	species	clade
Otu00001	Bathycoccus_prasinos	
Otu00002	Micromonas_bravo	Micromonas_Clade-B.E.3
Otu00004	Micromonas_bravo	Micromonas_Clade-B.E.3
Otu00010	Gymnodinium_sp.	
Otu00011	Heterocapsa_nei/rotundata	
Otu00013	Micromonas_bravo	Micromonas_Clade-B..4
Otu00015	Heterocapsa_pygmaea	
Otu00023	Micromonas_commoda	Micromonas_Clade-A.ABC.1-2
Otu00040	Micromonas_bravo	Micromonas_Clade-B.E.3
Otu00041	Micromonas_bravo	Micromonas_Clade-B.E.3
Otu00053	Gyrodinium_dominans	
Otu00074	Micromonas_bravo	Micromonas_Clade-B.E.3
Otu00078	Gyrodinium_fusiforme	
Otu00084	Micromonas_commoda	Micromonas_Clade-A.ABC.1-2
Otu00429	Pedinellales_X_sp.	

Description of the rhythmic bacteria ASVs (1/3)

Rhythmic bacteria

OTU number	Percentage of total sequences	Number of sequences	PNmax	Week of maximum occurrence	Kingdom	Phylum/Division	Class	Order	Family	Genus	Blast
Otu00003	2.673	116177	19.059	17	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	Ascidiaceihabitans	
Otu00006	2.093	93879	17.407	16	Bacteria	Cyanobacteria	Cyanobacteria	Cyanobacteria	Cyanobacteria	Synechococcus	
Otu00007	1.870	88757	15.989	50	Bacteria	Proteobacteria	Alphaproteobacteria	SAR11_clade	Surface_1	NA	SAR11Ia
Otu00009	1.816	67828	15.9	42	Bacteria	Cyanobacteria	Cyanobacteria	Cyanobacteria	Cyanobacteria	Synechococcus	
Otu00010	1.428	61890	16.169	27	Bacteria	Cyanobacteria	Cyanobacteria	Cyanobacteria	Cyanobacteria	Synechococcus	
Otu00013	1.212	50564	12.689	37	Bacteria	Cyanobacteria	Cyanobacteria	Cyanobacteria	Cyanobacteria	Synechococcus	
Otu00015	1.009	41422	12.137	33	Bacteria	Cyanobacteria	Cyanobacteria	Cyanobacteria	Cyanobacteria	Synechococcus	
Otu00019	0.670	36817	15.567	33	Bacteria	Proteobacteria	Gammaproteobacteria	Oceanospirillales	SAR86_clade	NA	
Otu00020	0.771	36579	40.015	49	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodospirillales	Rhodospirillaceae	AEGEAN-169_marine_group	
Otu00021	0.736	35853	11.333	24	Bacteria	Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	NS4_marine_group	
Otu00024	0.733	34027	25.95	49	Bacteria	Proteobacteria	Alphaproteobacteria	SAR11_clade	Surface_1	NA	SAR11Ib
Otu00025	0.743	33995	14.028	20	Bacteria	Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	NS5_marine_group	
Otu00028	0.585	30511	11.652	16	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	Amylibacter	
Otu00029	0.642	30408	24.165	1	Bacteria	Proteobacteria	Gammaproteobacteria	Oceanospirillales	SAR86_clade	NA	
Otu00031	0.641	27760	12.365	46	Bacteria	Proteobacteria	Alphaproteobacteria	SAR11_clade	Surface_1	NA	SAR11Ia
Otu00032	0.580	26799	12.817	24	Bacteria	Cyanobacteria	Cyanobacteria	Cyanobacteria	Cyanobacteria	Synechococcus	
Otu00033	0.658	25997	31.32	34	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodospirillales	Rhodospirillaceae	AEGEAN-169_marine_group	
Otu00034	0.607	25252	17.496	50	Bacteria	Proteobacteria	Alphaproteobacteria	SAR11_clade	Surface_1	NA	SAR11Ib
Otu00036	0.531	24223	32.179	6	Bacteria	Proteobacteria	Gammaproteobacteria	Oceanospirillales	SAR86_clade	NA	
Otu00037	0.579	24047	13.644	50	Bacteria	Proteobacteria	Alphaproteobacteria	SAR11_clade	Surface_1	NA	SAR11Ib
Otu00038	0.530	23735	12.744	1	Bacteria	Proteobacteria	Gammaproteobacteria	Oceanospirillales	SAR86_clade	NA	
Otu00039	0.439	23588	11.855	50	Bacteria	Proteobacteria	Alphaproteobacteria	SAR11_clade	Surface_2	NA	SAR11IIa
Otu00042	0.370	21129	11.026	37	Bacteria	Proteobacteria	Alphaproteobacteria	SAR11_clade	Surface_2	NA	SAR11IIa
Otu00044	0.409	18972	11.564	19	Bacteria	Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	NS5_marine_group	
Otu00046	0.390	18596	21.52	49	Bacteria	Cyanobacteria	Cyanobacteria	Cyanobacteria	Cyanobacteria	Prochlorococcus	
Otu00048	0.369	18276	17.47	10	Bacteria	Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	NS4_marine_group	
Otu00050	0.291	17702	19.425	8	Bacteria	Proteobacteria	Alphaproteobacteria	SAR11_clade	Surface_1	NA	SAR11Ia
Otu00052	0.385	17640	10.395	10	Bacteria	Bacteroidetes	Flavobacteriia	Flavobacteriales	Cryomorphaceae	NA	
Otu00054	0.341	16565	24.641	49	Bacteria	Proteobacteria	Alphaproteobacteria	SAR11_clade	Surface_4	NA	SAR11IV
Otu00056	0.363	15897	10.713	27	Bacteria	Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	NS5_marine_group	
Otu00059	0.389	14598	10.587	21	Bacteria	Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	NS4_marine_group	
Otu00062	0.294	13761	12.802	47	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	Ascidiaceihabitans	
Otu00063	0.255	13666	32.484	6	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	Nautella	
Otu00065	0.238	12921	10.986	26	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	Ascidiaceihabitans	
Otu00066	0.261	12748	21.873	37	Bacteria	Proteobacteria	Alphaproteobacteria	Rickettsiales	SAR116_clade	NA	
Otu00067	0.254	12635	17.183	50	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodospirillales	Rhodospirillaceae	AEGEAN-169_marine_group	

Description of the rhythmic bacteria ASVs (2/3)

OTU number	Percentage of total sequences	Number of sequences	PNmax	Week of maximum occurrence	Kingdom	Phylum/Division	Class	Order	Family	Genus	Blast
Otu00070	0.298	12110	18.9	28	Bacteria	Proteobacteria	Alphaproteobacteria	Rickettsiales	SAR116_clade	Candidatus_Puniceispirillum	
Otu00071	0.239	12103	10.121	47	Bacteria	Proteobacteria	Alphaproteobacteria	SAR11_clade	Surface_2	NA	SAR11IIa
Otu00073	0.230	11609	38.716	5	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	PS1_clade	NA	
Otu00074	0.263	11582	15.055	20	Bacteria	Proteobacteria	Gammaproteobacteria	Cellvibrionales	Halieaceae	OM60(NOR5)_clade	
Otu00076	0.193	11075	14.474	34	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	NA	
Otu00077	0.212	10761	26.004	6	Bacteria	Proteobacteria	Gammaproteobacteria	Oceanospirillales	ZD0405	NA	
Otu00078	0.209	10730	21.404	8	Bacteria	Proteobacteria	Gammaproteobacteria	Oceanospirillales	SAR86_clade	NA	
Otu00083	0.196	9869	29.838	8	Bacteria	Proteobacteria	Gammaproteobacteria	Oceanospirillales	SAR86_clade	NA	
Otu00084	0.188	9667	22.86	49	Bacteria	Proteobacteria	Alphaproteobacteria	SAR11_clade	Surface_1	NA	SAR11IIa
Otu00086	0.201	9431	14.051	41	Bacteria	Proteobacteria	Alphaproteobacteria	SAR11_clade	NA	NA	SAR11IIIa
Otu00089	0.197	9151	12.905	28	Bacteria	Proteobacteria	Alphaproteobacteria	Rickettsiales	SAR116_clade	NA	
Otu00091	0.177	8911	21.949	1	Bacteria	Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	NS2b_marine_group	
Otu00093	0.184	8464	18.773	46	Bacteria	Proteobacteria	Gammaproteobacteria	Oceanospirillales	SAR86_clade	NA	
Otu00095	0.135	7616	22.989	43	Bacteria	Proteobacteria	Gammaproteobacteria	Oceanospirillales	SAR86_clade	NA	
Otu00096	0.183	7609	15.753	46	Bacteria	Proteobacteria	Alphaproteobacteria	SAR11_clade	NA	NA	SAR11IIIa
Otu00103	0.143	7221	25.715	5	Bacteria	Proteobacteria	Gammaproteobacteria	Oceanospirillales	SAR86_clade	NA	
Otu00112	0.133	6827	32.989	50	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodospirillales	Rhodospirillaceae	OM75_clade	
Otu00115	0.091	6671	10.871	8	Bacteria	Proteobacteria	Alphaproteobacteria	SAR11_clade	Surface_1	NA	SAR11Ia
Otu00124	0.127	5986	10.63	12	Bacteria	Cyanobacteria	Cyanobacteria	Cyanobacteria	Cyanobacteria	Synechococcus	
Otu00126	0.120	5893	12.413	21	Bacteria	Proteobacteria	Alphaproteobacteria	Rickettsiales	SAR116_clade	NA	
Otu00138	0.103	4980	14.592	50	Bacteria	Actinobacteria	Acidimicrobiia	Acidimicrobiales	OM1_clade	Candidatus_Actinomarina	
Otu00145	0.101	4754	12.444	43	Bacteria	Proteobacteria	Gammaproteobacteria	KI89A_clade	NA	NA	
Otu00148	0.114	4680	15.138	37	Bacteria	Proteobacteria	Alphaproteobacteria	Rickettsiales	SAR116_clade	NA	
Otu00149	0.090	4638	12.952	47	Bacteria	Proteobacteria	Alphaproteobacteria	Rickettsiales	SAR116_clade	NA	
Otu00155	0.087	4495	13.97	16	Bacteria	Proteobacteria	Alphaproteobacteria	Rickettsiales	SAR116_clade	Candidatus_Puniceispirillum	
Otu00158	0.077	4345	10.305	49	Bacteria	Proteobacteria	Alphaproteobacteria	SAR11_clade	Surface_4	NA	SAR11IV
Otu00164	0.075	4227	13.172	43	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodospirillales	Rhodospirillaceae	AEGEAN-169_marine_group	
Otu00169	0.086	3988	10.65	26	Bacteria	Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	NS5_marine_group	
Otu00173	0.066	3815	17.406	5	Bacteria	Proteobacteria	Gammaproteobacteria	Oceanospirillales	OM182_clade	NA	
Otu00176	0.093	3774	10.754	5	Bacteria	Proteobacteria	Gammaproteobacteria	Oceanospirillales	SAR86_clade	NA	
Otu00177	0.072	3703	14.666	50	Bacteria	Proteobacteria	Alphaproteobacteria	Rickettsiales	Mitochondria	NA	
Otu00184	0.049	3589	10.111	50	Bacteria	Proteobacteria	Alphaproteobacteria	SAR11_clade	Surface_1	Candidatus_Pelagibacter	SAR11Ia
Otu00195	0.074	3321	17.779	5	Bacteria	Proteobacteria	Gammaproteobacteria	Salinisphaerales	Salinisphaeraceae	ZD0417_marine_group	
Otu00198	0.068	3287	11.921	48	Bacteria	Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	NS4_marine_group	
Otu00208	0.045	3016	18.844	8	Bacteria	Proteobacteria	Betaproteobacteria	Methylophilales	Methylophilaceae	OM43_clade	
Otu00210	0.051	2994	10.741	17	Bacteria	Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	Ulvibacter	
Otu00213	0.054	2961	13.903	48	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	NA	

Description of the rhythmic bacteria ASVs (3/3)

OTU number	Percentage of total sequences	Number of sequences	PNmax	Week of maximum occurrence	Kingdom	Phylum/Division	Class	Order	Family	Genus	Blast
Otu00232	0.053	2605	19.099	49	Bacteria	Proteobacteria	Gammaproteobacteria	Oceanospirillales	Oceanospirillaceae	Pseudohongiella	
Otu00244	0.046	2390	12.26	50	Bacteria	Bacteroidetes	Flavobacteriia	Flavobacteriales	Cryomorphaceae	Fluviicola	
Otu00266	0.037	2023	21.501	49	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodospirillales	Rhodospirillaceae	NA	
Otu00281	0.031	1909	11.451	17	Bacteria	Proteobacteria	Gammaproteobacteria	KI89A_clade	NA	NA	
Otu00285	0.033	1876	10.851	8	Bacteria	Proteobacteria	Alphaproteobacteria	SAR11_clade	Surface_1	NA	SAR11Ic
Otu00321	0.036	1614	12.36	1	Bacteria	Actinobacteria	Acidimicrobiia	Acidimicrobiales	Sva0996_marine_group	NA	
Otu00330	0.028	1558	10.049	3	Bacteria	Actinobacteria	Acidimicrobiia	Acidimicrobiales	Sva0996_marine_group	NA	
Otu00363	0.024	1303	14.168	50	Bacteria	Bacteroidetes	Flavobacteriia	Flavobacteriales	NS9_marine_group	NA	
Otu00375	0.021	1217	12.334	49	Bacteria	Proteobacteria	SPOTSOCT00m83	NA	NA	NA	
Otu00422	0.017	1008	13.579	49	Bacteria	Proteobacteria	Alphaproteobacteria	Rickettsiales	SHWN-night2	NA	
Otu00429	0.019	994	10.942	49	Bacteria	Proteobacteria	Alphaproteobacteria	Rickettsiales	S25-593	NA	
Otu00439	0.020	972	13.727	49	Bacteria	Chloroflexi	SAR202_clade	NA	NA	NA	
Otu00444	0.019	964	16.443	8	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	PS1_clade	NA	
Otu00486	0.018	815	12.093	49	Bacteria	Actinobacteria	Acidimicrobiia	Acidimicrobiales	Sva0996_marine_group	NA	
Otu00605	0.009	549	10.256	47	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	OCS116_clade	NA	

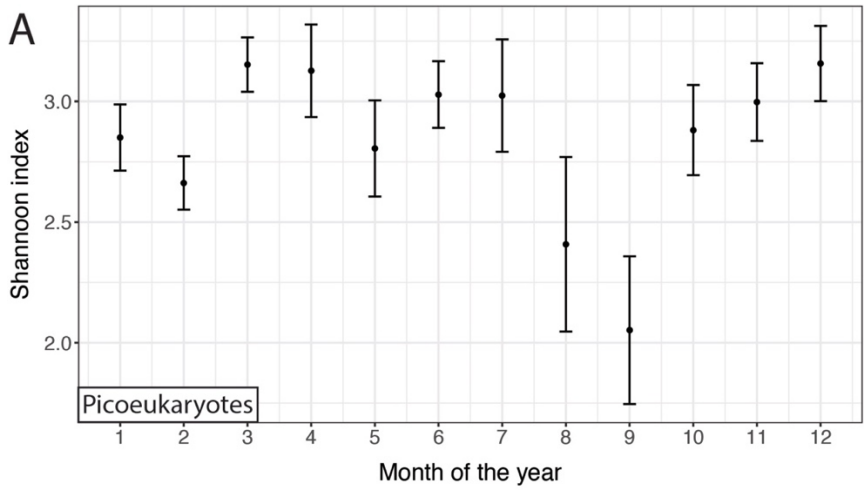
Description of the rhythmic archaea ASVs

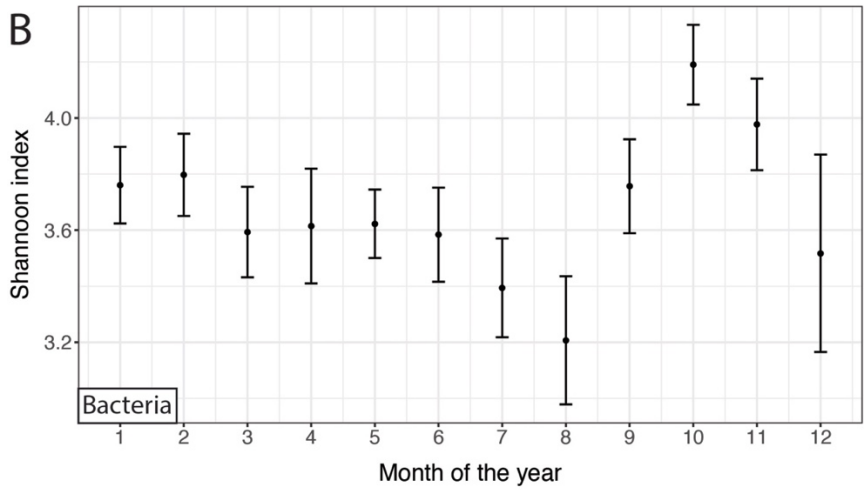
Rhythmic archaea

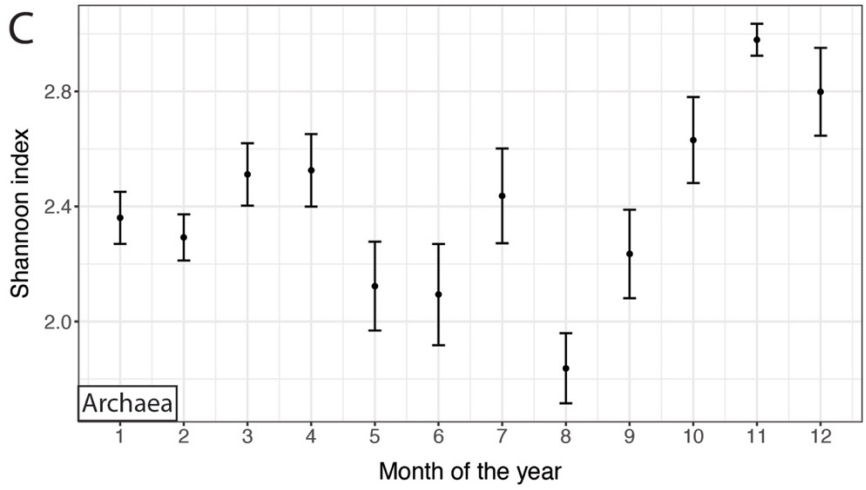
OTU number	Percentage	Number of sequences	PNmax	Week	Kingdom	Phylum/Division	Class	Order	Family	Blast
Otu00001	28.562	735140	22.6	34	Archaea	Thaumarchaeota	Marine_Group_I	Unknown_Order	Unknown_Family	Thaumarchaeota
Otu00002	11.791	276613	14.12	41	Archaea	Euryarchaeota	Thermoplasmata	Thermoplasmatales	Marine_Group_II	Marine_Group_II
Otu00004	4.806	124623	24.337	50	Archaea	Thaumarchaeota	Marine_Group_I	Unknown_Order	Unknown_Family	Thaumarchaeota
Otu00005	4.386	109298	16.304	23	Archaea	Thaumarchaeota	Marine_Group_I	Unknown_Order	Unknown_Family	Thaumarchaeota
Otu00006	5.160	100516	14.603	34	Archaea	Euryarchaeota	Thermoplasmata	Thermoplasmatales	Marine_Group_II	Marine_Group_II
Otu00007	3.499	83497	19.406	33	Archaea	Thaumarchaeota	Marine_Group_I	Unknown_Order	Unknown_Family	Thaumarchaeota
Otu00008	1.998	57863	18.824	6	Archaea	Euryarchaeota	Thermoplasmata	Thermoplasmatales	Marine_Group_II	Marine_Group_II
Otu00009	2.103	53542	12.871	41	Archaea	Euryarchaeota	Thermoplasmata	Thermoplasmatales	Marine_Group_II	Marine_Group_II
Otu00011	1.612	40958	16.212	50	Archaea	Thaumarchaeota	Marine_Group_I	Unknown_Order	Unknown_Family	Thaumarchaeota
Otu00012	1.327	34773	17.895	47	Archaea	Euryarchaeota	Thermoplasmata	Thermoplasmatales	Marine_Group_III	Marine_Group_III
Otu00013	1.252	33341	16.757	41	Archaea	Euryarchaeota	Thermoplasmata	Thermoplasmatales	Marine_Group_II	Marine_Group_II
Otu00014	1.149	33062	26.612	8	Archaea	Thaumarchaeota	Marine_Group_I	Unknown_Order	Unknown_Family	Thaumarchaeota
Otu00015	1.177	30593	26.118	26	Archaea	Thaumarchaeota	Marine_Group_I	Unknown_Order	Unknown_Family	Thaumarchaeota
Otu00016	0.987	26164	22.972	48	Archaea	Thaumarchaeota	Marine_Group_I	Unknown_Order	Unknown_Family	Thaumarchaeota
Otu00018	0.665	20681	16.385	48	Archaea	Thaumarchaeota	Marine_Group_I	Unknown_Order	Unknown_Family	Thaumarchaeota
Otu00019	0.865	19397	16.91	45	Archaea	Euryarchaeota	Thermoplasmata	Thermoplasmatales	Marine_Group_II	Marine_Group_II
Otu00022	0.524	15080	11.405	41	Archaea	Euryarchaeota	Thermoplasmata	Thermoplasmatales	Marine_Group_II	Marine_Group_II
Otu00024	0.633	14492	10.068	45	Archaea	Euryarchaeota	Thermoplasmata	Thermoplasmatales	Marine_Group_II	Marine_Group_II
Otu00025	0.550	14093	13.26	46	Archaea	Euryarchaeota	Thermoplasmata	Thermoplasmatales	Marine_Group_II	Marine_Group_II
Otu00028	0.410	11827	18.351	49	Archaea	Euryarchaeota	Thermoplasmata	Thermoplasmatales	Marine_Group_III	Marine_Group_III
Otu00029	0.407	11689	20.889	8	Archaea	Thaumarchaeota	Marine_Group_I	NA	NA	Thaumarchaeota
Otu00031	0.402	11330	15.611	48	Archaea	Euryarchaeota	Thermoplasmata	Thermoplasmatales	Marine_Group_II	Marine_Group_II
Otu00035	0.227	8114	10.73	43	Archaea	Euryarchaeota	Thermoplasmata	Thermoplasmatales	Marine_Group_II	Marine_Group_II
Otu00044	0.227	6282	15.13	3	Archaea	Euryarchaeota	Thermoplasmata	Thermoplasmatales	Marine_Group_II	Marine_Group_II
Otu00049	0.162	4870	10.255	48	Archaea	Thaumarchaeota	Marine_Group_I	Unknown_Order	Unknown_Family	Thaumarchaeota
Otu00050	0.141	4691	14.405	6	Archaea	Euryarchaeota	Thermoplasmata	Thermoplasmatales	Marine_Group_II	Marine_Group_II
Otu00057	0.119	3428	12.436	48	Archaea	Euryarchaeota	Thermoplasmata	Thermoplasmatales	Marine_Group_II	Marine_Group_II
Otu00058	0.119	3406	12.32	48	Archaea	Euryarchaeota	Thermoplasmata	Thermoplasmatales	Marine_Group_II	Marine_Group_II
Otu00066	0.105	2931	13.351	49	Archaea	Euryarchaeota	Thermoplasmata	Thermoplasmatales	Marine_Group_II	Marine_Group_II
Otu00069	0.074	2820	10.245	43	Archaea	Thaumarchaeota	Marine_Group_I	Unknown_Order	Unknown_Family	Thaumarchaeota
Otu00071	0.115	2643	10.542	36	Archaea	Euryarchaeota	Thermoplasmata	Thermoplasmatales	Marine_Group_II	Marine_Group_II

Supplementary Fig 1:

Average Shannon index, with the standard error, per month for picoeukaryotes (A), bacteria(B), archaea (C).

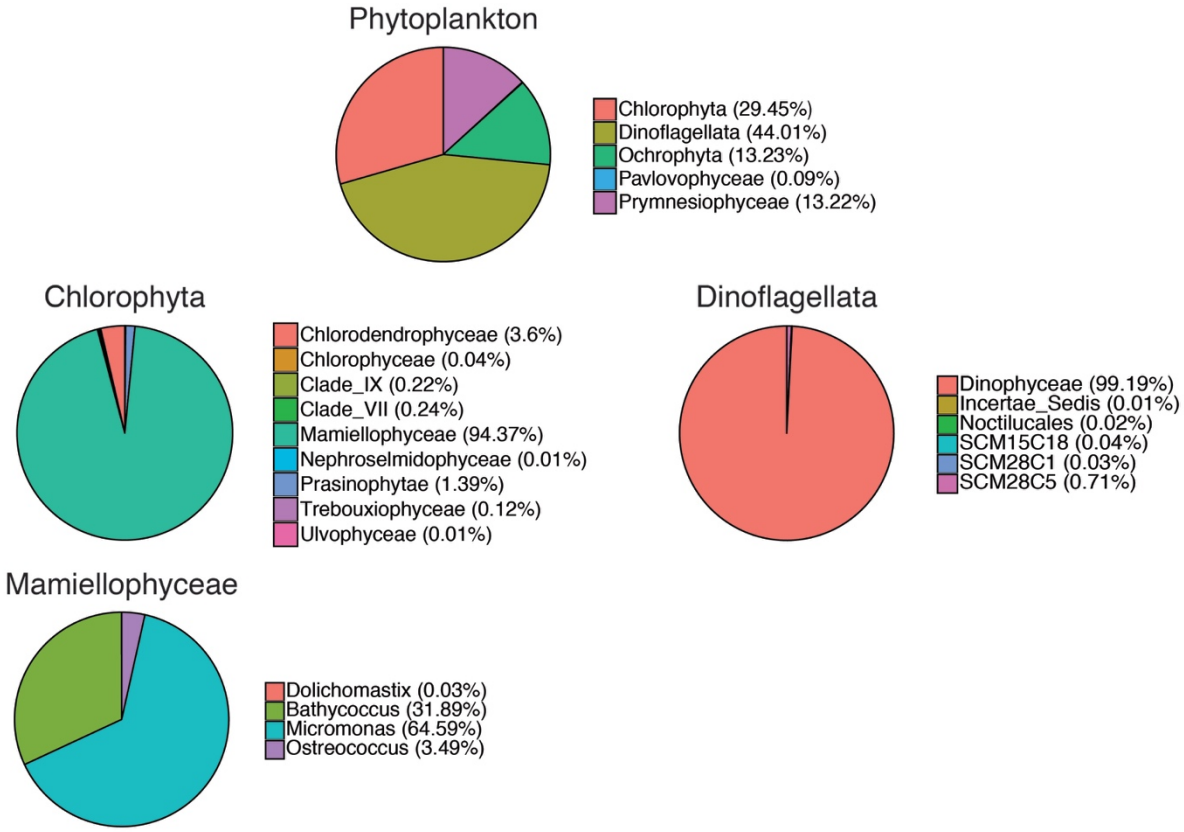






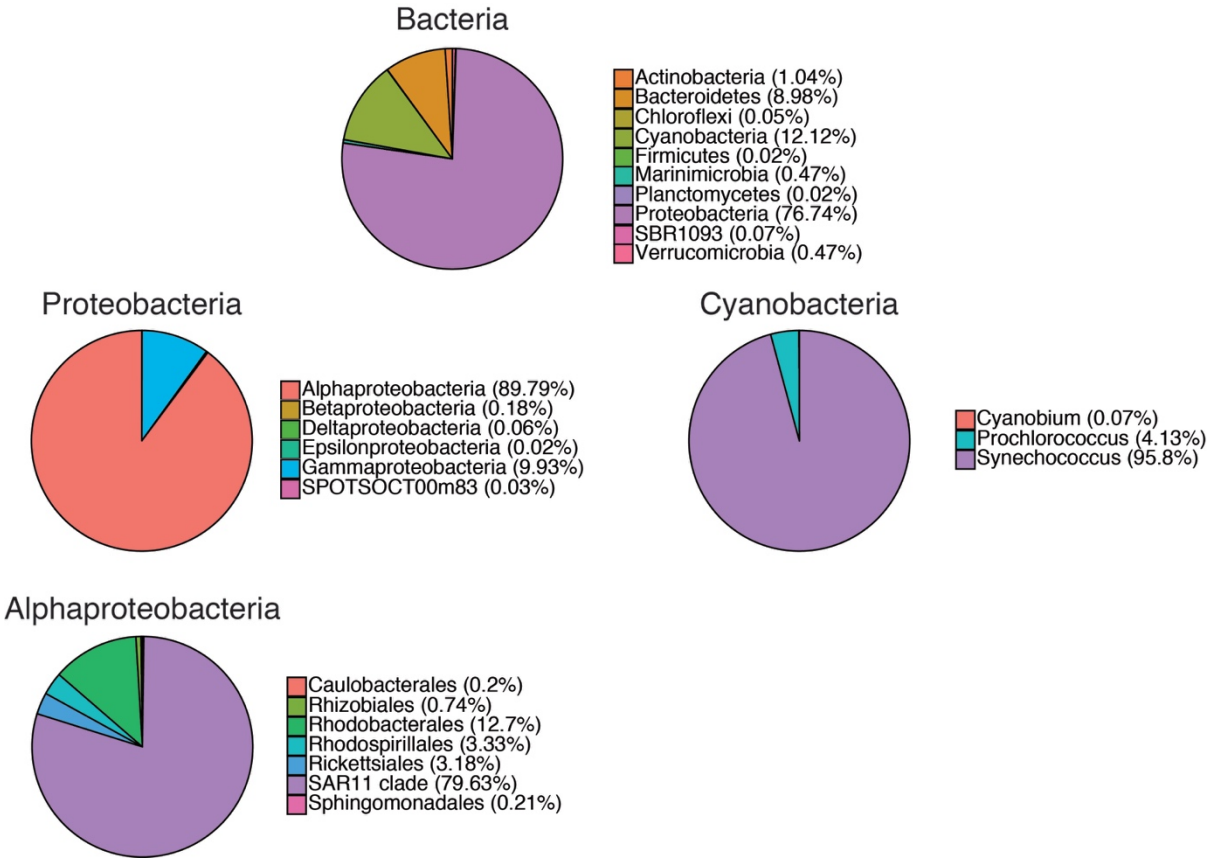
Supplementary Fig 2:

Overall proportion of phytoplankton divisions and classes at the SOLA station in the Banyuls Bay from 2007 to 2015.



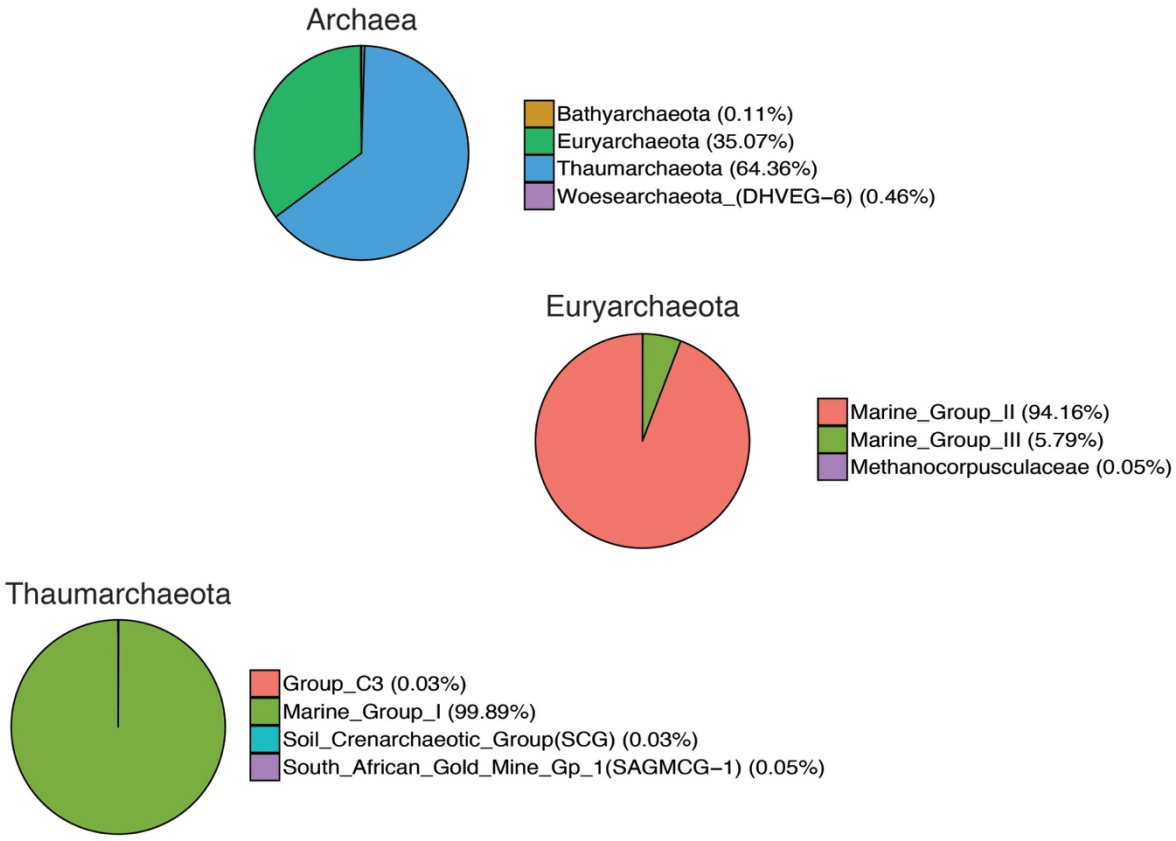
Supplementary Fig 3:

Overall proportion of bacteria phylums and classes at the SOLA station in the Banyuls Bay from 2007 to 2015.



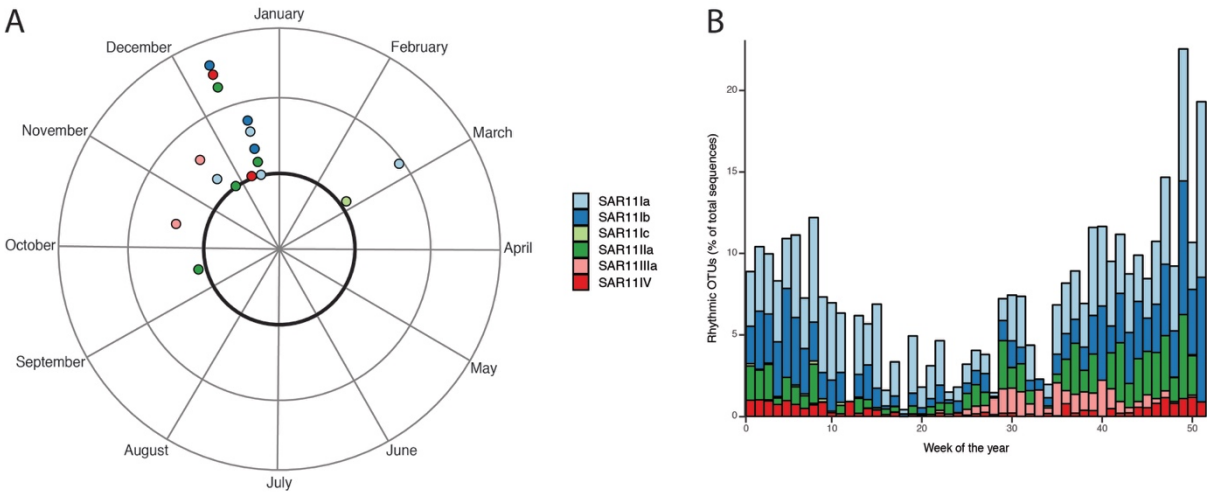
Supplementary Fig 4:

Overall proportion of archaea phylums and classes at the SOLA station in the Banyuls Bay from 2007 to 2015.



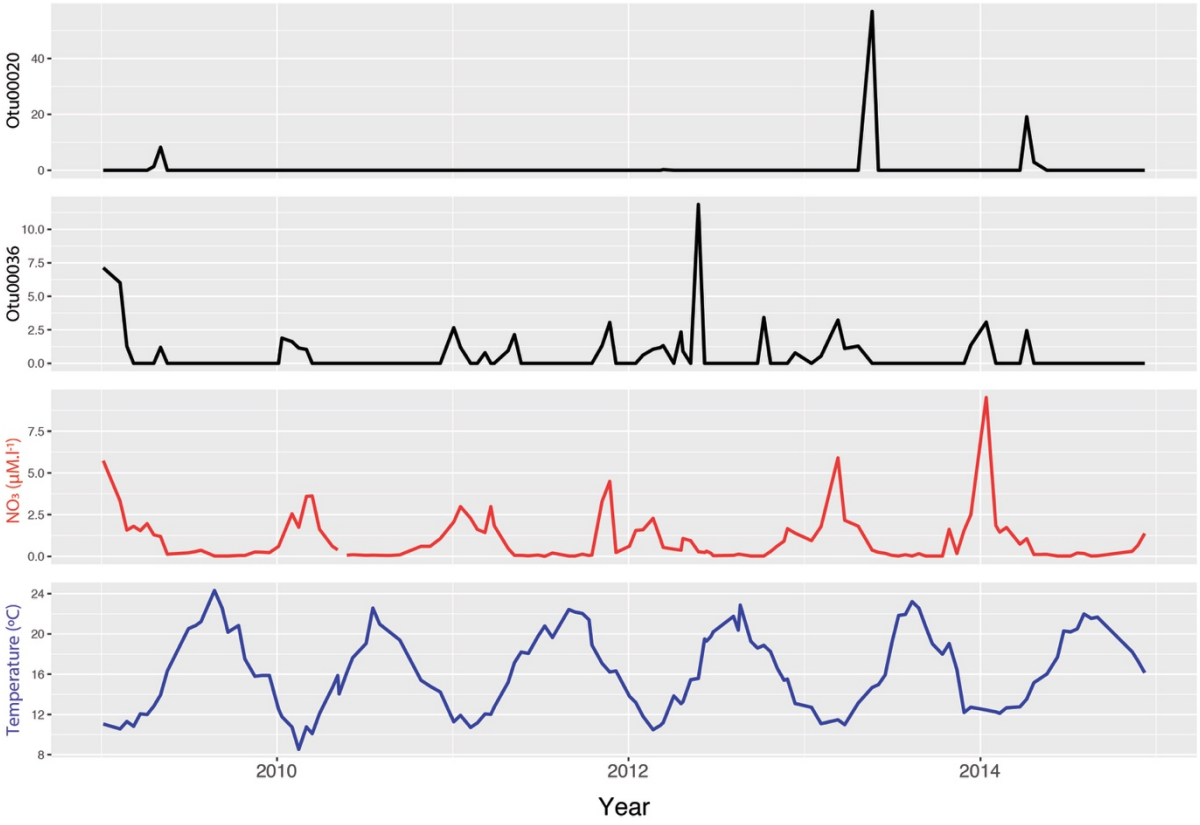
Supplementary Fig 5:

Polar plots representing the rhythmic SAR11 OTUs (A). The bar plots show the proportion of sequences belonging to rhythmic SAR11 OTUs averaged per week of the year (B).



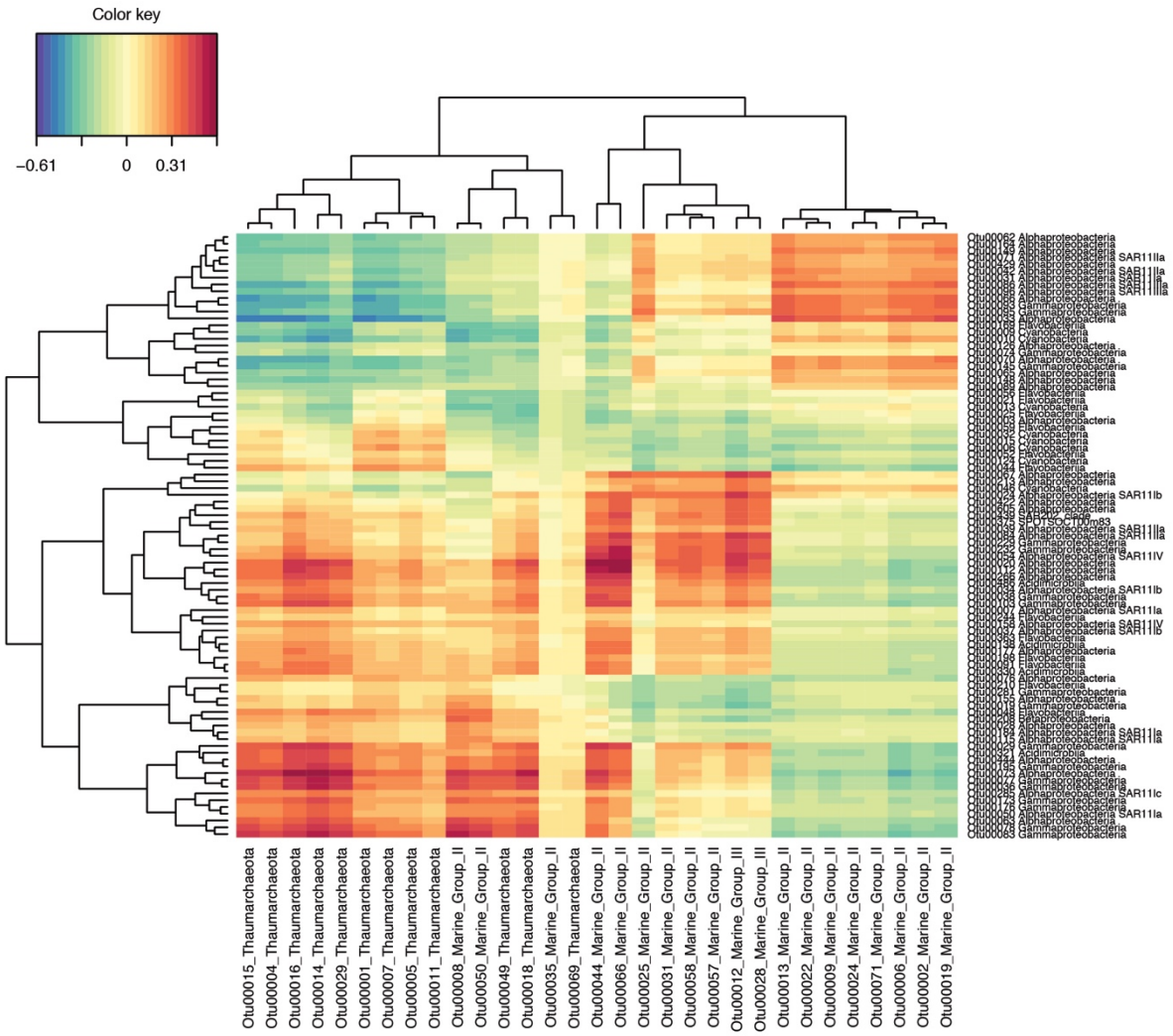
Supplementary Fig 6:

Temperature, nitrate (NO_3) and OTU abundance (percentage of total reads) of OTU00020 and OTU00036 from 2009 to 2014 at the SOLA sampling point in the Banyuls Bay.



Supplementary Fig 7:

Heatmap, based on a sPLS function that relate correlations between abundance matrixes, showing co-occurrences between rhythmic bacteria OTUs and archaea OTUs.



Addendum

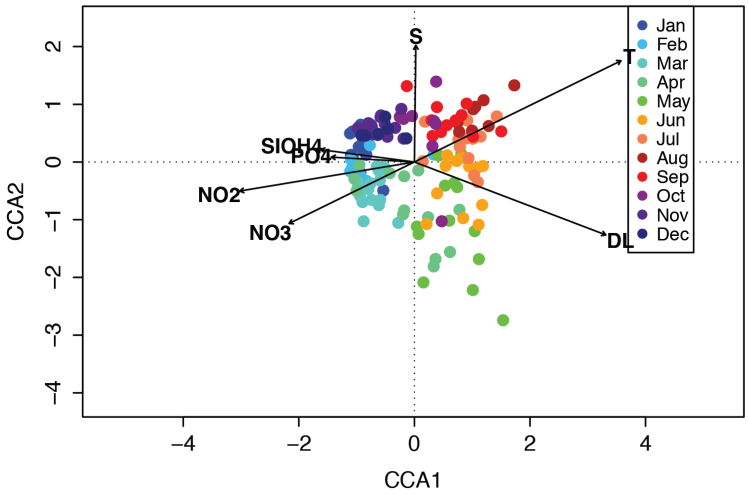
My contribution:

For this chapter I was in charge of the sequence analysis (firstly done with mothur, but redone with DADA2 because it was better suited to our needs), the management of the time series data (gathering and putting together the biological and environmental data), the exploitation of the time series data (finding and using the Lomb Scargle periodigram to investigate the rhythmicity of ASVs in an unenvenly sampled time series), setting up the figures and supplementary data and the writing of the article with the co-authors.
The seawater sampling and DNA extractions were done before I started my PhD by people from the observatory. The PCRs and sequencing was done by a private company. The environmental data was made available thanks to the SOMLIT.

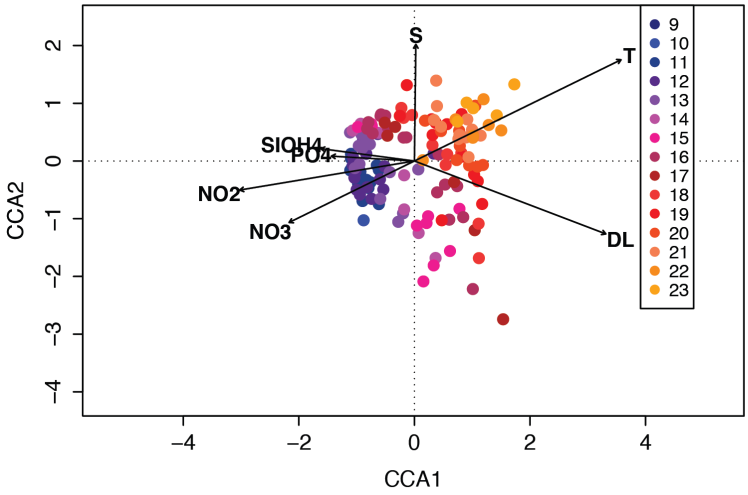
Corrections to the chapter:

Additionally, the reviewers suggested to look at different factors that could explain the seasonality. Here the CCAs were plotted but with a color coding corresponding to the sample's month of sampling, sea surface temperature or nitrate levels.
In the following figures we can see a gradient both in temperature and nutrients for the three data sets. The variations in temperature and nitrate follow the direction of their corresponding arrow. Indeed, temperature increases during the summer months which also correspond to low nutrient levels. During winter it is the opposite, we find high nutrient levels and low temperatures. However, certain communities found in the original CCA showed peculiar distributions. For example, in the eukaryotic phytoplankton data there is a point that was sampled in January (blue) but that groups with samples from March (turquoise). However, temperature and nutrient levels can explain why these communities group together since they have similar temperature as well as high nutrient values (the highest levels of the data set).
Another example is the October sample (purple) that groups with samples from the month of May (green). Unfortunately, temperature and nitrate levels are quite different between these samples (19°C vs 16°C and 1.5µmol l^{-1} vs 0µmol l^{-1} for October vs May, respectively).

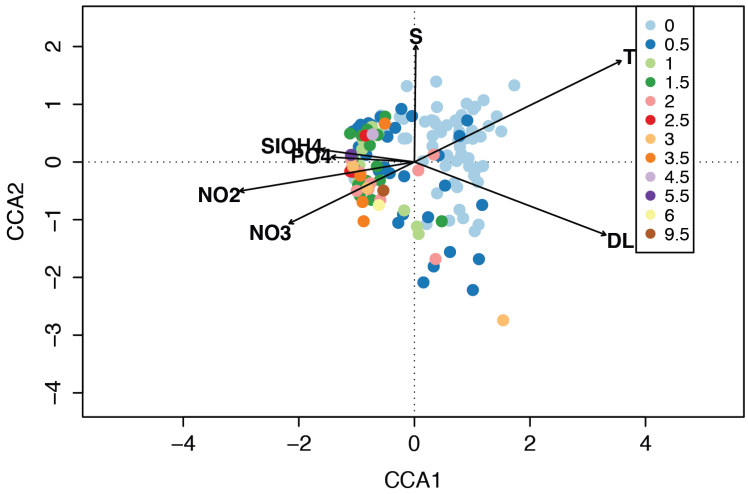
Other factors such as salinity or phosphate levels could help explain the distribution of this point.



Month

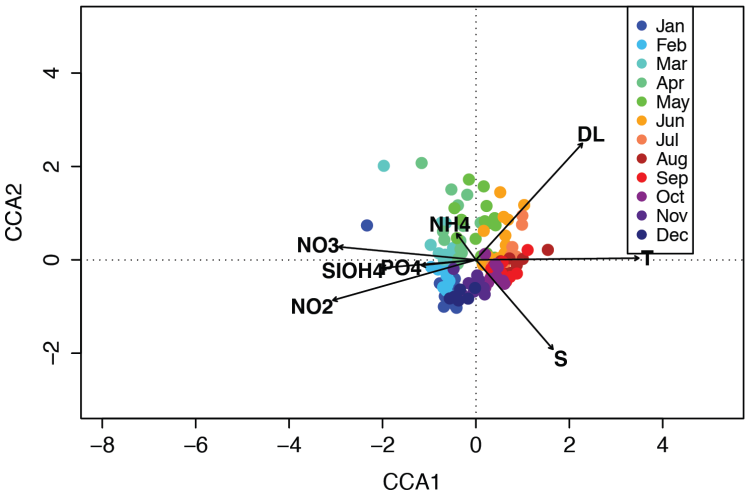


Temperature (°C)

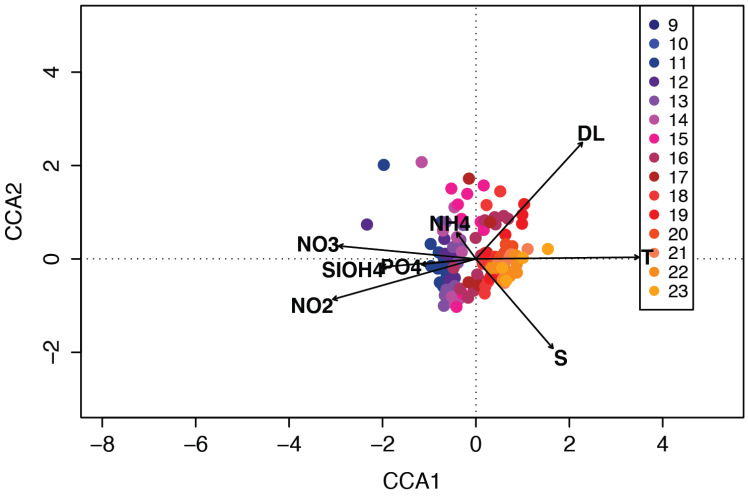


Nitrate (µmol l^{-1})

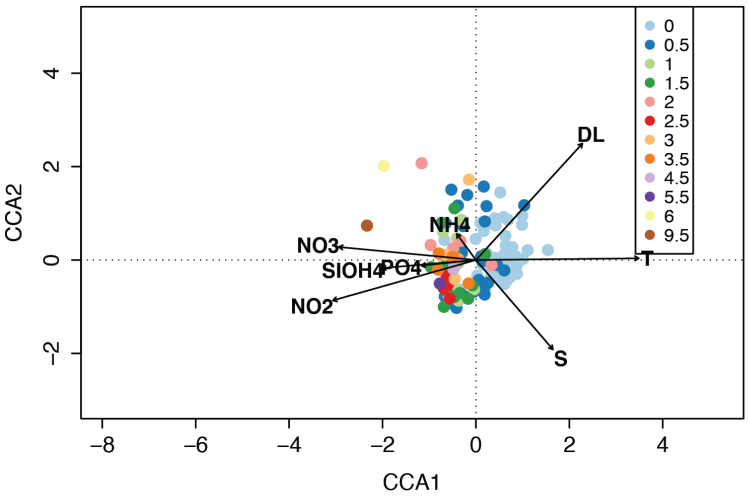
CCA of eukaryotic phytoplankton communities showing the samples month, temperature or nitrate levels



Month

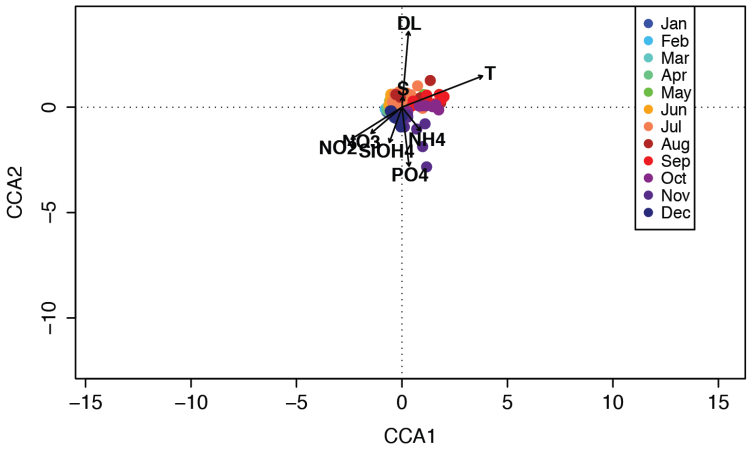


Temperature (°C)

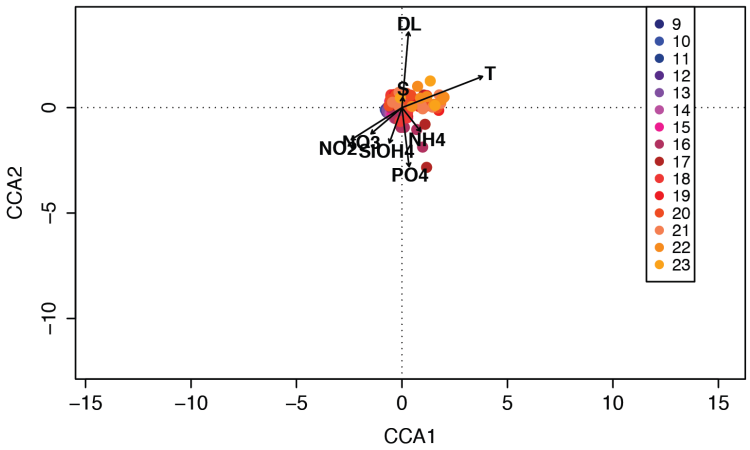


Nitrate (µmol l^{-1})

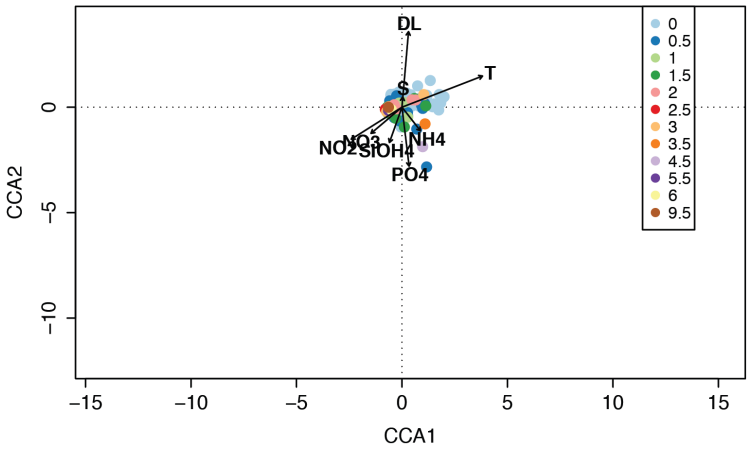
CCA of bacterial communities showing the samples month, temperature or nitrate levels



Month



Temperature (°C)



Nitrate (µmol l^{-1})

CCA of archaeal communities showing the samples month, temperature or nitrate levels

Chapter 3

Switching neighbors in environmentally challenged coastal marine microbes

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3.1 <3µm time series data

Prologue

Microbial interactions could have a high impact on community structure and dynamics. To grasp a better understanding of microbial interactions in the Bay of Banyuls, we increased the sampling frequency to twice a week. This was performed for three years, during the most productive months (January to March) to focus on the phytoplankton bloom period. Coastal environments are submitted to irregular freshwater influxes from nearby rivers and other meteorological events, which can amplify the impact of environmental factors on marine microbe interactions. We showed that salinity and nutrient concentrations at the SOLA sampling station were influenced by these freshwater influxes, of which the origin remains unclear. Bray Curtis dissimilarity analyses and principal coordinate analyses showed that community composition and abundance were mainly impacted by salinity and to a lesser extent by temperature. The Maximal Information Coefficient (MIC) correlation statistic was used to determine co-occurrences between individual ASVs, rendered as a network with Cytoscape. According to network analysis, salinity and temperature impacted community structure. Subnetworks analyses revealed that dominant ASVs, present throughout the three-year time series, switched their first neighbors depending on the environmental perturbations they faced. In addition, eukaryotes co-occurred preferentially with eukaryotes, and prokaryotes with prokaryotes. Overall, our study highlighted that increasing the sampling frequency allows for an improved understanding of microbial community dynamics, while long term lower resolution time series (*i.e.* Chapter I) help build a baseline that give appropriate context to future measurements. Time series will be, if they are not already, powerful tools to help monitor the impact of climate change on microbial communities.

This manuscript is currently in preparation.

Switching neighbors in environmentally challenged coastal marine microbes

Stefan Lambert[a], Jean-Claude Lozano[a], François-Yves Bouget[a], and Pierre E. Galand[b]

[a]CNRS, Laboratoire d'Océanographie Microbienne (LOMIC), Observatoire Océanologique de Banyuls, Sorbonne Université, Banyuls sur Mer, Paris, France; [b]CNRS, Laboratoire d'Ecogéochimie des Environnements Benthiques (LECOB), Observatoire Océanologique de Banyuls, Sorbonne Université, Banyuls sur Mer, Paris, France

Coastal ecosystems, that are subject to seasonal phytoplanktonic blooms and stochastic freshwater influxes from nearby rivers, magnify the impact of environmental factors on marine microbe interactions. Sampling twice a week, for three years, during the most productive months (January – March) allowed us to show that freshwater influxes strongly impacted salinity and nutrient concentrations at the SOLA sampling station and that community composition and abundance were impacted by environmental stresses. Cluster analysis of yearly networks showed that low salinity conditions could have influenced network structure. Despite these environmental perturbations, subnetworks showed persistent amplicon sequence variants that could switch their first neighbors when faced with different environmental challenges. Furthermore, eukaryotes showed a preferred co-occurrence with eukaryotes, whereas prokaryotes preferred to co-occur with prokaryotes. Long term, high resolution, sampling stations are critical in order to characterize marine microbial interactions. Implementing and maintaining time series, since they allow distinction between seasonal events and novel impacts to community dynamics, will improve the study of anthropological perturbations.

Keywords: Coastal time Series | High resolution | Microbial co-occurrences

Introduction

Microbial communities are complex systems of co-occurring species (1–5) formed by different types of relationships defined as, for example, parasitism, mutualism or predator-prey interactions (6, 7). Networks analysis have been used to study these interactions (5, 8–11) and have demonstrated the impact of environmental conditions (10), or hydrological factors (12) on community structures. The networks have also revealed specific ecological niches for bacterioplankton (9, 13) and time-lagged interactions (4, 5) detected with local similarity analysis (14).

Community compositions are shaped and impacted by changes in environmental conditions. These variations can be reoccurring yearly, such as seasonalsa temperature changes (11), day length transitions (10, 15), phytoplankton blooms forming during the winter to spring transition (16, 17) or seasonal mixing of the water column (11, 18). Furthermore, in temperate coastal ecosystems, microbial community composition depends on sudden and often dramatic events such as the influx of freshwater from land, sediment resuspension during storms (12), or short timescale events such as wind direction and precipitation (19). As the impact of environmental changes is increasing, exacerbated by anthropological pressure, the question remains whether communities, and their interactions, could remain stable over time, or if the predicted increases in temperature and precipitation could disrupt individual interactions within communities (20, 21).

Long term sampling stations have helped elucidate links between environment and microbial communities. They have shown the temporal reoccurrences of microbial communities (6, 22, 23) and allowed the discovery of major novel marine groups (24). Time series analyses are helping us understand and decipher yearly biological events and determine the prevalence of these events in different ecosystems (25). However, most long term study sites demonstrate a monthly sampling period (10, 13, 26) and inherently miss any event that lasted less than a month. It has been shown that weekly and even daily samplings are needed to observe short time scale events, such as species successions and associations during bloom periods (4, 27–29). Furthermore, multiyear time series studies are important for discriminating microbial community dynamics from stochastic events (16). With the recent access to cheap sequencing technology, and the development of powerful tools to

analyze the data produced, there have been multiple enhancements to time series analyses. There has been a shift from broad taxonomic resolution (10) to amplicon sequence variants (ASVs) (30), and a transition from single domain studies (13, 22) to studies focusing on multiple domains (3, 4, 31).

We have previously observed in a coastal site of North western Mediterranean Sea that about a third of picoeukaryotes and bacteria and 70% of archaea ASVs sequences displayed robust annual rhythms despite irregular environmental conditions (23). The aim of this study was to investigate at the same sampling point (SOLA, bay of Banyuls), with a higher sampling frequency, the effect of environmental perturbations on the microbial community composition and microbe-microbe co-occurrences. We sampled a coastal site (SOLA) weekly for 3 years, and increased the sampling frequency to twice a week during the most productive winter months (January-March). Sampling included an "average" year (2015) in terms of physical and chemical parameter seasonal dynamics (23) and two atypical years, 2016 and 2017, marked by strong environmental perturbations in terms of temperature and freshwater influx, respectively. We investigated the effect of these disturbances on both eukaryotes and prokaryotes diversity, by amplifying 18S and 16S rRNA genes respectively, and we used ASVs to resolve taxa.

Materials and Methods

Sampling. Surface water (3m) was collected from January 2015 to March 2017 at the Service d'Observation du Laboratoire Arago (SOLA) sampling station (42°31'N, 03°11'E) in the Bay of Banyuls, North Western Mediterranean Sea, France. Samples were collected twice a week during the periods of January – March 2015, January – April 2016 and December 2016 – March 2017 and roughly once a week otherwise. Niskin bottles were used to obtain seawater that was stored in 10 L carboys until arrival to the laboratory. 5 L of seawater were prefiltered through 3 μm pore-size polycarbonate filters (Merck-Millipore, Darmstadt, Germany), and the microbial biomass was collected on 0.22-μm pore-size GV Sterivex cartridges (Merck-Millipore) and stored at –80 °C until nucleic acid extraction. The physicochemical (temperature, salinity, nitrate) and biological (chlorophyll a) parameters were provided by the Service d'Observation en Milieu Littoral (SOMLIT). The levels of the nearby river, the Baillaury, were obtained online from the "Service Central d'Hydrométéorologie et d'Appui à la

Prévision des Inondations" (http://www.hydro.eaufrance.fr/)

DNA extraction, amplification and sequencing. The nucleic acid extraction followed protocols published earlier (23). To summarize, the sterivex filters were thawed on ice, followed by addition of lysis buffer (40nM EDTA, 50nM Tris, 0.75M sucrose) and 25 μL of lysozyme (20 mg mL^{-1}). The filters were then incubated for 45 minutes at 37°C on a rotary mixer. Subsequently, 8μL of Proteinase K (20mg mL^{-1}) and 26μL of sodium dodecyl sulfate (20% v/v) were added before incubating for 1 hour at 55°C. Total DNA extracted and purified with the Qiagen AllPrep kit (Qiagen, Hilden, Germany) following the kit's protocol.

Specific primers were used to target either the eukaryotic V4 region (TAReuk_F1 [5'-CCAGCASCYGCGGTAATTCC] and TAReuk_R [5'-ACTTTCGTTCTTGATYRATGA], (32)) or the prokaryotic V4-V5 region (515F-Y [5'-GTGYCAGCMGCCGCGGTAA] and 926R [5'-CCGYCAATTYMTTTRAGTTT], (33)). Sequencing was carried out by the Genotoul plateform (Toulouse, France), with the Illumina Miseq 2x250 bp kits.

Sequence analysis and preprocessing. The standard pipeline of the DADA2 package (https://benjjneb.github.io/dada2/index.html, version 1.6) in "R" (https://cran.r-project.org) was used to do the analysis of the raw sequences. The parameters used for the eukaryote dataset were: trimLeft=c(20, 21) ,truncLen=c(250,250), maxN=0, maxEE=c(2,2), truncQ=2. And for the prokaryote dataset: trimLeft=c(19, 20), truncLen=c(240,200), maxN=0, maxEE=c(2,5), truncQ=2. The prokaryote sequences were of slightly lower quality, which explains the shorter cutoff and the higher expected error parameter. We analyzed 141 and 142 samples for the eukaryote and prokaryote datasets respectively and obtained 3.8 and 3.4 million total reads respectively, which is an average of ca. 27000 and 24000 reads per sample respectively (Supplementary Table 1). The taxonomy assignments were done with PR2 v.4.10.0 database (https://github.com/vaulot/pr2database/releases) for the eukaryote dataset and with SILVA v.128 database (https://www.arb-silva.de/documentation/release128/) for the prokaryote dataset. The "assignTaxonomy" function in DADA2 implements the RDP naive Bayesian classifier method described in Wang et al (34).

Taxa belonging to the supergroup "Opisthokonta" were removed from the eukaryote dataset.

Comparably, taxa belonging to eukaryotes were removed from the prokaryote dataset. Samples containing less than 5000 reads and 9000 reads were removed from the eukaryote and prokaryote dataset respectively. A total of 139 and 137 samples remained for the eukaryotes and the prokaryotes respectively. These preprocessing steps were done with the "R" package "Phyloseq" (https://joey711.github.io/phyloseq/). Sequence counts for both datasets were normalized with the "DESeq2" package (https://github.com/mikelove/DESeq2).

Statistics. The Bray-Curtis (BC) dissimilarity index was calculated between community composition of two successive samples (t vs t_{+1}) within each dataset with the "vegdist" function of the "Vegan" package in "R" (https://cran.r-project.org/web/packages/vegan/). Similarity between samples based on Bray Curtis similarity was visualized in a principal coordinate analysis (PCoA) for each dataset with the "Phyloseq" package in "R".

The Maximal Information Coefficient (MIC), (35) was computed for the 20 most abundant ASVs per sample and per year. The output of the MIC was then used to build a network in Cytoscape (36). The full network was pruned to visualize ASV interactions that had a Pearson linear regression > 0 and a MIC > 0.75 (Selected ASVs found in Supplementary Table 2). The layout chosen for the network was edge-weighted spring embedded, using the MIC parameter. Each ASV is represented by a node in the network, which size is proportional to the abundance of the ASV that year. The sample of maximum abundance per ASV was determined, and the corresponding environmental parameters were used for the subsequent color codding of the network. Cluster analyses were done with the CytoCluster app (37) for Cytoscape, using the HC-PIN clustering algorithm with the default parameters (Weak, Threshold: 2.0 and ComplexSize Threshold: 3) (Results in Supplementary Table 3). Network analysis was done using the NetworkAnalyzer tool included in Cytoscape (Results in Supplementary Table 4). The networks were treated as undirected.

The Venn diagram was made using an online tool (http://bioinformatics.psb.ugent.be/webtools/Venn/) and applying it to the ASVs pruned from the main network (Supplementary Table 2).

Radar plots were made with the "fmsb" package (https://cran.r-project.org/web/packages/fmsb) in "R".

Results

Environmental conditions. *Chlorophyll a*, temperature, salinity and nitrate were measured at the sampling point SOLA (Fig. 1). When focusing on the most productive winter months, average *chlorophyll a* concentrations varied from 0.58 μg L^{-1} to 1.37 μg L^{-1}. In 2014-2015, *chlorophyll a* concentrations were under average during the whole winter, except during the month of March, which was above average. During 2015-2016, *chlorophyll a* levels stayed rather close to the average. There were two increases, one at the end of December and ended mid-January and the second during the month of April. Comparably, 2016-2017 *chlorophyll a* concentrations were close to average most of the year, except for two major increases, during the month of December 2016 (2.11 μg L^{-1}) and February 2017 (3.74 μg L^{-1}).

The average temperature over 8 years varied from 11.4°C to 15.1°C during the period of December to April with lowest values at the end of February. Temperature in winter 2014-2015 was warmer than average in December (+1.65°C) and January (+0.59°C). The following months were close to average. Water temperature in winter 2015-2016 remained, on average, warmer in January (+1.25°C) and February (+1.52°C). Minimum values were observed at the beginning of March, when it finally returned closer to the 8-year average. Winter 2016-2017 showed a different pattern by staying close to the average temperature from December to February, and then 1°C warmer than average in March and April.

The average salinity over 8 years varied from 37.38 psu to 37.84 psu during the winter period. Winter 2014-2015 showed salinity close to average values with the only exception at the end of March (36.44 psu). Salinity in 2015-2016 remained above average throughout most of the winter. The winter of 2016-2017 displayed two marked decreases in salinity, in December 2016 (35.54 psu) and February 2017 (35.30 psu). Those decreases lasted almost the full months. On the other hand, the salinities of January and March were slightly above average, and April was close to the average.

Nitrate followed similar patterns as salinity. Average nitrate varied from 0.72 μmol L^{-1} to 2.68 μmol L^{-1}. Nitrate concentrations in 2014-2015 and 2015-2016 remained under average most of the year. However, 2016-2017 had two major increases in nitrate levels during the months of December

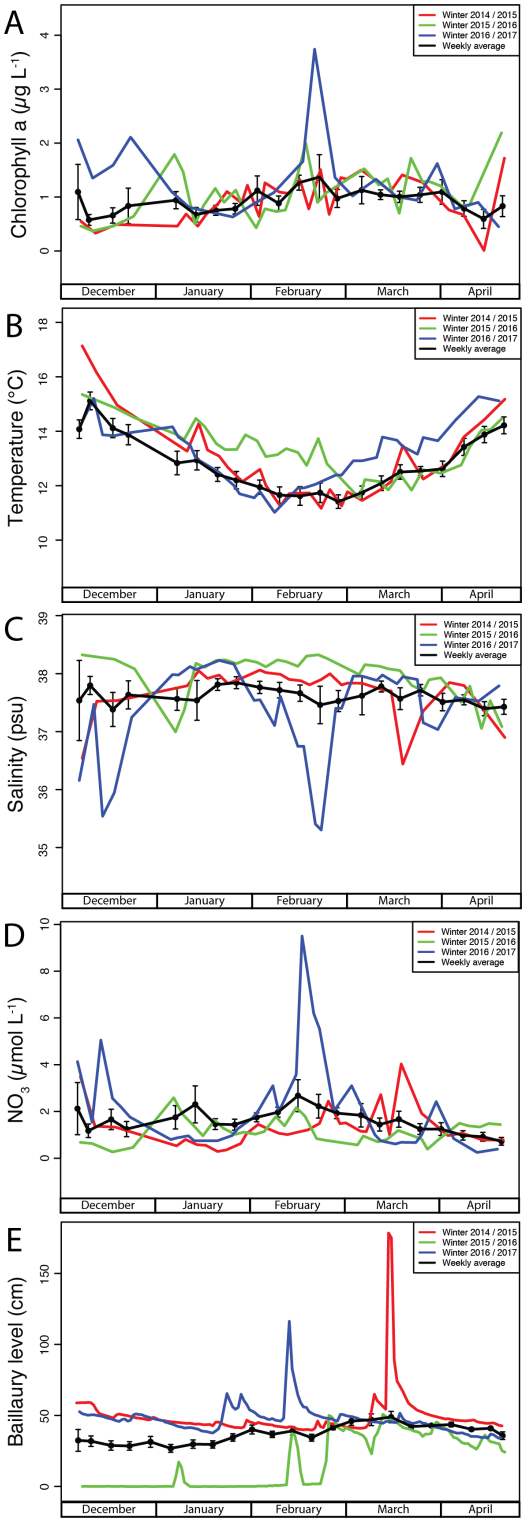

Fig. 1. : *Chlorophyll a*, temperature, salinity, nitrate (NO_3), and the level of the nearby river (The Baillaury) from December to April at the SOLA station in the Bay of Banyuls. The black line depicts the average value (with s.e.) from 2007 to 2015 (2008 to 2015 for the Baillaury level). The red, green and blue line show the values for the winter of 2014/2015, 2015/2016 and 2016/2017, respectively.

(5.05 μmol L^{-1}) and February (9.50 μmol L^{-1}).

On average the water levels of the nearby river, the Baillaury, varied from 26.8 cm to 48.8 cm between 2008 to 2018. (Fig. 1E). The height of the Baillaury remained fairly stable throughout the 2014-2015 winter, apart from the month of March that displayed a very important increase (178.35 cm). The Baillaury was mostly dry from December 2015 to February 2016. It then had a sudden increase at the end of February. The levels thereafter were close to the average. In 2017, the height of the Baillaury had two increases, one during January (64.87 cm) and another mid-February (116.23 cm).

Pairwise changes in community composition. To compare the community composition of successive samples (i.e. t sample vs t_{+1} sample), the Bray-Curtis (BC) dissimilarity indices were calculated for the periods of January – March 2015, January – April 2016 and December 2016 – March 2017. Salinity corresponding to the t_{+1} samples were plotted with the BC scores for eukaryotes (Fig. 2A) and prokaryotes (Fig. 2B). Regardless of the dataset, high BC scores between successive samples, which indicate changes in community composition, were observed when salinity decreased. During the sharp decrease of salinity at the end of March 2015, the BC scores increased. This was more visible in the prokaryote dataset than in the eukaryote dataset. The progressive decrease in salinity from March to April 2016 was mirrored by the high BC scores at the same moment in both datasets. Both major decreases in salinity in December 2016 and February 2017 were echoed in the BC scores for the same samples in both data sets as well. Even during the early onset of the decrease in salinity (beginning of February) there was an increase of the BC score of the corresponding sample.

Similarly, temperature, corresponding to the t_{+1} sample, was plotted with BC dissimilarity index between succeeding samples for the eukaryote (Fig. 3A) and the prokaryote (Fig. 3B) data sets. Variations in temperature did not fit the variations of BC scores as well as salinity.

Seasonal community composition. Principal coordinates analysis (PCoA) were carried out on both the eukaryote and prokaryote datasets so as to compare the composition of communities during the sampling period (Fig. 4). Communities grouped generally by month of sampling, but March communities were more variable, regardless of the year. In addition, December 2016 and February

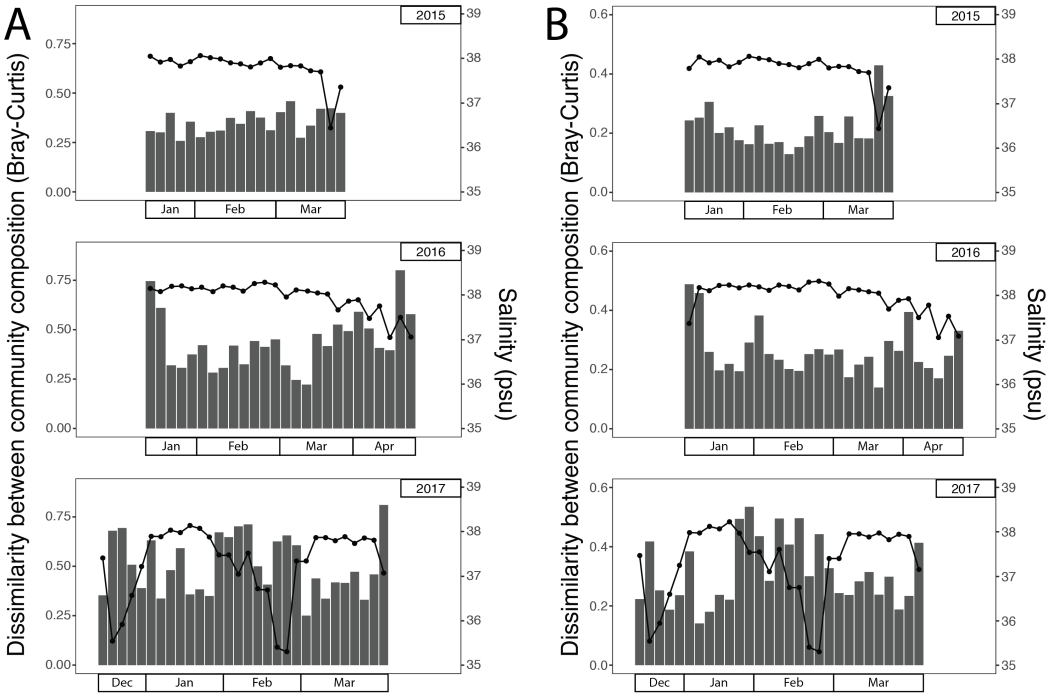

Fig. 2. : Bray-Curtis dissimilarity index between two succeeding samples, separated by year of sampling for eukaryotes (A) and prokaryotes (B). The line represents seawater salinity.

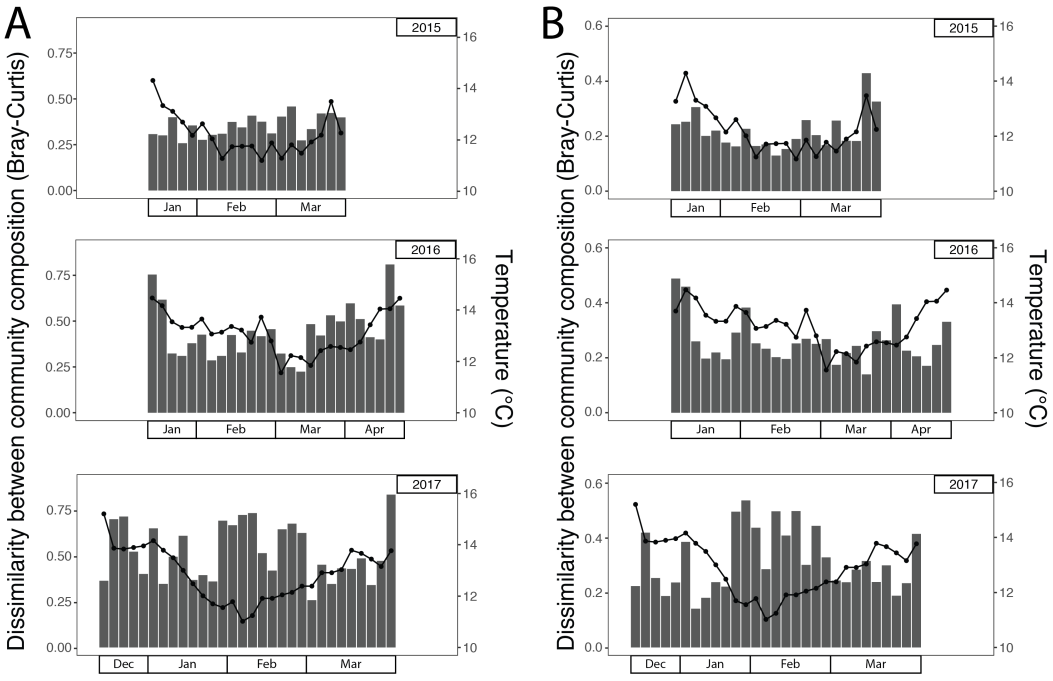

Fig. 3. : Bray-Curtis dissimilarity scores between two succeeding samples, separated by year of sampling for eukaryotes (A) and prokaryotes (B). The line represents seawater temperature.

2017 also displayed more diverging communities. The axis of the PCoAs had a higher explanation percentage for the prokaryote dataset (28.4% and 19.7%) than the eukaryote dataset (17.5% and 10.4%). Overall, a seasonal dynamic was visible for both domains. The eukaryote communities went from the top left of their PCoA to the bottom part of the graph with time, whereas the prokaryote communities went from the bottom left to the top left of the graph.

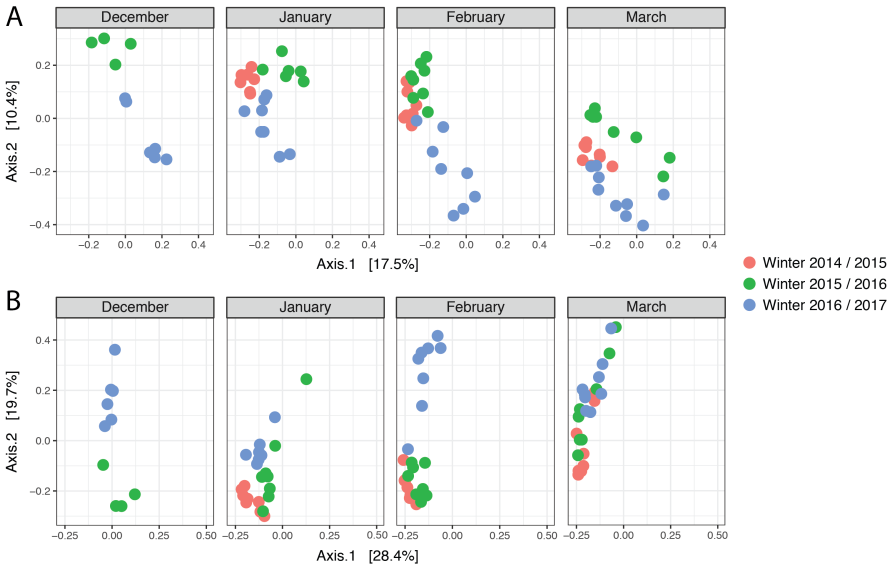

Fig. 4. : Principal Coordinate Analysis (PCoA) for eukaryote (A) and prokaryote community composition (B).

Abundance at the class level. With the aim of visualizing possible changes in abundances, ASVs counts, after being grouped at the class level, were separated according to month and year of sampling. The eukaryotic dataset (Fig. 5A) was divided into four main classes, *Bacillariophyta*, *Dinophyceae*, *Mamiellophyceae* and *Syndiniales*. *Bacillariophyta* showed relatively low levels of average abundance throughout the sampling period. January 2016 had higher levels of average abundance than 2015 and 2017, whereas February 2017 showed a higher level of average abundance compared to the two other years. Concerning March, 2017 had a high level of average abundance. However, *Bacillariophyta* were nearly not present during March 2016. Average abundance in the *Dinophyceae* class remained relatively stable during the three years of sampling, with higher levels in January and February 2017. On the other hand, *Mamiellophyceae* had more variable average abundances. For the month of January, 2015 had the highest average abundance. The rest of 2015 was quite stable. Average abundance of *Mamiellophyceae* showed a progressive increase with time in

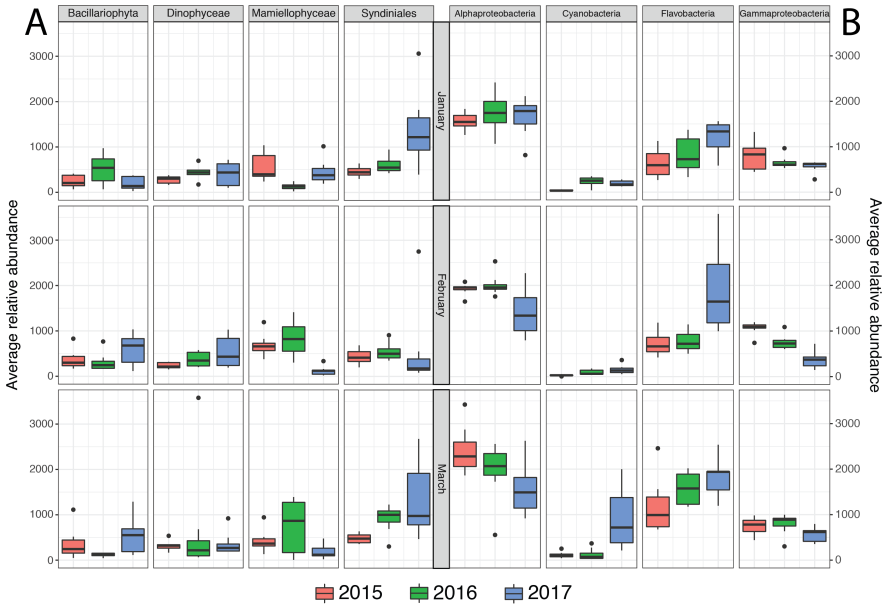

Fig. 5. : Average relative abundance of eukaryote (A) and prokaryote (B) classes according to year of sampling.

2016, starting off at very low levels in January, and ending up at the highest level for that class in March. *Mamiellophyceae* decreased from January 2017 to relatively low levels in February and March 2017. *Syndiniales* showed consistent levels of average abundance in 2015. This was also true in 2016, with a slight increase in March. However, in 2017, the average abundance showed high levels in January and March, but February had low levels.

The Average relative abundance was calculated for the primary classes of the prokaryote dataset, i.e. *Alphaproteobacteria*, *Cyanobacteria*, *Flavobacteria* and *Gammaproteobacteria*. *Alphaproteobacteria* relative average abundances, per year, remained similar during the sampling period. Decreases in abundance only happened in February and March 2017. *Cyanobacteria* average abundances were relatively low and stable during the three years of sampling, except for a sharp increase in March 2017. Average abundance for *Flavobacteria* in 2015 and 2016 was fairly stable, with an increase in March for both years. However, *Flavobacteria* in 2017 had a higher than average relative abundance, particularly in February. Finally, *Gammaproteobacteria* demonstrated relatively stable abundances for each year, respectively.

Co-occurrences of amplicon sequence variants. In order to investigate co-occurrences between ASVs, their Maximal Information Coefficients (MICs) were calculated and MICs > 0.75 were

represented in yearly networks (Fig. 6). Nodes depict ASVs and their size correspond to the abundance of the ASV for each year (Fig. 6). Cluster analysis (Supplementary Table 3) revealed that the 2015 network was composed of 2 main clusters, with a modularity of 12.4 and 5.7, respectively, as well as 4 smaller clusters (modularity < 1), and had an average number of neighbors (ANN, summarized in Supplementary Table 4) of 11.6. The 2016 network consisted of 3 clusters with a modularity of 14.5, 6 and 3, respectively, and had an ANN of 8.1. Finally, the 2017 network formed 8 low modularity clusters (modularity between 4.4 and 0.5) with an ANN of 5.5. The ASVs found in the 2015 network were mainly associated with an average salinity (37 psu). Whereas the ASVs in the 2016 network were mainly related to high salinity (38 psu). The 2017 network demonstrated high, average and low salinity ASVs (38, 37 and 35 psu) (Fig. 6A). The 2015 network did not show any discernible pattern for temperature distribution. The 2016 network was separated into a low temperature (11-12°C) group and a high temperature (13-14°C) group. In contrast, the 2017 network showed a central structure (13°C), with lower temperature fringes (12°C) (Fig. 6B). The 2015 and 2016 network depicted a temporal transition from January to March. As with other parameters, the 2017 network showed dispersed ASVs, especially considering the month of February, that was dispersed all over the network (Fig. 6C).

Additionally, we color coded the ASVs according to trophic group for eukaryotes and taxonomical classification for prokaryotes (Fig. 7). Details concerning this color coding can be found in supplementary table 7 and 8 for the eukaryotes and prokaryotes, respectively. This figure shows complex co-occurrences between different groups. Even though there is no general discernible pattern between the tree networks, we can observe that in 2015, for example, there is are multiple co-occurrences between autotrophs and parasites. On the other hand, in 2016, there are multiple co-occurrences between autotrophs and grazers. And in 2017 we can see that the low salinity ASVs correspond mainly to *flavobacteria*.

With the purpose of visualizing shared and year-specific ASVs between the networks, a Venn diagram was created (Fig. 8). A total of 42 ASVs were shared amongst the three winters of sampling. Year 2016 had a higher number of specific ASVs (51). Year 2015 and 2017 both had 45 year-specific ASVs. The ASVs that were shared between two years were consistent as well.

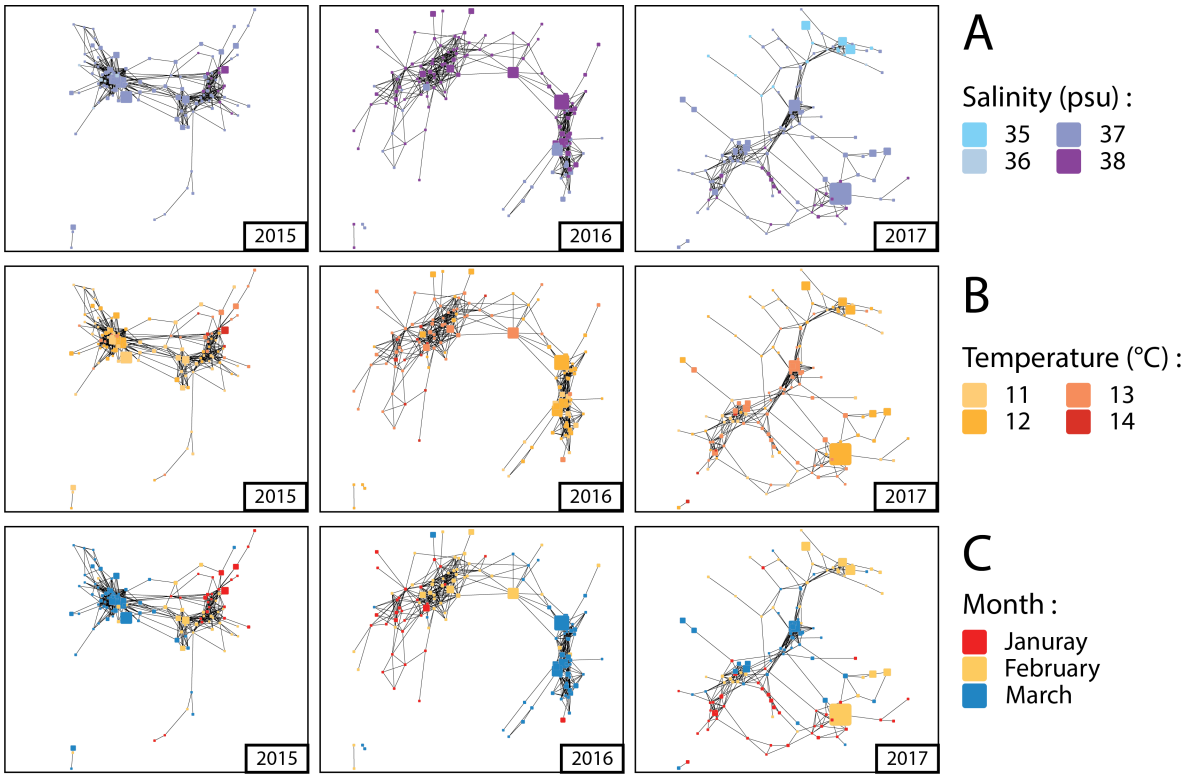

Fig. 6. : Networks showing co-occurrences between ASVs calculated with the MIC statistic. The size of the node is proportional to the sequence abundance of its corresponding ASV. The color coding of each ASV reflects either salinity (A), water temperature (B) or the month of sampling (C).

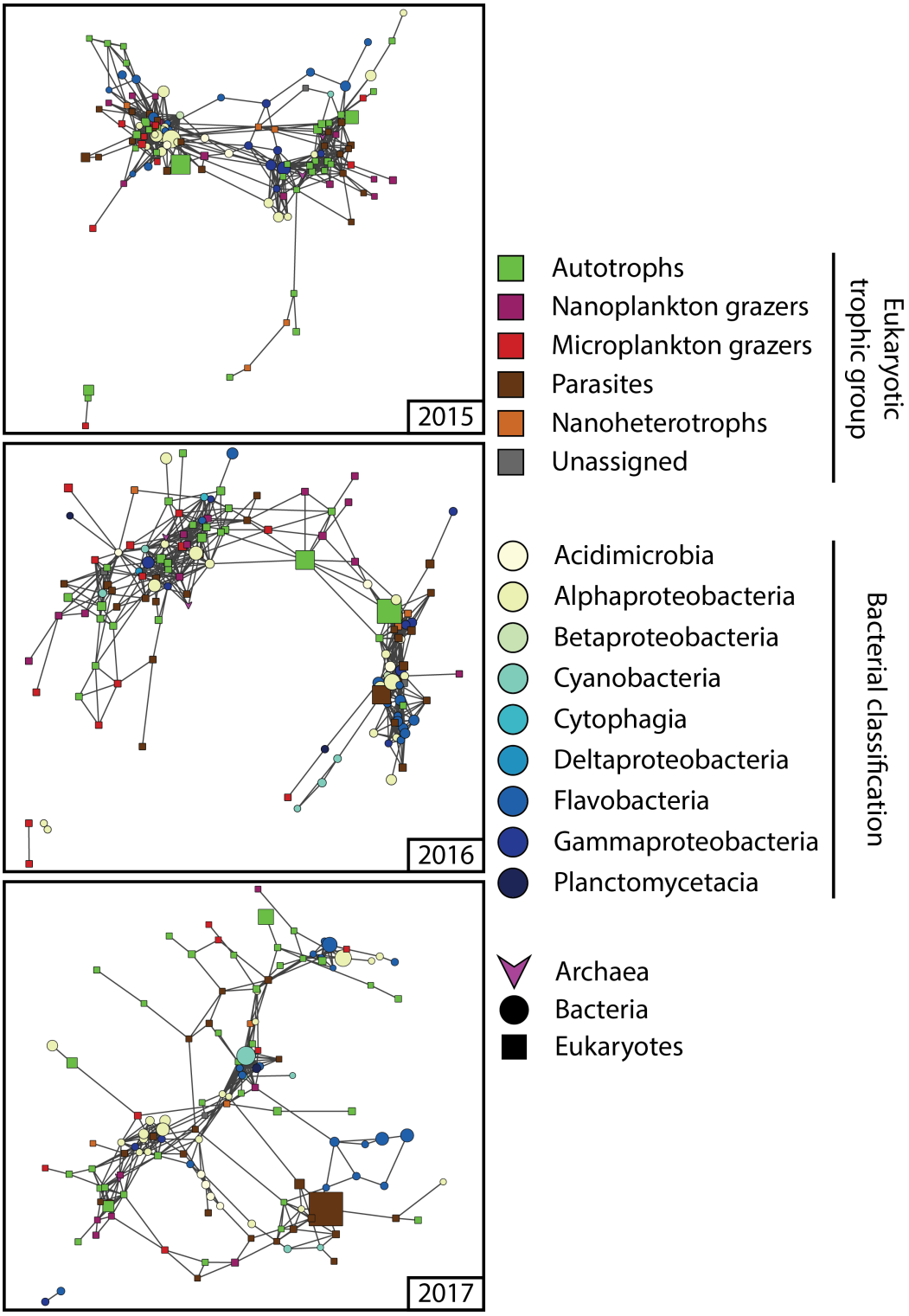

Fig. 7. : (***Addenum***) Yearly networks showing co-occurrences between ASVs calculated with the MIC statistic. The size of the node is proportional to the sequence abundance of its corresponding ASV. The color coding of each ASV reflects either the trophic group for eukaryotes or taxonomical classification for prokayotes

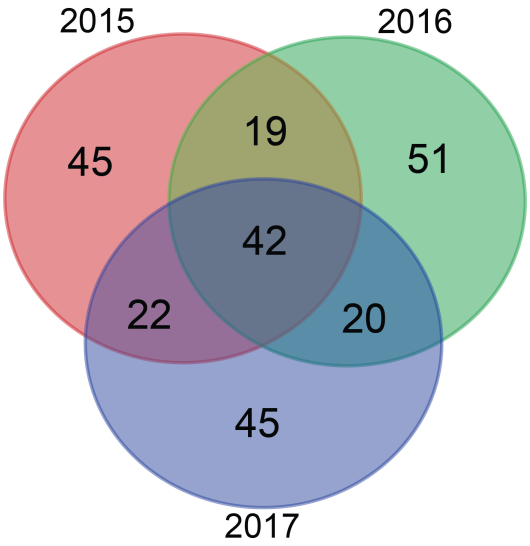

Fig. 8. : Venn diagram showing the shared and/or specific eukaryotic and bacterial ASVs per year.

Changes of ASVs co-occurrences. In order to assess co-occurrences at a fine taxonomical level, we selected five ASVs, among the most abundant that were found every year (Supplementary Table 6), to build subnetworks. Yearly co-occurrences with other ASVs for each subnetwork were visualized in radar plots (Fig. 9). The radius corresponds to the MIC score between the central ASV (described under each respective plot) and its first ASV neighbors. We selected two *mamiellophyceae*, one *cryptophyceae*, one *pelagophyceae* and one *dinophyceae* to represent the eukaryotes. They shared similar number of total first neighbors (TFN), ranging from 14 to 22. For the prokaryotes, three *alphaproteobacteria*, one *flavobacteria* and one *cyanobacteria* were selected. TFN numbers were divided into two groups, *Rhodobacteraceae*, *SAR11 surface 2* and *Tenacibaculum* had 14, 20 and 19 TFNs respectively, whereas *SAR11 surface 1* and *Synechococcus* had 44 and 42 TFNs, each. In these subnetworks, each ASV predominately co-occurred with members of the same domain of life, except for *Synechococcus* that had similar numbers of eukaryote and prokaryote first neighbors (Supplementary Table 5).

The *mamiellophyceae* and *dinophyceae* had more first neighbors in 2016, whereas the *cryptophyceae* had more first neighbors in 2015. The *pelagophyceae* had similar number of co-occurrences every year. Concerning the prokaryotes, *rhodobacteria*, *SAR11 surface 1* and *synechococcus* had more first neighbors in 2015. *SAR11 surface 2* had more first neighbors in 2016, whereas *Tenacinaculum* had a balanced number of first neighbors every year. Most ASVs had specific first neighbors depending

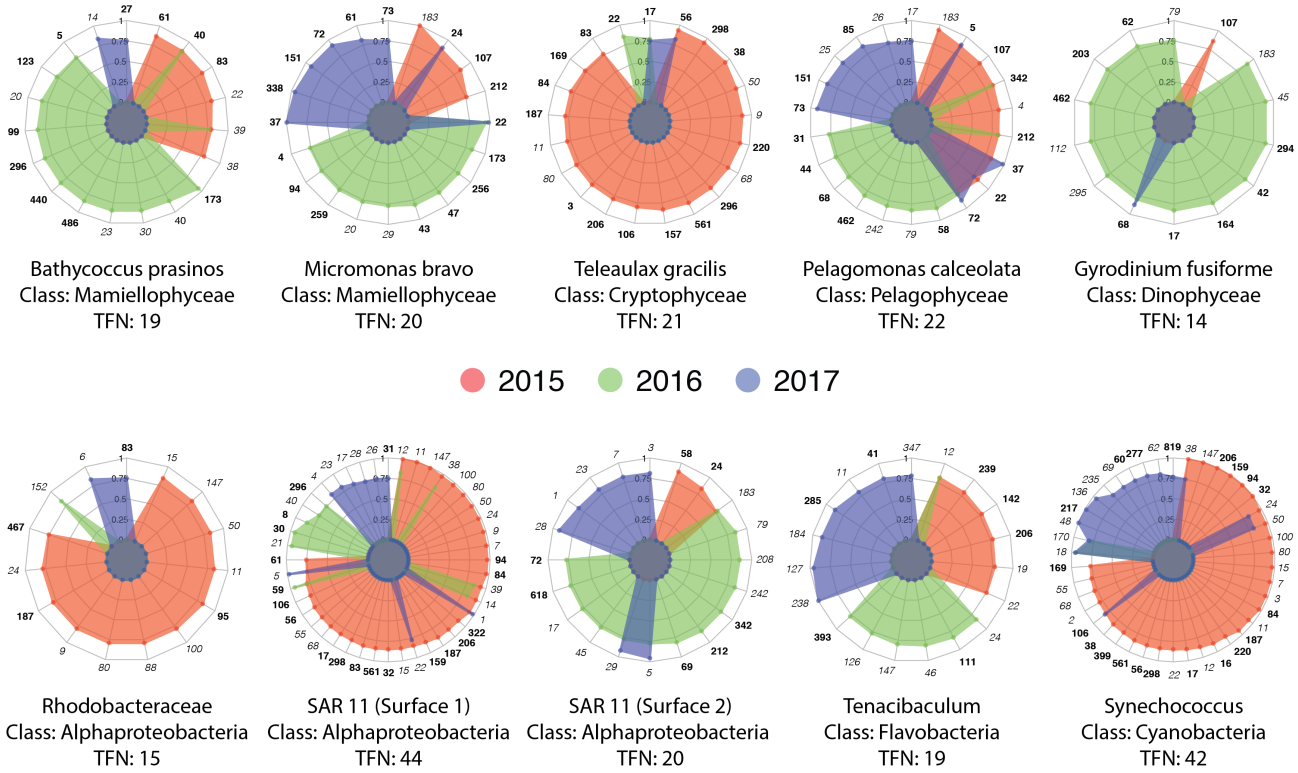

Fig. 9. : Radar plot depicting the first neighbors, per year, of selected ASVs (Supplementary Table 5). The radius corresponds to the MIC score between the central ASV (described under each plot) and its first neighbor.

on the year of sampling. Few ASVs demonstrated conserved partners between sampling periods.

Discussion

We compared the average environmental conditions in winter at the SOLA station (2007 - 2017) to data from 2015-2017. It showed that for all environmental variables, 2015 remained close to the weekly average throughout the sampling period and could thus be considered as a "standard year". Year 2016 displayed a higher temperature during most of the sampling period and 2017 demonstrated sharp salinity decreases at times with strong co-occurring nutrient and chlorophyll increases. 2016 and 2017 were thus defined as years with unusual environmental events.

The high sampling frequency (every 3 to 4 days) revealed biological variations at a high resolution. In both the eukaryote and prokaryote dataset, when there was a decrease in salinity, abrupt (December 2016 and February 2017) or progressive (March to April 2016), there was a marked increase in community dissimilarity between two successive samples (Fig. 2). Similarly, both datasets

showed increased differences in community composition between samples (Fig. 4) during the low salinity periods. This was particularly visible for samples taken in December 2016 and February 2017. The class abundance data also showed responses to decreases in salinity (Fig. 5). The eukaryotes displayed an increase in diatoms and a decrease in *mamiellophyceae* during the month of February 2017. However, *syndiniales* showed an important increase in abundance after the decrease in salinity (i.e. January and March 2017). In the prokaryote dataset, the months that displayed a decrease in salinity also showed a decrease in *Alphaproteobacteria* abundance that seems to be compensated by an increase in *Flavobacteria* and *Cyanobacteria*.

At the SOLA station, salinity drop and nutrient concentration increase are certainly due to the freshwater influx from the nearby Baillaury river triggered punctually by local precipitations (38). Large rivers further up the coast and low salinity lenses from the Rhone River have also been shown to impact the study site (39). Freshwater can impact marine community composition through different mechanisms, either by increasing nutrient concentrations and lowering salinity, which leads to a highly productive system that can, for example, lead to a prevalence of *Flavobacteria* (12), or by the physical movement of the freshwater acting as a transporter for allochthones taxa (12). It should be noted that distinguishing the separate physical effects of salinity and the biological effect of nutrients *in situ* is challenging. However, sampling across the salinity gradient of the Baltic Sea has demonstrated the importance of salinity itself in shaping community composition (40, 41). Co-occurring changes in nutrient levels have also been shown to impact community composition (15).

For this study, the MIC statistic (35) was determined to be the most appropriate tool to calculate correlations between different ASVs (42) in order to build yearly networks (Fig. 6). Microbial networks can demonstrate many thousands of possible interactions. The MIC was designed for rapid exploration of large date sets. It is a non-parametric method that can identify important relationships and was designed to give similar scores to equally noisy relationships regardless of the type of relationship (such as linear or exponential). Cluster analysis done with the CytoCluster app revealed that the three winters had different network structures. Year 2015 was dominated by two main modules surrounded by smaller ones. 2016 was composed of three main modules, and

2017 consisted of eight small modules (Supplementary Table 3). Modules could suggest different ecological processes that shape the overall co-occurrences in the network or represent specialized niches (43). Hence, module characteristics in 2017 could indicate harsher environmental conditions that lead to an increase in ecological niches. For this same reason, the 2015 and 2016 network could display broader ecological niches, since they show larger modules. However, the origin of these modules demand more investigation and, in any case, drawing conclusions from networks alone is not evident (43). Earlier network analysis have however demonstrated that bacterioplankton successions were determined directly by short term phytoplankton bloom and indirectly by temperature (11). Using wavelet-based identification of pairwise associations, daily changes and rapid transition in community composition were presented in a 93-consecutive-day time series (28). Networks spanning multiple years have been used to investigate co-occurrences between community members, either by using the local similarity analysis (14) at a single study site (5) but also to create a meta-analysis of publicly available 16S data (8).

Regardless of the environmental perturbations, some ASVs reoccur every year (23). After determining representative ASVs in both datasets (Supplementary Table 6), we identified their first neighbors within each network (Fig. 9, Supplementary Table 5). It appears that these resident ASVs changed co-occurring partners when they were faced with fluctuating environmental factors. An increase in salinity probably created a stress that promoted specific species to dominate the system (44). Salinity and pH stresses can have an impact on community composition and resilience (45). Furthermore, changes in salinity facilitates the establishment of specialist species (46). Hence salinity and/or temperature stresses observed during the sampling period could promote the establishment of resident species observed in this study.

For the prokaryote dataset, we observed resident ASVs belonging to the *Alphaproteobacteria*, *Flavobacteria* and *Cyanobacteria* classes. These same classes have already been found to be persistent in other time series analysis (13). Moreover, the *Flavobacteria Tenacibaculum* was observed to co-occur with different diatoms in 2015 and 2017. This co-occurrence has been shown in a daily sampling study off the coast of San Pedro (4). However, the same study site showed a correlation between *Rhodobacteraceae* and diatoms (47), but our study showed that the *Rhodobacteraceae* co-occurred with

dinoflagellates (Supplementary Table 5). Furthermore, it was shown that Eukaryota sequences had weak correlations with other domains of life (47, 48) and that bacterial taxa had stronger correlations with themselves than with eukaryotes (10). Similarly, Steele et al. (5) demonstrated that subnetworks centered on bacteria contained more bacteria and that eukaryotes co-occurred more with eukaryotes. Similar results were found in our study, except for *synechococcus*, which displayed an equal number of eukaryote and prokaryote first neighbors (Fig. 9, Supplementary Table 5). *Bathycoccus prasinos* have been shown earlier to co-occur with *Alpha-* and *Gammaproteobacteria* (23), and *micromonas* to co-occur *SAR11*. However, in the present study, both *SAR11* ASVs (Supplementary Table 5) co-occurred mainly with dinoflagellates and diatoms, and not with *micromonas*. This could be because we did not use the same primers between both studies, thus modifying the observable community.

In summary, environmental factors have an impact on community composition and abundance of coastal marine microbes. Network analysis revealed that low salinity conditions could impact co-occurrences between ASVs. Despite these impacts, certain persistent ASVs demonstrated different co-occurrences when faced with environmental perturbations. To fully investigate community composition and functional adaptations when facing environmental changes would require increases in time series prevalence and outputs. Indeed, impacts of major environmental events can only be appreciated when the average or the baseline has been characterized. It has been shown that after a mild winter there was a loss of spring bloom, and thus its carbon export. Even the summer cyanobacteria bloom was not capable of compensating this loss (21). Microcosms experiments corroborate the fact that future predicted environmental conditions could lead to lower carbon exports (20). Considering the changes to come, having well established time series to monitor anthropological impact seems essential.

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Supplementary information
Type of files:
Tables: MS Excel spreadsheet documents (.xlsx)

Supplementary table 1: Eukaryotic and bacteria ASV tables containing reference sequences, taxonomy and proportional abundance in the different samples.

Supplementary table 2: Description of ASVs found in the networks. As described in the material and methods, these ASVs met the selection criteria of Pearson linear regression > 0 and MIC > 0.75. The table contains 3 sheets, one for each network. Furthermore, each sheet contains the taxonomy and size (per network) of each selected ASV. The temperature, salinity and month correspond to sample of maximum abundance per ASV.

Supplementary table 1 and 2 are available here:
https://figshare.com/s/a5b98411644d492bc623

Supplementary table 3:

Results of the cluster analysis done with the HC-PIN clustering algorithm in Cytoscpae

Cluster details	Rank	Nodes	Edges	Modularity	InDeg	OutDeg
2015	1	50	1225	12.405	459	37
	2	39	741	5.714	200	35
	3	6	15	0.727	8	11
	4	3	3	0.667	2	3
	5	3	3	0.667	2	3
	6	3	3	0	2	3
2016	1	47	1081	14.471	246	17
	2	51	1275	6	228	38
	3	3	3	3	3	1
2017	1	23	253	4.429	93	21
	2	21	210	3.37	91	27
	3	19	171	3.167	38	12
	4	10	45	2.25	27	12
	5	3	3	2	2	1
	6	4	6	1.25	5	4
	7	6	15	1.222	11	9
	8	3	3	0.5	3	6

Supplementary table 4:

Results of the network analysis done with
the NetworkAnalyzer tool included in Cytoscape

	2015	2016	2017
Clustering coefficient	0.476612146	0.383111978	0.33652604
Connected componentes	2	3	2
diameter	9	10	11
radius	1	1	1
centralization	0.195350581	0.092425132	0.091350886
Shortest path	15506	16260	16004
Characteristic path length	3.228298723	4.091266913	4.627343164
Avg. number of neighbors (ANN)	11.578125	8.075757576	5.488372093
Number of nodes	128	132	129
density	0.091166339	0.061647004	0.042877907
heterogeneity	0.855399364	0.643538144	0.718183882
Isolated Nodes	0	0	0
Number of self-loops	0	0	0
Multi-edge node pairs	0	0	0
Analysis time (sec)	0.073	0.033	0.023

MIC value and taxonomy of first neighbors (1/10)

	First neighbors of:	steri_euk_Otu00004	Eukaryota	Chlorophyta	Mamiellophyceae	Mamiellales	Bathycoccaceae	Bathycoccus	Bathycoccus_prasinos
2015	**2016**	**2017**	**Name**	**Kingdom**	**Division**	**Class**	**Order**	**Family**	**Genus**	**Species**
	0.7574		steri_euk_Otu00005	Eukaryota	Chlorophyta	Mamiellophyceae	Mamiellales	Mamiellaceae	Micromonas	Micromonas_Clade-B.E.3
		0.75029	steri_euk_Otu00027	Eukaryota	Dinoflagellata	Dinophyceae	Peridiniales	NA	NA	NA
0.85754	0.84535		steri_euk_Otu00040	Eukaryota	Dinoflagellata	Syndiniales	Dino-Group-II	Dino-Group-II-Clade-13	Dino-Group-II-Clade-13_X	Dino-Group-II-Clade-13_X_sp.
0.86791			steri_euk_Otu00061	Eukaryota	Ciliophora	Spirotrichea	Choreotrichida	Choreotrichida_X	Choreotrichida_XX	Choreotrichida_XX_sp.
0.85754			steri_euk_Otu00083	Eukaryota	Dinoflagellata	Syndiniales	Dino-Group-II	Dino-Group-II-Clade-6	Dino-Group-II-Clade-6_X	Dino-Group-II-Clade-6_X_sp.
	0.84535		steri_euk_Otu00099	Eukaryota	Dinoflagellata	Syndiniales	Dino-Group-II	Dino-Group-II-Clade-1	Dino-Group-II-Clade-1_X	Dino-Group-II-Clade-1_X_sp.
	0.77322		steri_euk_Otu00123	Eukaryota	Dinoflagellata	Syndiniales	Dino-Group-II	Dino-Group-II-Clade-3	Dino-Group-II-Clade-3_X	Dino-Group-II-Clade-3_X_sp.
	0.94566		steri_euk_Otu00173	Eukaryota	Ochrophyta	Pelagophyceae	Pelagomonadales	Pelagomonadaceae	Pelagomonadaceae_clade_A	Pelagomonadaceae_clade_A_sp.
	0.84535		steri_euk_Otu00296	Eukaryota	Stramenopiles_X	MAST	MAST-6	MAST-6_X	MAST-6_XX	MAST-6_XX_sp.
	0.84535		steri_euk_Otu00440	Eukaryota	Dinoflagellata	Syndiniales	Dino-Group-II	Dino-Group-II-Clade-17	Dino-Group-II-Clade-17_X	Dino-Group-II-Clade-17_X_sp.
	0.84535		steri_euk_Otu00486	Eukaryota	Stramenopiles_X	MAST	MAST-3	MAST-3F	MAST-3F_X	MAST-3F_X_sp.
		0.83333	steri_prok_Otu00014	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	Amylibacter	
	0.82272		steri_prok_Otu00020	Bacteria	Actinobacteria	Acidimicrobiia	Acidimicrobiales	OM1_clade	Candidatus_Actinomarina	
0.82454			steri_prok_Otu00022	Bacteria	Actinobacteria	Acidimicrobiia	Acidimicrobiales	OM1_clade	Candidatus_Actinomarina	
	0.84535		steri_prok_Otu00023	Bacteria	Proteobacteria	Gammaproteobacteria	Oceanospirillales	SAR86_clade	NA	
	0.84535		steri_prok_Otu00030	Bacteria	Actinobacteria	Acidimicrobiia	Acidimicrobiales	OM1_clade	Candidatus_Actinomarina	
0.78755			steri_prok_Otu00038	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	Ascidiaceihabitans	
0.79101	0.7574		steri_prok_Otu00039	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	NA	
	0.84535		steri_prok_Otu00040	Bacteria	Proteobacteria	Gammaproteobacteria	Oceanospirillales	SAR86_clade	NA	

MIC value and taxonomy of first neighbors (2/10)

	First neighbors of:	steri_euk_Otu00005	Eukaryota	Chlorophyta	Mamiellophyceae	Mamiellales	Mamiellaceae	Micromonas	Micromonas_Clade-B.E.3
2015	**2016**	**2017**	**Name**	**Kingdom**	**Division**	**Class**	**Order**	**Family**	**Genus**	**Species**
	0.7574		steri_euk_Otu00004	Eukaryota	Chlorophyta	Mamiellophyceae	Mamiellales	Bathycoccaceae	Bathycoccus	Bathycoccus_prasinos
	0.94566	0.98083	steri_euk_Otu00022	Eukaryota	Chlorophyta	Mamiellophyceae	Mamiellales	Mamiellaceae	Micromonas	Micromonas_Clade-B.E.3
0.85754		0.88019	steri_euk_Otu00024	Eukaryota	Ochrophyta	Pelagophyceae	Pelagomonadales	Pelagomonadaceae	Pelagomonas	Pelagomonas_calceolata
		0.99498	steri_euk_Otu00037	Eukaryota	Chlorophyta	Mamiellophyceae	Mamiellales	Mamiellaceae	Micromonas	Micromonas_Clade-B..4
	0.81194		steri_euk_Otu00043	Eukaryota	Ciliophora	Spirotrichea	Strombidiida_G	Strombidiida_G_X	Strombidiida_G_XX	Strombidiida_G_XX_sp.
	0.81194		steri_euk_Otu00047	Eukaryota	Ciliophora	Spirotrichea	Strombidiida_F	Strombidiida_F_X	Strombidiida_F_XX	Strombidiida_F_XX_sp.
		0.81127	steri_euk_Otu00061	Eukaryota	Ciliophora	Spirotrichea	Choreotrichida	Choreotrichida_X	Choreotrichida_XX	Choreotrichida_XX_sp.
		0.91829	steri_euk_Otu00072	Eukaryota	Ciliophora	Spirotrichea	Strombidiida	Tontoniidae_B	Pseudotontonia	Pseudotontonia_simplicidens
		0.75029	steri_euk_Otu00073	Eukaryota	Cryptophyta	Cryptophyceae	Cryptophyceae_X	Cryptomonadales	Geminigera	Geminigera_cryophila
	0.76284		steri_euk_Otu00094	Eukaryota	Ciliophora	Spirotrichea	Strombidiida	NA	NA	NA
0.8466			steri_euk_Otu00107	Eukaryota	Ochrophyta	Dictyochophyceae	ctyochophyceae_X	Florenciellales	Florenciella	Florenciella_parvula
		0.91829	steri_euk_Otu00151	Eukaryota	Dinoflagellata	Syndiniales	Dino-Group-II	Dino-Group-II-Clade-7	Dino-Group-II-Clade-7_X	Dino-Group-II-Clade-7_X_sp.
	0.82995		steri_euk_Otu00173	Eukaryota	Ochrophyta	Pelagophyceae	Pelagomonadales	Pelagomonadaceae	Pelagomonadaceae_clade_A	Pelagomonadaceae_clade_A_sp.
0.75908			steri_euk_Otu00212	Eukaryota	Dinoflagellata	Syndiniales	Dino-Group-II	Dino-Group-II-Clade-36	Dino-Group-II-Clade-36_X	Dino-Group-II-Clade-36_X_sp.
	0.81194		steri_euk_Otu00256	Eukaryota	Dinoflagellata	Syndiniales	Dino-Group-II	Dino-Group-II-Clade-26	Dino-Group-II-Clade-26_X	Dino-Group-II-Clade-26_X_sp.
	0.77322		steri_euk_Otu00259	Eukaryota	Ochrophyta	Pelagophyceae	Pelagophyceae_X	Pelagophyceae_XX	Pelagophyceae_XXX	Pelagophyceae_XXX_sp.
		0.95443	steri_euk_Otu00338	Eukaryota	Dinoflagellata	Syndiniales	Dino-Group-II	Dino-Group-II-Clade-7	Dino-Group-II-Clade-7_X	Dino-Group-II-Clade-7_X_sp.
	0.77322		steri_prok_Otu00020	Bacteria	Actinobacteria	Acidimicrobiia	Acidimicrobiales	OM1_clade	Candidatus_Actinomarina	
	0.77322		steri_prok_Otu00029	Bacteria	Proteobacteria	Alphaproteobacteria	SAR11_clade	Surface_2	NA	
0.99863			steri_prok_Otu00183	Archaea	Euryarchaeota	Thermoplasmata	hermoplasmatales	Marine_Group_II	NA	

MIC value and taxonomy of first neighbors (3/10)

	First neighbors of:	steri_euk_Otu00016	Eukaryota	Cryptophyta	Cryptophyceae	Cryptophyceae_X	Cryptomonadales	Teleaulax	Teleaulax_gracilis
2015	**2016**	**2017**	**Name**	**Kingdom**	**Division**	**Class**	**Order**	**Family**	**Genus**	**Species**
0.79172			steri_euk_Otu00003	Eukaryota	Dinoflagellata	Syndiniales	Dino-Group-I	Dino-Group-I-Clade-1	Dino-Group-I-Clade-1_X	Dino-Group-I-Clade-1_X_sp.
	0.77322	0.75029	steri_euk_Otu00017	Eukaryota	Cryptophyta	Cryptophyceae	Cryptophyceae_X	Cryptomonadales	Plagioselmis	Plagioselmis_prolonga
	0.85079		steri_euk_Otu00022	Eukaryota	Chlorophyta	Mamiellophyceae	Mamiellales	Mamiellaceae	Micromonas	Micromonas_Clade-B.E.3
0.93211			steri_euk_Otu00038	Eukaryota	Dinoflagellata	Dinophyceae	Gymnodiniales	Gymnodiniaceae	Gymnodinium	Gymnodinium_sp.
0.93211		0.81922	steri_euk_Otu00056	Eukaryota	Stramenopiles_X	MAST	MAST-3	MAST-3E	MAST-3E_X	MAST-3E_X_sp.
0.76676			steri_euk_Otu00083	Eukaryota	Dinoflagellata	Syndiniales	Dino-Group-II	Dino-Group-II-Clade-6	Dino-Group-II-Clade-6_X	Dino-Group-II-Clade-6_X_sp.
0.79101			steri_euk_Otu00084	Eukaryota	Dinoflagellata	Syndiniales	Dino-Group-II	roup-II-Clade-10-and-11	ɔ-Group-II-Clade-10-and-11_X	ɔ-Group-II-Clade-10-and-11_X_sp.
0.79958			steri_euk_Otu00106	Eukaryota	Dinoflagellata	Dinophyceae	Dinophyceae_X	Tovelliaceae	Woloszynskia	Woloszynskia_halophila
0.83075			steri_euk_Otu00157	Eukaryota	Dinoflagellata	Syndiniales	Dino-Group-III	Dino-Group-III_X	Dino-Group-III_XX	Dino-Group-III_XX_sp.
0.76676			steri_euk_Otu00169	Eukaryota	Ciliophora	Spirotrichea	Strombidiida	Tontoniidae_A	Spirotontonia	Spirotontonia_sp.
0.79101			steri_euk_Otu00187	Eukaryota	Dinoflagellata	Dinophyceae	Peridiniales	Heterocapsaceae	Heterocapsa	Heterocapsa_nei/rotundata
0.79172			steri_euk_Otu00206	Eukaryota	Dinoflagellata	Syndiniales	Dino-Group-II	NA	NA	NA
0.88654			steri_euk_Otu00220	Eukaryota	Ochrophyta	Dictyochophyceae	ctyochophyceae_X	Dictyochales	Dictyocha	Dictyocha_speculum
0.8466			steri_euk_Otu00296	Eukaryota	Stramenopiles_X	MAST	MAST-6	MAST-6_X	MAST-6_XX	MAST-6_XX_sp.
0.93211			steri_euk_Otu00298	Eukaryota	Dinoflagellata	Syndiniales	Dino-Group-I	Dino-Group-I-Clade-5	Dino-Group-I-Clade-5_X	Dino-Group-I-Clade-5_X_sp.
0.84515			steri_euk_Otu00561	Eukaryota	Ciliophora	Litostomatea	Cyclotrichia	Cyclotrichia_X	Cyclotrichia_XX	Cyclotrichia_XX_sp.
0.88654			steri_prok_Otu00009	Bacteria	Cyanobacteria	Cyanobacteria	SubsectionI	FamilyI	Synechococcus	
0.79101			steri_prok_Otu00011	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	Planktomarina	
0.88654			steri_prok_Otu00050	Bacteria	Planctomycetes	Planctomycetacia	Planctomycetales	Planctomycetaceae	Blastopirellula	
0.85754			steri_prok_Otu00068	Bacteria	Proteobacteria	Alphaproteobacteria	Rickettsiales	SAR116_clade	Candidatus_Puniceispirillum	
0.79101			steri_prok_Otu00080	Bacteria	Bacteroidetes	Flavobacteriia	Flavobacteriales	Cryomorphaceae	NA	

MIC value and taxonomy of first neighbors (4/10)

	First neighbors of:		steri_euk_Otu00024	Eukaryota	Ochrophyta	Pelagophyceae	Pelagomonadales	Pelagomonadaceae	Pelagomonas	Pelagomonas_calceolata
2015	**2016**	**2017**	**Name**	**Kingdom**	**Division**	**Class**	**Order**	**Family**	**Genus**	**Species**
0.85754		0.88019	steri_euk_Otu00005	Eukaryota	Chlorophyta	Mamiellophyceae	Mamiellales	Mamiellaceae	Micromonas	Micromonas_Clade-B.E.3
0.82454		0.76541	steri_euk_Otu00022	Eukaryota	Chlorophyta	Mamiellophyceae	Mamiellales	Mamiellaceae	Micromonas	Micromonas_Clade-B.E.3
	0.77322		steri_euk_Otu00031	Eukaryota	Ochrophyta	Bacillariophyta	Bacillariophyta_X	Raphid-pennate	Pseudo-nitzschia	Pseudo-nitzschia_galaxiae
0.82454		0.97986	steri_euk_Otu00037	Eukaryota	Chlorophyta	Mamiellophyceae	Mamiellales	Mamiellaceae	Micromonas	Micromonas_Clade-B..4
	0.77322		steri_euk_Otu00044	Eukaryota	Dinoflagellata	Syndiniales	Dino-Group-III	Dino-Group-III_X	Dino-Group-III_XX	Dino-Group-III_XX_sp.
	0.84652		steri_euk_Otu00058	Eukaryota	Stramenopiles_X	MOCH	MOCH-2	MOCH-2_X	MOCH-2_XX	MOCH-2_XX_sp.
	0.77322		steri_euk_Otu00068	Eukaryota	Ochrophyta	Bacillariophyta	Bacillariophyta_X	Raphid-pennate	NA	NA
0.79172	0.79815	0.88521	steri_euk_Otu00072	Eukaryota	Ciliophora	Spirotrichea	Strombidiida	Tontoniidae_B	Pseudotontonia	Pseudotontonia_simplicidens
		0.91829	steri_euk_Otu00073	Eukaryota	Cryptophyta	Cryptophyceae	Cryptophyceae_X	Cryptomonadales	Geminigera	Geminigera_cryophila
		0.85977	steri_euk_Otu00085	Eukaryota	Ciliophora	Spirotrichea	Strombidiida_B	Strombidiida_B_X	Strombidiida_B_XX	Strombidiida_B_XX_sp.
0.85754			steri_euk_Otu00107	Eukaryota	Ochrophyta	Dictyochophyceae	ctyochophyceae_X	Florenciellales	Florenciella	Florenciella_parvula
		0.88521	steri_euk_Otu00151	Eukaryota	Dinoflagellata	Syndiniales	Dino-Group-II	Dino-Group-II-Clade-7	Dino-Group-II-Clade-7_X	Dino-Group-II-Clade-7_X_sp.
0.82454	0.81818		steri_euk_Otu00212	Eukaryota	Dinoflagellata	Syndiniales	Dino-Group-II	Dino-Group-II-Clade-36	Dino-Group-II-Clade-36_X	Dino-Group-II-Clade-36_X_sp.
0.8466	0.81818		steri_euk_Otu00342	Eukaryota	Dinoflagellata	Syndiniales	Dino-Group-II	Dino-Group-II-Clade-7	Dino-Group-II-Clade-7_X	Dino-Group-II-Clade-7_X_sp.
	0.81675		steri_euk_Otu00462	Eukaryota	Ciliophora	Spirotrichea	Strombidiida	Strombidiidae_J	Strombidiidae_J_X	Strombidiidae_J_X_sp.
0.83075			steri_prok_Otu00004	Bacteria	Proteobacteria	Alphaproteobacteria	SAR11_clade	Surface_2	NA	
		0.75029	steri_prok_Otu00017	Bacteria	Proteobacteria	Gammaproteobacteria	Oceanospirillales	ZD0405	NA	
		0.86508	steri_prok_Otu00025	Archaea	Thaumarchaeota	Marine_Group_I	Unknown_Order	Unknown_Family	Candidatus_Nitrosopumilus	
		0.76541	steri_prok_Otu00026	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	PS1_clade	NA	
	0.82851		steri_prok_Otu00079	Bacteria	Cyanobacteria	Cyanobacteria	SubsectionI	FamilyI	Prochlorococcus	
0.93211			steri_prok_Otu00183	Archaea	Euryarchaeota	Thermoplasmata	hermoplasmatales	Marine_Group_II	NA	
	0.81675		steri_prok_Otu00242	Bacteria	Proteobacteria	Deltaproteobacteria	SAR324_clade	NA	NA	

MIC value and taxonomy of first neighbors (5/10)

	First neighbors of:	steri_euk_Otu00131	Eukaryota	Dinoflagellata	Dinophyceae	Gymnodiniales	Gymnodiniaceae	Gyrodinium	Gyrodinium_fusiforme
2015	**2016**	**2017**	**Name**	**Kingdom**	**Division**	**Class**	**Order**	**Family**	**Genus**	**Species**
	0.82995		steri_euk_Otu00017	Eukaryota	Cryptophyta	Cryptophyceae	Cryptophyceae_X	Cryptomonadales	Plagioselmis	Plagioselmis_prolonga
	0.8688		steri_euk_Otu00042	Eukaryota	Dinoflagellata	Dinophyceae	Gymnodiniales	Gymnodiniaceae	NA	NA
	0.78776		steri_euk_Otu00062	Eukaryota	Haptophyta	Prymnesiophyceae	Isochrysidales	Noelaerhabdaceae	Gephyrocapsa	Gephyrocapsa_oceanica
	0.79815	0.87086	steri_euk_Otu00068	Eukaryota	Ochrophyta	Bacillariophyta	Bacillariophyta_X	Raphid-pennate	NA	NA
0.85754			steri_euk_Otu00107	Eukaryota	Ochrophyta	Dictyochophyceae	ctyochophyceae_X	Florenciellales	Florenciella	Florenciella_parvula
	0.84652		steri_euk_Otu00164	Eukaryota	Haptophyta	Prymnesiophyceae	ophyceae_Clade_D	esiophyceae_Clade_D_X	mnesiophyceae_Clade_D_XX	mnesiophyceae_Clade_D_XX_sp.
	0.7942		steri_euk_Otu00203	Eukaryota	Ciliophora	Spirotrichea	Choreotrichida	Strobilidiidae_A	Strobilidiidae_A_X	Strobilidiidae_A_X_sp.
	0.90239		steri_euk_Otu00294	Eukaryota	Ochrophyta	Dictyochophyceae	ctyochophyceae_X	Florenciellales	Florenciellales_X	Florenciellales_X_sp.
	0.79815		steri_euk_Otu00462	Eukaryota	Ciliophora	Spirotrichea	Strombidiida	Strombidiidae_J	Strombidiidae_J_X	Strombidiidae_J_X_sp.
	0.90239		steri_prok_Otu00045	Bacteria	Proteobacteria	Alphaproteobacteria	SAR11_clade	Surface_1	NA	
	0.75535		steri_prok_Otu00079	Bacteria	Cyanobacteria	Cyanobacteria	SubsectionI	FamilyI	Prochlorococcus	
	0.79815		steri_prok_Otu00112	Bacteria	Bacteroidetes	Cytophagia	Cytophagales	Flammeovirgaceae	Marinoscillum	
	0.90239		steri_prok_Otu00183	Archaea	Euryarchaeota	Thermoplasmata	hermoplasmatales	Marine_Group_II	NA	
	0.79815		steri_prok_Otu00295	Bacteria	Bacteroidetes	Flavobacteriia	Flavobacteriales	NS9_marine_group	NA	

MIC value and taxonomy of first neighbors (6/10)

	First neighbors of:		steri_prok_Otu00002	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	Ascidiaceihabitans		
2015	**2016**	**2017**	**Name**	**Kingdom**	**Division**	**Class**	**Order**	**Family**	**Genus**	**Species**
		0.75029	steri_euk_Otu00083	Eukaryota	Dinoflagellata	Syndiniales	Dino-Group-II	Dino-Group-II-Clade-6	Dino-Group-II-Clade-6_X	Dino-Group-II-Clade-6_X_sp.
0.82805			steri_euk_Otu00095	Eukaryota	Dinoflagellata	Syndiniales	Dino-Group-I	Dino-Group-I-Clade-1	Dino-Group-I-Clade-1_X	Dino-Group-I-Clade-1_X_sp.
0.79101			steri_euk_Otu00187	Eukaryota	Dinoflagellata	Dinophyceae	Peridiniales	Heterocapsaceae	Heterocapsa	Heterocapsa_nei/rotundata
0.75537			steri_euk_Otu00467	Eukaryota	Dinoflagellata	Syndiniales	Dino-Group-II	Dino-Group-II-Clade-32	Dino-Group-II-Clade-32_X	Dino-Group-II-Clade-32_X_sp.
		0.82946	steri_prok_Otu00006	Bacteria	Cyanobacteria	Cyanobacteria	SubsectionI	FamilyI	Synechococcus	
0.79101			steri_prok_Otu00009	Bacteria	Cyanobacteria	Cyanobacteria	SubsectionI	FamilyI	Synechococcus	
0.82805			steri_prok_Otu00011	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	Planktomarina	
0.8466			steri_prok_Otu00015	Bacteria	Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	Formosa	
0.78556			steri_prok_Otu00024	Bacteria	Bacteroidetes	Flavobacteriia	Flavobacteriales	Cryomorphaceae	Fluviicola	
0.82805			steri_prok_Otu00050	Bacteria	Planctomycetes	Planctomycetacia	Planctomycetales	Planctomycetaceae	Blastopirellula	
0.79101			steri_prok_Otu00080	Bacteria	Bacteroidetes	Flavobacteriia	Flavobacteriales	Cryomorphaceae	NA	
0.79101			steri_prok_Otu00088	Bacteria	Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	NS3a_marine_group	
0.79101			steri_prok_Otu00100	Bacteria	Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	NS4_marine_group	
0.82805			steri_prok_Otu00147	Bacteria	Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	NA	
	0.82851		steri_prok_Otu00152	Bacteria	Bacteroidetes	Flavobacteriia	Flavobacteriales	Cryomorphaceae	NA	

MIC value and taxonomy of first neighbors (7/10)

	First neighbors of:	steri_prok_Otu00003	Bacteria	Proteobacteria	Alphaproteobacteria	SAR11_clade	Surface_1	Candidatus_Pelagibacter	
2015	**2016**	**2017**	**Name**	**Kingdom**	**Division**	**Class**	**Order**	**Family**	**Genus**	**Species**
	0.84652		steri_euk_Otu00008	Eukaryota	Dinoflagellata	Syndiniales	Dino-Group-I	Dino-Group-I-Clade-1	Dino-Group-I-Clade-1_X	Dino-Group-I-Clade-1_X_sp.
0.83075			steri_euk_Otu00017	Eukaryota	Cryptophyta	Cryptophyceae	Cryptophyceae_X	Cryptomonadales	Plagioselmis	Plagioselmis_prolonga
	0.94566		steri_euk_Otu00030	Eukaryota	Foraminifera	NA	NA	NA	NA	NA
		0.75029	steri_euk_Otu00031	Eukaryota	Ochrophyta	Bacillariophyta	Bacillariophyta_X	Raphid-pennate	Pseudo-nitzschia	Pseudo-nitzschia_galaxiae
0.8466			steri_euk_Otu00032	Eukaryota	Ochrophyta	Bacillariophyta	Bacillariophyta_X	r-centric-Mediophyceae	Chaetoceros	Chaetoceros_sp
0.82454			steri_euk_Otu00056	Eukaryota	Stramenopiles_X	MAST	MAST-3	MAST-3E	MAST-3E_X	MAST-3E_X_sp.
0.79101	0.94566		steri_euk_Otu00059	Eukaryota	Dinoflagellata	Syndiniales	Dino-Group-II	roup-II-Clade-10-and-11	ɔ-Group-II-Clade-10-and-11_X	ɔ-Group-II-Clade-10-and-11_X_sp.
0.75537			steri_euk_Otu00061	Eukaryota	Ciliophora	Spirotrichea	Choreotrichida	Choreotrichida_X	Choreotrichida_XX	Choreotrichida_XX_sp.
0.84585			steri_euk_Otu00083	Eukaryota	Dinoflagellata	Syndiniales	Dino-Group-II	Dino-Group-II-Clade-6	Dino-Group-II-Clade-6_X	Dino-Group-II-Clade-6_X_sp.
0.96563			steri_euk_Otu00084	Eukaryota	Dinoflagellata	Syndiniales	Dino-Group-II	roup-II-Clade-10-and-11	ɔ-Group-II-Clade-10-and-11_X	ɔ-Group-II-Clade-10-and-11_X_sp.
0.96563			steri_euk_Otu00094	Eukaryota	Ciliophora	Spirotrichea	Strombidiida	NA	NA	NA
0.79958			steri_euk_Otu00106	Eukaryota	Dinoflagellata	Dinophyceae	Dinophyceae_X	Tovelliaceae	Woloszynskia	Woloszynskia_halophila
0.85754			steri_euk_Otu00159	Eukaryota	Ochrophyta	Bacillariophyta	Bacillariophyta_X	r-centric-Mediophyceae	Chaetoceros	Chaetoceros_sp.
0.87867			steri_euk_Otu00187	Eukaryota	Dinoflagellata	Dinophyceae	Peridiniales	Heterocapsaceae	Heterocapsa	Heterocapsa_nei/rotundata
0.87885			steri_euk_Otu00206	Eukaryota	Dinoflagellata	Syndiniales	Dino-Group-II	NA	NA	NA
	0.75444		steri_euk_Otu00296	Eukaryota	Stramenopiles_X	MAST	MAST-6	MAST-6_X	MAST-6_XX	MAST-6_XX_sp.
0.83075			steri_euk_Otu00298	Eukaryota	Dinoflagellata	Syndiniales	Dino-Group-I	Dino-Group-I-Clade-5	Dino-Group-I-Clade-5_X	Dino-Group-I-Clade-5_X_sp.
0.88654			steri_euk_Otu00322	Eukaryota	Ochrophyta	Chrysophyceae	Chrysophyceae_X	Chrysophyceae_XX	Chrysophyceae_XXX	Chrysophyceae_XXX_sp.
0.84585			steri_euk_Otu00561	Eukaryota	Ciliophora	Litostomatea	Cyclotrichia	Cyclotrichia_X	Cyclotrichia_XX	Cyclotrichia_XX_sp.
0.93211		0.97986	steri_prok_Otu00001	Bacteria	Proteobacteria	Alphaproteobacteria	SAR11_clade	Surface_1	NA	
		0.8132	steri_prok_Otu00004	Bacteria	Proteobacteria	Alphaproteobacteria	SAR11_clade	Surface_2	NA	
0.75537		0.97986	steri_prok_Otu00005	Bacteria	Proteobacteria	Alphaproteobacteria	SAR11_clade	Surface_1	NA	
0.96563			steri_prok_Otu00007	Bacteria	Proteobacteria	Alphaproteobacteria	SAR11_clade	Surface_1	Candidatus_Pelagibacter	
0.96563			steri_prok_Otu00009	Bacteria	Cyanobacteria	Cyanobacteria	SubsectionI	FamilyI	Synechococcus	
0.99863			steri_prok_Otu00011	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	Planktomarina	
0.99863	0.82995		steri_prok_Otu00012	Bacteria	Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	NS4_marine_group	
0.93211	0.81675		steri_prok_Otu00014	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	Amylibacter	
0.8466			steri_prok_Otu00015	Bacteria	Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	Formosa	
		0.77324	steri_prok_Otu00017	Bacteria	Proteobacteria	Gammaproteobacteria	Oceanospirillales	ZD0405	NA	
	0.94566		steri_prok_Otu00021	Bacteria	Proteobacteria	Gammaproteobacteria	Oceanospirillales	SAR86_clade	NA	
0.8466		0.77324	steri_prok_Otu00022	Bacteria	Actinobacteria	Acidimicrobiia	Acidimicrobiales	OM1_clade	Candidatus_Actinomarina	
		0.81127	steri_prok_Otu00023	Bacteria	Proteobacteria	Gammaproteobacteria	Oceanospirillales	SAR86_clade	NA	
0.96563			steri_prok_Otu00024	Bacteria	Bacteroidetes	Flavobacteriia	Flavobacteriales	Cryomorphaceae	Fluviicola	
		0.75029	steri_prok_Otu00026	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	PS1_clade	NA	
		0.75029	steri_prok_Otu00028	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodospirillales	Rhodospirillaceae	AEGEAN-169_marine_group	
0.96563	0.81194		steri_prok_Otu00038	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	Ascidiaceihabitans	
0.93211	0.84652		steri_prok_Otu00039	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	NA	
	0.7574		steri_prok_Otu00040	Bacteria	Proteobacteria	Gammaproteobacteria	Oceanospirillales	SAR86_clade	NA	
0.96563			steri_prok_Otu00050	Bacteria	Planctomycetes	Planctomycetacia	Planctomycetales	Planctomycetaceae	Blastopirellula	
0.82472			steri_prok_Otu00055	Bacteria	Proteobacteria	Betaproteobacteria	Methylophilales	Methylophilaceae	OM43_clade	
0.82805			steri_prok_Otu00068	Bacteria	Proteobacteria	Alphaproteobacteria	Rickettsiales	SAR116_clade	Candidatus_Puniceispirillum	
0.96563			steri_prok_Otu00080	Bacteria	Bacteroidetes	Flavobacteriia	Flavobacteriales	Cryomorphaceae	NA	
0.96563			steri_prok_Otu00100	Bacteria	Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	NS4_marine_group	
0.98769			steri_prok_Otu00147	Bacteria	Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	NA	

MIC value and taxonomy of first neighbors (8/10)

	First neighbors of:		steri_prok_Otu00004	Bacteria	Proteobacteria	Alphaproteobacteria	SAR11_clade	Surface_2	NA	
2015	**2016**	**2017**	**Name**	**Kingdom**	**Division**	**Class**	**Order**	**Family**	**Genus**	**Species**
0.83075			steri_euk_Otu00024	Eukaryota	Ochrophyta	Pelagophyceae	Pelagomonadales	Pelagomonadaceae	Pelagomonas	Pelagomonas_calceolata
0.88654			steri_euk_Otu00058	Eukaryota	Stramenopiles_X	MOCH	MOCH-2	MOCH-2_X	MOCH-2_XX	MOCH-2_XX_sp.
	0.81194		steri_euk_Otu00069	Eukaryota	Ochrophyta	Bacillariophyta	Bacillariophyta_X	Araphid-pennate	Asterionellopsis	Asterionellopsis_glacialis
	0.77322		steri_euk_Otu00072	Eukaryota	Ciliophora	Spirotrichea	Strombidiida	Tontoniidae_B	Pseudotontonia	Pseudotontonia_simplicidens
	0.82851		steri_euk_Otu00212	Eukaryota	Dinoflagellata	Syndiniales	Dino-Group-II	Dino-Group-II-Clade-36	Dino-Group-II-Clade-36_X	Dino-Group-II-Clade-36_X_sp.
	0.82851		steri_euk_Otu00342	Eukaryota	Dinoflagellata	Syndiniales	Dino-Group-II	Dino-Group-II-Clade-7	Dino-Group-II-Clade-7_X	Dino-Group-II-Clade-7_X_sp.
	0.77322		steri_euk_Otu00618	Eukaryota	Dinoflagellata	Syndiniales	Dino-Group-II	Dino-Group-II-Clade-20	Dino-Group-II-Clade-20_X	Dino-Group-II-Clade-20_X_sp.
		0.82946	steri_prok_Otu00001	Bacteria	Proteobacteria	Alphaproteobacteria	SAR11_clade	Surface_1	NA	
		0.8132	steri_prok_Otu00003	Bacteria	Proteobacteria	Alphaproteobacteria	SAR11_clade	Surface_1	Candidatus_Pelagibacter	
	0.77322	0.95443	steri_prok_Otu00005	Bacteria	Proteobacteria	Alphaproteobacteria	SAR11_clade	Surface_1	NA	
		0.81922	steri_prok_Otu00007	Bacteria	Proteobacteria	Alphaproteobacteria	SAR11_clade	Surface_1	Candidatus_Pelagibacter	
	0.77322		steri_prok_Otu00017	Bacteria	Proteobacteria	Gammaproteobacteria	Oceanospirillales	ZD0405	NA	
		0.81922	steri_prok_Otu00023	Bacteria	Proteobacteria	Gammaproteobacteria	Oceanospirillales	SAR86_clade	NA	
		0.91829	steri_prok_Otu00028	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodospirillales	Rhodospirillaceae	AEGEAN-169_marine_group	
	0.77322	0.91829	steri_prok_Otu00029	Bacteria	Proteobacteria	Alphaproteobacteria	SAR11_clade	Surface_2	NA	
	0.77322		steri_prok_Otu00045	Bacteria	Proteobacteria	Alphaproteobacteria	SAR11_clade	Surface_1	NA	
	0.85249		steri_prok_Otu00079	Bacteria	Cyanobacteria	Cyanobacteria	SubsectionI	FamilyI	Prochlorococcus	
0.75455	0.77322		steri_prok_Otu00183	Archaea	Euryarchaeota	Thermoplasmata	hermoplasmatales	Marine_Group_II	NA	
	0.85079		steri_prok_Otu00208	Bacteria	Proteobacteria	Gammaproteobacteria	Oceanospirillales	SAR86_clade	NA	
	0.84535		steri_prok_Otu00242	Bacteria	Proteobacteria	Deltaproteobacteria	SAR324_clade	NA	NA	

MIC value and taxonomy of first neighbors (9/10)

	First neighbors of:	steri_prok_Otu00008	Bacteria	Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	Tenacibaculum		
2015	**2016**	**2017**	**Name**	**Kingdom**	**Division**	**Class**	**Order**	**Family**	**Genus**	**Species**
		0.80403	steri_euk_Otu00041	Eukaryota	Ochrophyta	Bacillariophyta	Bacillariophyta_X	r-centric-Mediophyceae	Thalassiosira	Thalassiosira_profunda
	0.82995		steri_euk_Otu00111	Eukaryota	Stramenopiles_X	Labyrinthulea	Thraustochytriales	Thraustochytriaceae	Thraustochytriaceae_X	Thraustochytriaceae_X_sp.
0.78095			steri_euk_Otu00142	Eukaryota	Ochrophyta	Bacillariophyta	Bacillariophyta_X	r-centric-Mediophyceae	Thalassiosira	Thalassiosira_sp.
0.77749			steri_euk_Otu00206	Eukaryota	Dinoflagellata	Syndiniales	Dino-Group-II	NA	NA	NA
0.79862			steri_euk_Otu00239	Eukaryota	Ciliophora	Spirotrichea	Strombidiida	Tontoniidae_A	Laboea	Laboea_strobila
		0.87086	steri_euk_Otu00285	Eukaryota	Dinoflagellata	Dinophyceae	NA	NA	NA	NA
	0.77322		steri_euk_Otu00393	Eukaryota	Stramenopiles_X	Oomycota	Oomycota_X	Oomycota_XX	Oomycota_XXX	Oomycota_XXX_sp.
		0.80403	steri_prok_Otu00011	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	Planktomarina	
0.81377	0.81194		steri_prok_Otu00012	Bacteria	Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	NS4_marine_group	
0.77517			steri_prok_Otu00019	Bacteria	Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	Tenacibaculum	
0.75537			steri_prok_Otu00022	Bacteria	Actinobacteria	Acidimicrobiia	Acidimicrobiales	OM1_clade	Candidatus_Actinomarina	
	0.82995		steri_prok_Otu00024	Bacteria	Bacteroidetes	Flavobacteriia	Flavobacteriales	Cryomorphaceae	Fluviicola	
	0.81194		steri_prok_Otu00046	Bacteria	Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	NS4_marine_group	
	0.77322		steri_prok_Otu00126	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	NA	
		0.95443	steri_prok_Otu00127	Bacteria	Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	Polaribacter_1	
	0.79815		steri_prok_Otu00147	Bacteria	Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	NA	
		0.88019	steri_prok_Otu00184	Bacteria	Bacteroidetes	Flavobacteriia	Flavobacteriales	Cryomorphaceae	NA	
		0.99498	steri_prok_Otu00238	Bacteria	Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	NA	
		0.78308	steri_prok_Otu00347	Bacteria	Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	NS2b_marine_group	

MIC value and taxonomy of first neighbors (10/10)

	First neighbors of:		steri_prok_Otu00009	Bacteria	Cyanobacteria	Cyanobacteria	SubsectionI	FamilyI	Synechococcus		
2015	**2016**	**2017**	**Name**	**Kingdom**	**Division**	**Class**	**Order**	**Family**	**Genus**	**Species**	
0.88654			steri_euk_Otu00016	Eukaryota	Cryptophyta	Cryptophyceae	Cryptophyceae_X	Cryptomonadales	Teleaulax	Teleaulax_gracilis	
0.85754			steri_euk_Otu00017	Eukaryota	Cryptophyta	Cryptophyceae	Cryptophyceae_X	Cryptomonadales	Plagioselmis	Plagioselmis_prolonga	
0.99863			steri_euk_Otu00032	Eukaryota	Ochrophyta	Bacillariophyta	Bacillariophyta_X	r-centric-Mediophyceae	Chaetoceros	_P._quinquecorne_endosymbiont	
0.82805			steri_euk_Otu00038	Eukaryota	Dinoflagellata	Dinophyceae	Gymnodiniales	Gymnodiniaceae	Gymnodinium	Gymnodinium_sp.	
0.8466			steri_euk_Otu00056	Eukaryota	Stramenopiles_X	MAST	MAST-3	MAST-3E	MAST-3E_X	MAST-3E_X_sp.	
		0.82946	steri_euk_Otu00060	Eukaryota	Ciliophora	Spirotrichea	Strombidiida	Strombidiidae_M	Strombidiidae_M_X	Strombidiidae_M_X_sp.	
0.96563			steri_euk_Otu00084	Eukaryota	Dinoflagellata	Syndiniales	Dino-Group-II	roup-II-Clade-10-and-11	ɔ-Group-II-Clade-10-and-11_X	ɔ-Group-II-Clade-10-and-11_X_sp.	
0.99863			steri_euk_Otu00094	Eukaryota	Ciliophora	Spirotrichea	Strombidiida	NA	NA	NA	
0.79958		0.81922	steri_euk_Otu00106	Eukaryota	Dinoflagellata	Dinophyceae	Dinophyceae_X	Tovelliaceae	Woloszynskia	Woloszynskia_halophila	
0.99863			steri_euk_Otu00159	Eukaryota	Ochrophyta	Bacillariophyta	Bacillariophyta_X	r-centric-Mediophyceae	Chaetoceros	Chaetoceros_sp.	
0.76676			steri_euk_Otu00169	Eukaryota	Ciliophora	Spirotrichea	Strombidiida	Tontoniidae_A	Spirotontonia	Spirotontonia_sp.	
0.93211			steri_euk_Otu00187	Eukaryota	Dinoflagellata	Dinophyceae	Peridiniales	Heterocapsaceae	Heterocapsa	Heterocapsa_nei/rotundata	
0.99863			steri_euk_Otu00206	Eukaryota	Dinoflagellata	Syndiniales	Dino-Group-II	NA	NA	NA	
		0.97986	steri_euk_Otu00217	Eukaryota	Haptophyta	Prymnesiophyceae	Prymnesiales	Chrysochromulinaceae	Chrysochromulina	Chrysochromulina_sp.	
0.88654			steri_euk_Otu00220	Eukaryota	Ochrophyta	Dictyochophyceae	ctyochophyceae_X	Dictyochales	Dictyocha	Dictyocha_speculum	
		0.82831	steri_euk_Otu00277	Eukaryota	Stramenopiles_X	MAST	MAST-1	MAST-1C	MAST-1C_X	MAST-1C_X_sp.	
0.8466			steri_euk_Otu00298	Eukaryota	Dinoflagellata	Syndiniales	Dino-Group-I	Dino-Group-I-Clade-5	Dino-Group-I-Clade-5_X	Dino-Group-I-Clade-5_X_sp.	
0.82805			steri_euk_Otu00399	Eukaryota	Dinoflagellata	Dinophyceae	NA	NA	NA	NA	
0.84585			steri_euk_Otu00561	Eukaryota	Ciliophora	Litostomatea	Cyclotrichia	Cyclotrichia_X	Cyclotrichia_XX	Cyclotrichia_XX_sp.	
		0.76789	steri_euk_Otu00819	Eukaryota	Dinoflagellata	Syndiniales	Dino-Group-I	Dino-Group-I-Clade-1	Dino-Group-I-Clade-1_X	Dino-Group-I-Clade-1_X_sp.	
0.79101			steri_prok_Otu00002	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	Ascidiaceihabitans		
0.96563			steri_prok_Otu00003	Bacteria	Proteobacteria	Alphaproteobacteria	SAR11_clade	Surface_1	Candidatus_Pelagibacter		
0.96563			steri_prok_Otu00007	Bacteria	Proteobacteria	Alphaproteobacteria	SAR11_clade	Surface_1	Candidatus_Pelagibacter		
0.93211			steri_prok_Otu00011	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	Planktomarina		
0.86791			steri_prok_Otu00012	Bacteria	Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	NS4_marine_group		
0.96563			steri_prok_Otu00015	Bacteria	Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	Formosa		
	0.94566	0.95443	steri_prok_Otu00018	Bacteria	Cyanobacteria	Cyanobacteria	SubsectionI	FamilyI	Synechococcus		
0.8466			steri_prok_Otu00022	Bacteria	Actinobacteria	Acidimicrobiia	Acidimicrobiales	OM1_clade	Candidatus_Actinomarina		
0.96563		0.81922	steri_prok_Otu00024	Bacteria	Bacteroidetes	Flavobacteriia	Flavobacteriales	Cryomorphaceae	Fluviicola		
0.99863		0.75029	steri_prok_Otu00038	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	Ascidiaceihabitans		
		0.99498	steri_prok_Otu00048	Bacteria	Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	Formosa		
0.96563		0.81127	steri_prok_Otu00050	Bacteria	Planctomycetes	Planctomycetacia	Planctomycetales	Planctomycetaceae	Blastopirellula		
0.77657			steri_prok_Otu00055	Bacteria	Proteobacteria	Betaproteobacteria	Methylophilales	Methylophilaceae	OM43_clade		
		0.81127	steri_prok_Otu00062	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	Ascidiaceihabitans		
0.78556			steri_prok_Otu00068	Bacteria	Proteobacteria	Alphaproteobacteria	Rickettsiales	SAR116_clade	Candidatus_Puniceispirillum		
		0.82946	steri_prok_Otu00069	Bacteria	Bacteroidetes	Flavobacteriia	Flavobacteriales	NS9_marine_group	NA		
0.96563			steri_prok_Otu00080	Bacteria	Bacteroidetes	Flavobacteriia	Flavobacteriales	Cryomorphaceae	NA		
0.96563			steri_prok_Otu00100	Bacteria	Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	NS4_marine_group		
		0.95443	steri_prok_Otu00136	Bacteria	Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	NS4_marine_group		
0.99863			steri_prok_Otu00147	Bacteria	Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	NA		
	0.77322	0.81127	steri_prok_Otu00170	Bacteria	Cyanobacteria	Cyanobacteria	SubsectionI	FamilyI	Synechococcus		
		0.83247	steri_prok_Otu00235	Bacteria	Proteobacteria	Alphaproteobacteria	Caulobacterales	Hyphomonadaceae	Hellea		

Supplementary table 6:

Taxonomy of the ASVs common to the three networks

Selected for subnetwork	Name	Kingdom	Division	Class	Order	Family	Genus	Species
	steri_euk_Otu00003	Eukaryota	Dinoflagellata	Syndiniales	Dino-Group-I	Dino-Group-I-Clade-1	Dino-Group-I-Clade-1_X	Dino-Group-I-Clade-1_X_sp.
Selected	**steri_euk_Otu00004**	**Eukaryota**	**Chlorophyta**	**Mamiellophyceae**	**Mamiellales**	**Bathycoccaceae**	**Bathycoccus**	**Bathycoccus_prasinos**
Selected	**steri_euk_Otu00005**	**Eukaryota**	**Chlorophyta**	**Mamiellophyceae**	**Mamiellales**	**Mamiellaceae**	**Micromonas**	**Micromonas_Clade-B.E.3**
	steri_euk_Otu00008	Eukaryota	Dinoflagellata	Syndiniales	Dino-Group-I	Dino-Group-I-Clade-1	Dino-Group-I-Clade-1_X	Dino-Group-I-Clade-1_X_sp.
Selected	**steri_euk_Otu00016**	**Eukaryota**	**Cryptophyta**	**Cryptophyceae**	**Cryptophyceae_X**	**Cryptomonadales**	**Teleaulax**	**Teleaulax_gracilis**
	steri_euk_Otu00017	Eukaryota	Cryptophyta	Cryptophyceae	Cryptophyceae_X	Cryptomonadales	Plagioselmis	Plagioselmis_prolonga
	steri_euk_Otu00022	Eukaryota	Chlorophyta	Mamiellophyceae	Mamiellales	Mamiellaceae	Micromonas	Micromonas_Clade-B.E.3
Selected	**steri_euk_Otu00024**	**Eukaryota**	**Ochrophyta**	**Pelagophyceae**	**Pelagomonadales**	**Pelagomonadaceae**	**Pelagomonas**	**Pelagomonas_calceolata**
	steri_euk_Otu00037	Eukaryota	Chlorophyta	Mamiellophyceae	Mamiellales	Mamiellaceae	Micromonas	Micromonas_Clade-B..4
	steri_euk_Otu00040	Eukaryota	Dinoflagellata	Syndiniales	Dino-Group-II	Dino-Group-II-Clade-13	Dino-Group-II-Clade-13_X	Dino-Group-II-Clade-13_X_sp.
	steri_euk_Otu00042	Eukaryota	Dinoflagellata	Dinophyceae	Gymnodiniales	Gymnodiniaceae	NA	NA
	steri_euk_Otu00044	Eukaryota	Dinoflagellata	Syndiniales	Dino-Group-III	Dino-Group-III_X	Dino-Group-III_XX	Dino-Group-III_XX_sp.
	steri_euk_Otu00056	Eukaryota	Stramenopiles_X	MAST	MAST-3	MAST-3E	MAST-3E_X	MAST-3E_X_sp.
	steri_euk_Otu00068	Eukaryota	Ochrophyta	Bacillariophyta	Bacillariophyta_X	Raphid-pennate	NA	NA
	steri_euk_Otu00072	Eukaryota	Ciliophora	Spirotrichea	Strombidiida	Tontoniidae_B	Pseudotontonia	Pseudotontonia_simplicidens
	steri_euk_Otu00085	Eukaryota	Ciliophora	Spirotrichea	Strombidiida_B	Strombidiida_B_X	Strombidiida_B_XX	Strombidiida_B_XX_sp.
Selected	**steri_euk_Otu00131**	**Eukaryota**	**Dinoflagellata**	**Dinophyceae**	**Gymnodiniales**	**Gymnodiniaceae**	**Gyrodinium**	**Gyrodinium_fusiforme**
Selected	**steri_prok_Otu00002**	**Bacteria**	**Proteobacteria**	**Alphaproteobacteria**	**Rhodobacterales**	**Rhodobacteraceae**	**Ascidiaceihabitans**	
Selected	**steri_prok_Otu00003**	**Bacteria**	**Proteobacteria**	**Alphaproteobacteria**	**SAR11_clade**	**Surface_1**	**Candidatus_Pelagibacter**	
Selected	**steri_prok_Otu00004**	**Bacteria**	**Proteobacteria**	**Alphaproteobacteria**	**SAR11_clade**	**Surface_2**	**NA**	
	steri_prok_Otu00005	Bacteria	Proteobacteria	Alphaproteobacteria	SAR11_clade	Surface_1	NA	
	steri_prok_Otu00007	Bacteria	Proteobacteria	Alphaproteobacteria	SAR11_clade	Surface_1	Candidatus_Pelagibacter	
Selected	**steri_prok_Otu00008**	**Bacteria**	**Bacteroidetes**	**Flavobacteriia**	**Flavobacteriales**	**Flavobacteriaceae**	**Tenacibaculum**	
Selected	**steri_prok_Otu00009**	**Bacteria**	**Cyanobacteria**	**Cyanobacteria**	**SubsectionI**	**FamilyI**	**Synechococcus**	
	steri_prok_Otu00010	Bacteria	Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	NS2b_marine_group	
	steri_prok_Otu00011	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	Planktomarina	
	steri_prok_Otu00012	Bacteria	Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	NS4_marine_group	
	steri_prok_Otu00014	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	Amylibacter	
	steri_prok_Otu00016	Bacteria	Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	NS5_marine_group	
	steri_prok_Otu00017	Bacteria	Proteobacteria	Gammaproteobacteria	Oceanospirillales	ZD0405	NA	
	steri_prok_Otu00019	Bacteria	Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	Tenacibaculum	
	steri_prok_Otu00020	Bacteria	Actinobacteria	Acidimicrobiia	Acidimicrobiales	OM1_clade	Candidatus_Actinomarina	
	steri_prok_Otu00021	Bacteria	Proteobacteria	Gammaproteobacteria	Oceanospirillales	SAR86_clade	NA	
	steri_prok_Otu00022	Bacteria	Actinobacteria	Acidimicrobiia	Acidimicrobiales	OM1_clade	Candidatus_Actinomarina	
	steri_prok_Otu00023	Bacteria	Proteobacteria	Gammaproteobacteria	Oceanospirillales	SAR86_clade	NA	
	steri_prok_Otu00024	Bacteria	Bacteroidetes	Flavobacteriia	Flavobacteriales	Cryomorphaceae	Fluviicola	
	steri_prok_Otu00026	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	PS1_clade	NA	
	steri_prok_Otu00030	Bacteria	Actinobacteria	Acidimicrobiia	Acidimicrobiales	OM1_clade	Candidatus_Actinomarina	
	steri_prok_Otu00038	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	Ascidiaceihabitans	
	steri_prok_Otu00039	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	NA	
	steri_prok_Otu00046	Bacteria	Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	NS4_marine_group	
	steri_prok_Otu00050	Bacteria	Planctomycetes	Planctomycetacia	Planctomycetales	Planctomycetaceae	Blastopirellula	

Supplementary table 7: Trophic type for eukaryote ASVs found in the yearly network

Kingdom	Class	Ecological function
Eukaryota	Bacillariophyta	Autotrophs
Eukaryota	Chlorodendrophyceae	Autotrophs
Eukaryota	Chrysophyceae	Autotrophs
Eukaryota	Colpodea	Nanoplankton grazers
Eukaryota	Cryptophyceae	Autotrophs
Eukaryota	Dictyochophyceae	Autotrophs
Eukaryota	Dinophyceae	Microplankton grazers
Eukaryota	Euglenozoa	Parasites
Eukaryota	Eustigmatophyceae	Autotrophs
Eukaryota	Filosa-Sarcomonadea	Parasites
Eukaryota	Filosa-Thecofilosea	Parasites
Eukaryota	Florideophyceae	Autotrophs
Eukaryota	Labyrinthulea	Parasites
Eukaryota	Litostomatea	Nanoplankton grazers
Eukaryota	Mamiellophyceae	Autotrophs
Eukaryota	MAST	Nanoheterotrophs
Eukaryota	MOCH	Autotrophs
Eukaryota	NA	Unassigned
Eukaryota	Oomycota	Parasites
Eukaryota	Pelagophyceae	Autotrophs
Eukaryota	Phaeodarea	Parasites
Eukaryota	Picozoa_X	Autotrophs
Eukaryota	Prasino-Clade-9	Autotrophs
Eukaryota	Prasino-Clade-VII	Autotrophs
Eukaryota	Prymnesiophyceae	Autotrophs
Eukaryota	RAD-B	Nanoplankton grazers
Eukaryota	Spirotrichea	Nanoplankton grazers
Eukaryota	Stramenopiles_XX	Unassigned
Eukaryota	Syndiniales	Parasites
Eukaryota	Telonemia_X	Unassigned
Eukaryota	Trebouxiophyceae	Autotrophs

Supplementary table 8: Taxonomical classification for prokaryote ASVs found in the yearly network

Kingdom	Class	Legend
Archaea	Marine_Group_I	Archaea
Archaea	Thermoplasmata	Archaea
Bacteria	Acidimicrobiia	Acidimicrobia
Bacteria	Alphaproteobacteria	Alphaproteobacteria
Bacteria	Betaproteobacteria	Betaproteobacteria
Bacteria	Cyanobacteria	Cyanobacteria
Bacteria	Cytophagia	Cytophagia
Bacteria	Deltaproteobacteria	Deltaproteobacteria
Bacteria	Flavobacteriia	Flavobacteria
Bacteria	Gammaproteobacteria	Gammaproteobacteria
Bacteria	Planctomycetacia	Planctomycetacia

Addendum

My contribution:

Concerning this chapter, I participated in the biweekly sampling during the winter months, as well as the weekly sampling during the rest of the year. I helped fill the carboys with sea water on the boat and filter the sea water once back at the laboratory. I also did the DNA extractions and the PCRs (under the supervision of team members). The DNA sequencing was done by the Genotoul sequencing platform. Regarding the data analysis, I was in charge of sequence analysis, investigating and exploiting the data sets and creating the figures. Finally, I helped write the article with the co-authors.

Corrections to the chapter:

The reviewers suggested adding a description of the community composition for this chapter:

We can see here that, during the winter, the eukaryotic data set is dominated by Syndiniales (26.97%), Mamiellophyceae (16.56%), Dinophyceae (14,24%) and Baccillariophyta (12.94%) (Table 1). Syndiniales had similar number of reads in 2015 and 2016, but had a higher number of reads in 2017. Mamiellophyceae on the other hand, showed similar number of reads and trend in 2015 and 2016. They were abundant at the beginning of the year, but then were less abundant and the end of the sampling period. Surprisingly, in 2017 Mamiellophyceae showed relatively low number of reads (Figure 1).
Additionally, the prokaryote data set was composed mainly of Alphaproteobacteria (38.97%), Flavobacteria (25.82%) and Gammaproteobacteria (15.18%) (Table 1). Alphaproteobacteria showed high number of reads during the 3 winters of sampling. Flavobacteria had similar number of reads in 2015 and 2016, but displayed a higher number of reads in 2017. Moreover, Gammaproteobacteria had a higher number of reads in 2015 than 2016 or 2017 (Figure 1).

Furthermore, the reviewers asked for other clarifications. Here is a list of these clarifications and were to find them in the chapter:

- Investigating ecological functions in the networks: page 14 (of this chapter)

- Better explanation of the MIC: page 17 (of this chapter)

Table 1: Number and percentages of reads per group

Eukaryotes			**Prokaryotes**		
Class	**Number of reads**	%	**Class**	**Number of reads**	%
Syndiniales	53096	26.97	Alphaproteobacteria	129596	38.97
Mamiellophyceae	32609	16.56	Flavobacteria	85860	25.82
Dinophyceae	28045	14.24	Gammaproteobacteria	50490	15.18
Bacillariophyta	25473	12.94	Cyanobacteria	16059	4.83
Spirotrichea	16179	8.22	Acidimicrobiia	15204	4.57
Cryptophyceae	7837	3.98	Marine Group I	6605	1.99
MAST	7205	3.66	Planctomycetacia	5606	1.69
Prymnesiophyceae	3471	1.76	Thermoplasmata	4229	1.27
Pelagophyceae	2834	1.44	Betaproteobacteria	4169	1.25
Labyrinthulea	2249	1.14	Deltaproteobacteria	2099	0.63
Dictyochophyceae	2085	1.06	Verrucomicrobiae	2066	0.62
Chrysophyceae	1230	0.62	Cytophagia	1857	0.56
MOCH	1039	0.53	Opitutae	1441	0.43
Other	13535	6.87	Other	7285	2.19

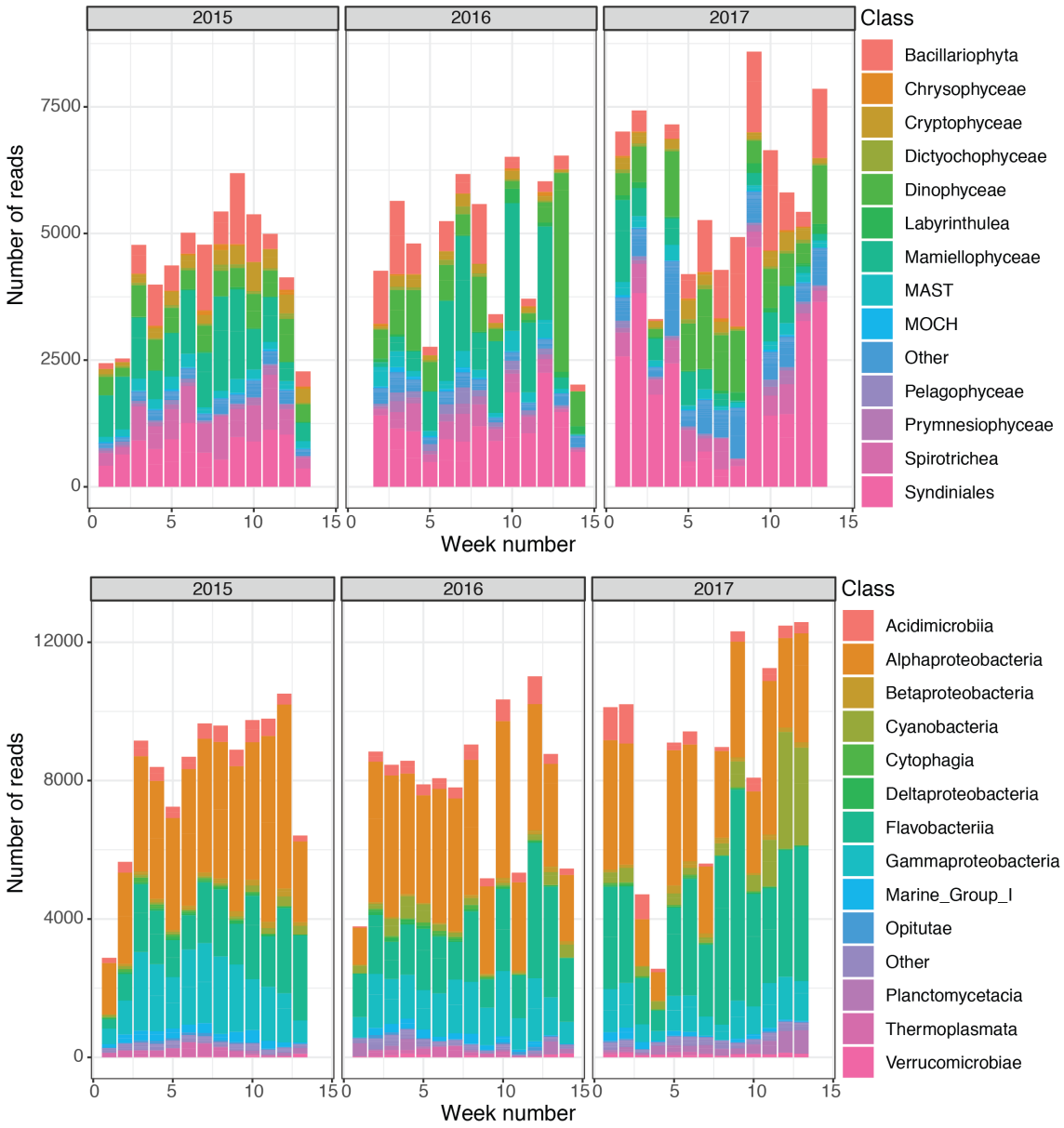


Figure 1: Number of normalized reads per year per group for Eukaryotes (top) and Prokaryotes (bottom).

3.2 >3µm time series data

My contribution: Concerning the 3µm filters data, I regrouped the samples from 2013 to 2017, carried out multiple tests to allow for sufficient DNA extractions. Indeed, the QUIAGEN kit, used for the sterivex of the time series, did not yield enough DNA for the 3µm filters. Once the DNA extraction protocol optimized, I carried out the DNA extractions and the PCRs (with the help of Jean-Claude Lozano). The DNA sequencing was done by the Genotoul sequencing platform. Similarly to the sterivex, I was in charge of the sequence analysis and I did some initial data analysis on the 3µm filter data set. Originally, the 3µm filter data (2015-2017) was supposed to be analyzed with the sterivex data (2015-2017), but this complicated the message of the chapter.

Context

In order to have a better understanding of the microbial ecology at SOLA, it was decided, in 2013, to keep the 3µm pre-filters. This would allow to look at a larger picture of marine microbes. Indeed, before 2013 only the "free-living" (between 3µm and 0.22µm) fraction was kept and analyzed, which can be quite limiting when one studies microbial eukaryotes or particle attached prokaryotes. In this part of the chapter I present preliminary results concerning the 3µm filter data from 2013 to 2017. The DNA was extracted from these filters and the sequencing allowed to yield data concerning eukaryotes and prokaryotes.

Material and methods

Sampling Surface water (3m) was collected at the Service d'Observation du Laboratoire Arago (SOLA) sampling station in the Bay of Banyuls, North Western Mediterranean Sea, France. Samples were collected roughly once a week from January 2015 to March 2017. However, during the periods of January – March 2015, January – April 2016 and December 2016 – March 2017 the sampling frequency was increased to twice a week. However, during 2013 and 2014 sampling was done twice a month on average. Niskin bottles were used to obtain seawater that was stored in 10 L carboys until arrival to

the laboratory. 5 L of seawater were prefiltered through 3 µm pore-size polycarbonate filters (Merck-Millipore, Darmstadt, Germany), and the microbial biomass was collected on 0.22-µm pore-size GV Sterivex cartridges (Merck-Millipore). Both sets of filters were stored at –80 °C until nucleic acid extraction.

DNA extraction and sequence analysis: The samples were extracted with the Nucleospin plant II kit (Macherey-Nagel). Specific primers were used to target either the eukaryotic V4 region (TAReuk_F1 [5'-CCAGCASCYGCGGTAATTCC] and TAReuk_R [5'-ACTTTCGTTCTTGATYRATGA]) or the prokaryotic V4-V5 region (515F-Y [5'-GTGYCAGCMGCCGCGGTAA] and 926R [5'-CCGYCAATTYMTTTRAGTTT]). The standard pipeline of the DADA2 (version 1.6) was used to do the analysis of the raw sequences. The parameters used for eukaryotes were: trimLeft=c(20, 21) ,truncLen=c(250,220), maxN=0, maxEE=c(2,5), truncQ=2. And for prokaryotes: trimLeft =c(19, 20) ,truncLen=c(240,200),maxN=0, maxEE=c(2,5), truncQ=2. The taxonomy assignments were done with PR2 v.4.10.0 database for eukaryotes and with SILVA v.128 database for prokaryotes. Taxa belonging to the supergroup "Opisthokonta" were removed from the eukaryote dataset. Comparably, taxa belonging to eukaryotes were removed from the prokaryote dataset. Samples containing less than 10000 reads and 4500 reads were removed from the eukaryote (3 samples removed) and prokaryote (3 samples removed) dataset respectively.

Results

As explained in the methods section of the second and third chapter, the time series 0.22µm filters were actually pre-filtered on 3µm filters. These pre-filters contain valuable information on community composition and seasonality of large eukaryotic and prokaryotic cells. Unfortunately, before 2013 the pre-filters were discarded after the filtration step of the protocol. For prokaryotes, the size fraction $>$ 3µm is often considered the "particle attached" fraction. As prokaryotes tend to be smaller than 3µm, they need to stick together or to a particle in order to accumulate on the 3µm filters.

The sequence abundance data reveals that there is a dominance of large eukaryotic

cells such as dinoflagellates (41.74%), syndiniales (22.27%) and diatoms (12.88%). Smaller cells, such as mamiellophyceae (dominant in the < 3µm size fraction), make up only a small fraction (3.75%) of the total sequence abundance in this dataset (Table 1).

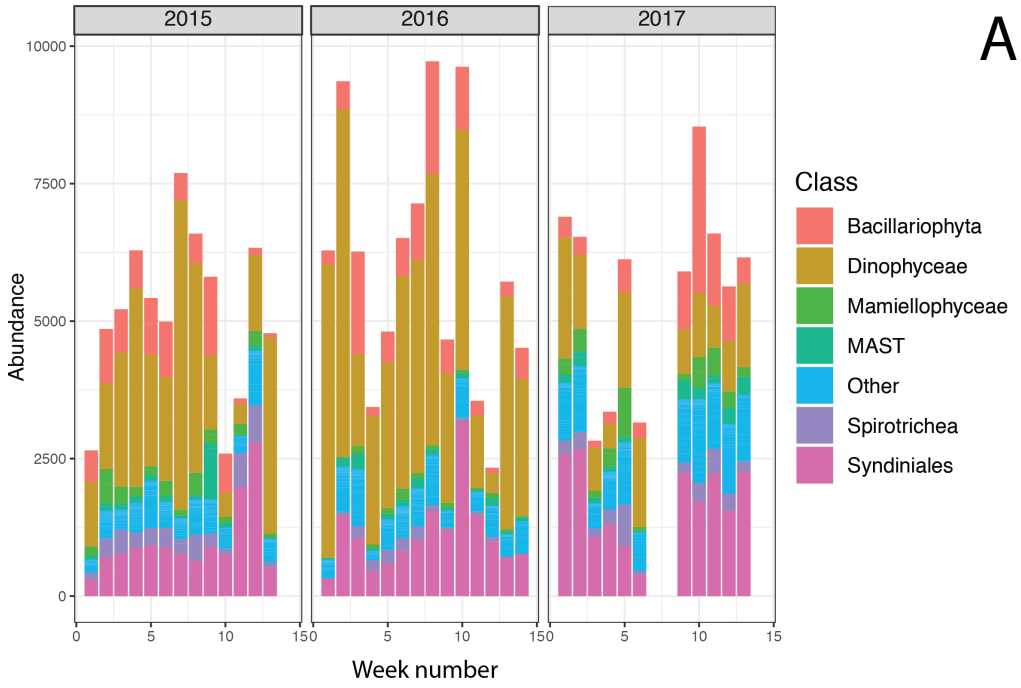
Concerning the prokaryote dataset, the dominant groups are flavobacteria (34.92%), alphaproteobacteria (19.14%) and planctomycetacia (13.32%) (Table 1).

For both the eukaryotes and prokaryotes, there are no major differences of sequence abundance between samples (Fig. 3). The main difference can probably be attributed to the different depth of sequencing between the samples (i.e. the overall total number of reads per sample).

Principal Coordinates Analyses (PCoAs) were done on both data sets (Fig. 4). The months of November to April of the eukaryote communities displayed a higher dispersal compared to the warmer months of May to October. On the other hand, the prokaryotic communities grouped together during most months of the year. The axis show a better explanation percentage for the prokaryote dataset (24% and 12.5%) than the eukaryote dataset (20.3% and 9%) (Fig. 4).

Table 1: Number and percentages of reads per group found during winter on the 3µm filters

Eukaryotes			**Prokaryotes**		
Class	**Number of reads**	**%**	**Class**	**Number of reads**	**%**
Dinophyceae	88667	41.74	Flavobacteria	75374	34.92
Syndiniales	47305	22.27	Alphaproteobacteria	41321	19.14
Bacillariophyta	27356	12.88	Planctomycetacia	28747	13.32
Spirotrichea	9243	4.35	Gammaproteobacteria	20146	9.33
Mamiellophyceae	7964	3.75	Cyanobacteria	17918	8.30
MAST	5355	2.52	Verrucomicrobiae	8013	3.71
Other	26537	12.49	Acidimicrobia	7610	3.53
			Other	16729	7.75



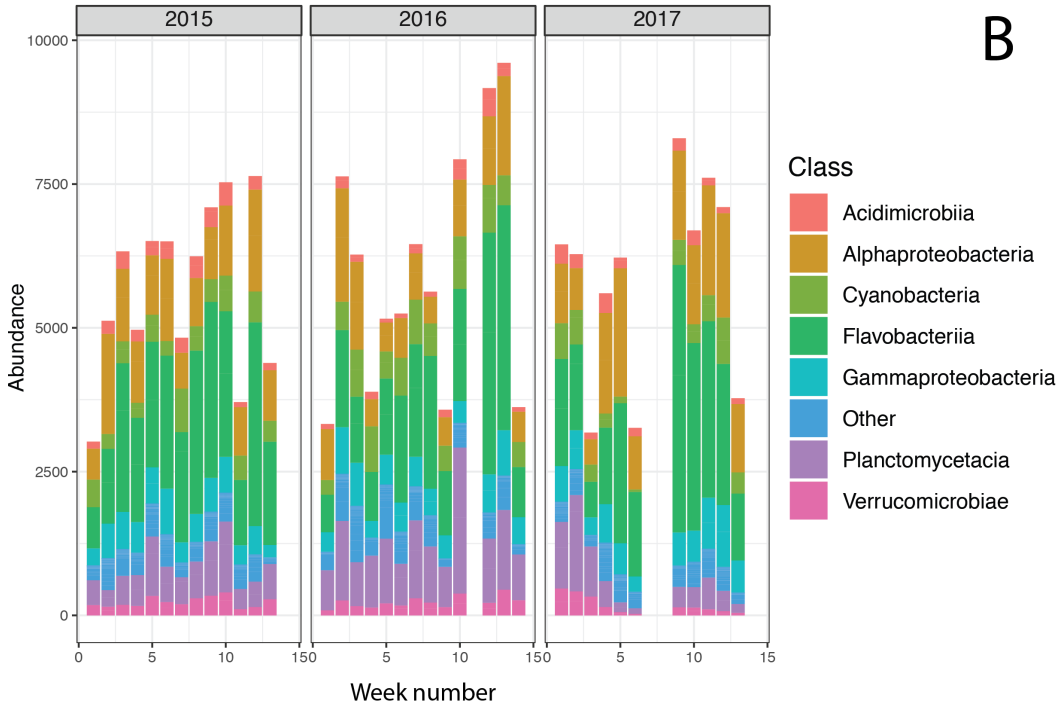


Figure 3: Sequence abundance for 3µm filters during winter from 2015 to 2017 for the eukaryotes (A) and prokaryotes (B).

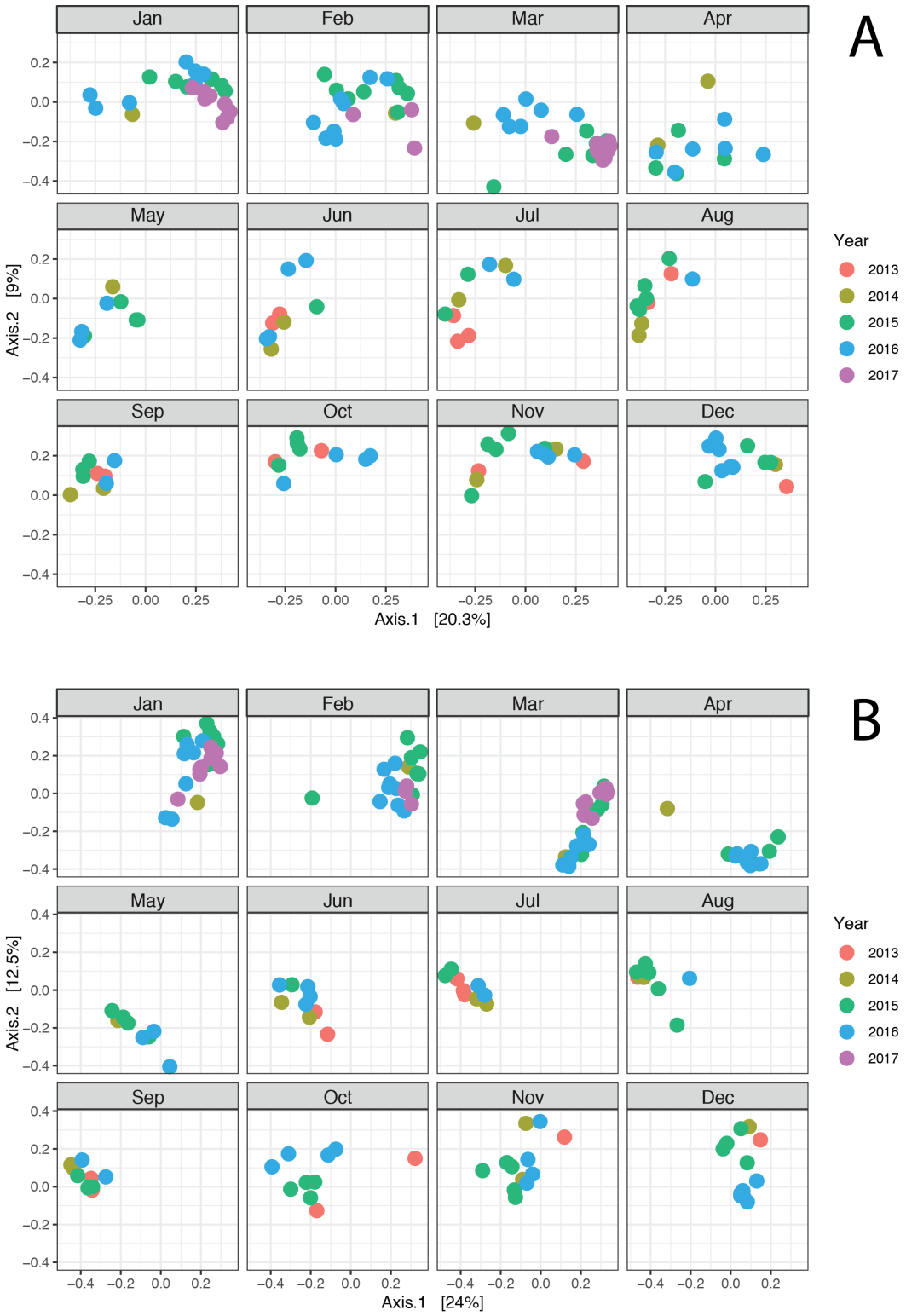


Figure 4: Principal Coordinate Analysis (PCoA) for eukaryote (A) and prokaryote community composition (B) found on 3µm filters from 2013 to 2017.

Chapter 4

Results of microcosms experiments

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Prologue

This chapter is a compilation of microcosms results acquired during my PhD that, due to multiple factors, were not exploited during that time.

My contribution: Concerning the microcosms, I participated in the sea water sampling. Once the sea water arrived in the laboratory, I was in charge of pre-filtering (3µm filters) the samples, managing the incubators, sub-sampling each condition daily and, at the end of the incubation period, the filtration on sterivex (0.22µm). Furthermore, I carried out the flow-cytometry, DNA extraction and sequence analysis for all the microcosm samples. All of this, as well as designing the experiments was done under supervision of my PhD advisor.

All things considered, this chapter represents a large amount of data: 30 weeks of microcosm flow cytometry and DNA analysis data. Unfortunately, due to an initial lack of significant results for the flow cytometry data, we did not have the resources to properly exploit this data at the time.

Microcosms experiments

Context

The initial objective of the microcosms was to individually test the effect of temperature and photoperiod variations on natural microbial communities. With time, we also tested the effects of light attenuation and the enrichment in different vitamin conditions.

The microcosms experiments were divided into four categories:

• **Solstice:** The solstice experiments are named this way because they were carried out during the four yearly solstices (spring, summer, fall, winter). We carried out the same experiment at multiple moments of the year to test *(i)* the response of different initial populations to identical light and temperature conditions and *(ii)* to investigate moments of nutrient limitations. The average day length and sea surface temperature were calculated for the months of March, June, September and December. These values were used to recreate the conditions of March, June, September and December in the incubators, regardless of the moment of sampling.

• **Depth:** These experiments were designed to test the effect of light attenuation on microbial communities. After measuring light attenuation at several depth at SOLA, we recreated similar light attenuation levels in the incubators. The conditions tested were simulations of 0, 3, 12 and 24 meters of depth with light intensities of 100%, 50%, 12.5% and 0.5%, respectively.

• **Temperature and photoperiod:** Here we wanted to test the effect of temperature variations and increases in photoperiod. Temperature variations were done by either increasing or lowering the temperature of the incubators by 2°C increments. Additionally, increases in photoperiod were done by selecting the light intensity of the following month or more. For example if the sampling was done in March, we would test the effects of photoperiod of March, April and May.

• **Vitamin:** Another condition that we tested was the effect of different vitamins and precursors on microbial communities. We tested the addition, at two concentrations(1nM and 1µM), of vitamins B1, B12, or both, or the addition of cHET, HMP,

or both. The compounds cHET (2-(2-carboxy-4-methylthiazol-5-yl)ethyl) and HMP (4-amino-5-hydroxymethyl-2-methylpyrimidine) are precursors to vitamin B1.

Here is a list of the different microcosm experiments carried out during my PhD:

Table 1: Summary of microcosms experiments

Conditions	Solstice	Depth	Temperature and photoperiod	Vitamin
Experimental replicates	8	3	10	6
Number of sterivex	56	24	90	69
Approx. number of flow cytometry samples	1350	580	2160	1660

Material and methods

3µm filtration: Seawater was sampled at the SOLA sampling station at a depth of 3 meters. This water was then taken back to the laboratory and filtered on 3µm filters (millipore) to remove predators. 400 mL of filtered seawater was transferred to cell culture flasks which were then placed in light and temperature controlled incubators. Experiments were carried out in biological triplicates.

Microbial abundance: Microbial abundance was determined by flow cytometry (Accuri C6 sampler). Daily samples of 1.5 ml were fixed with 60µl of glutaraldehyde then stored at -80°C awaiting flow cytometry analysis.

0.2µm filtration: At the end of the incubation period, triplicates were pooled together and filtrated out on sterivex filters (0.2µm) to accumulate the microbial biomass and were stored at -80°C until DNA extraction. The reason for pooling the triplicates was to get sufficient microbial biomass for the DNA extractions.

DNA extraction and sequence analysis: The samples were extracted with the Nucleospin plant II kit (Macherey-Nagel). Specific primers were used to target either the eukaryotic V4 region (TAReuk_F1 [5'-CCAGCASCYGCGGTAATTCC] and TAReuk_R

[5'-ACTTTCGTTCTTGATYRATGA]) or the prokaryotic V4-V5 region (515F-Y [5'-GTGYCAGCMGCCGCGGTAA] and 926R [5'-CCGYCAATTYMTTTRAGTTT]). The standard pipeline of the DADA2 (version 1.6) was used to do the analysis of the raw microcosm sequences. The parameters used for eukaryotes were: trimLeft=c(20, 21) ,truncLen=c(280,230), maxN=0, maxEE=c(2,2), truncQ=2. And for prokaryotes: trimLeft =c(19, 20) ,truncLen=c(240,200),maxN=0, maxEE=c(2,5), truncQ=2. The taxonomy assignments were done with PR2 v.4.10.0 database for eukaryotes and with SILVA v.128 database for prokaryotes. Taxa belonging to the supergroup "Opisthokonta" were removed from the eukaryote dataset. Comparably, taxa belonging to eukaryotes were removed from the prokaryote dataset. Samples containing less than 1000 reads were removed from the eukaryote (1 sample removed) and prokaryote (3 samples removed) datasets.

Results

Unfortunately, only few of the microcosm experiments showed interesting results. As an example, a temperature variation experiment, done during the month of March, will be discussed here, but it should be noted that similar flow cytometry and sequencing data exists for all the microcosm experiments.

We observed here that temperature promotes growth (Fig. 1) for both cyanobacteria and picoeukaryotes. However, at lower temperatures, there seemed to be a limitation in growth for picoeukaryotes. It can also be noted that similar results were obtained in both normal and enriched conditions ($+NO_3/PO_4$). This suggests that the environment was not limited in nutrients during the initial sampling.

With the sequence abundance data we can observe a different story. The chlorophyta demonstrated an increase in sequence abundance at low temperature in both the normal and enriched conditions (Fig. 2A). However, at higher temperatures the ochrophyta (diatoms) showed an increase in sequence abundance, especially in non-enriched conditions.

The flow cytometry data and the sequencing data do not show similar results. Indeed, we observed very little growth at low temperature, but an increase of sequence abun-

dance. This can be explained by different factors. First of all this could result from the normalization of the sequencing data. Or this could come from a bias in the amplification step before the sequencing. Furthermore, DNA sequencing can not inform us if the cells are active, but only if the DNA was present. These reasons could explain why we do not observe a similar trend in these results.

Overall, picoeukaryotes seemed to be better suited to survive at lower temperatures compared to diatoms, for example.

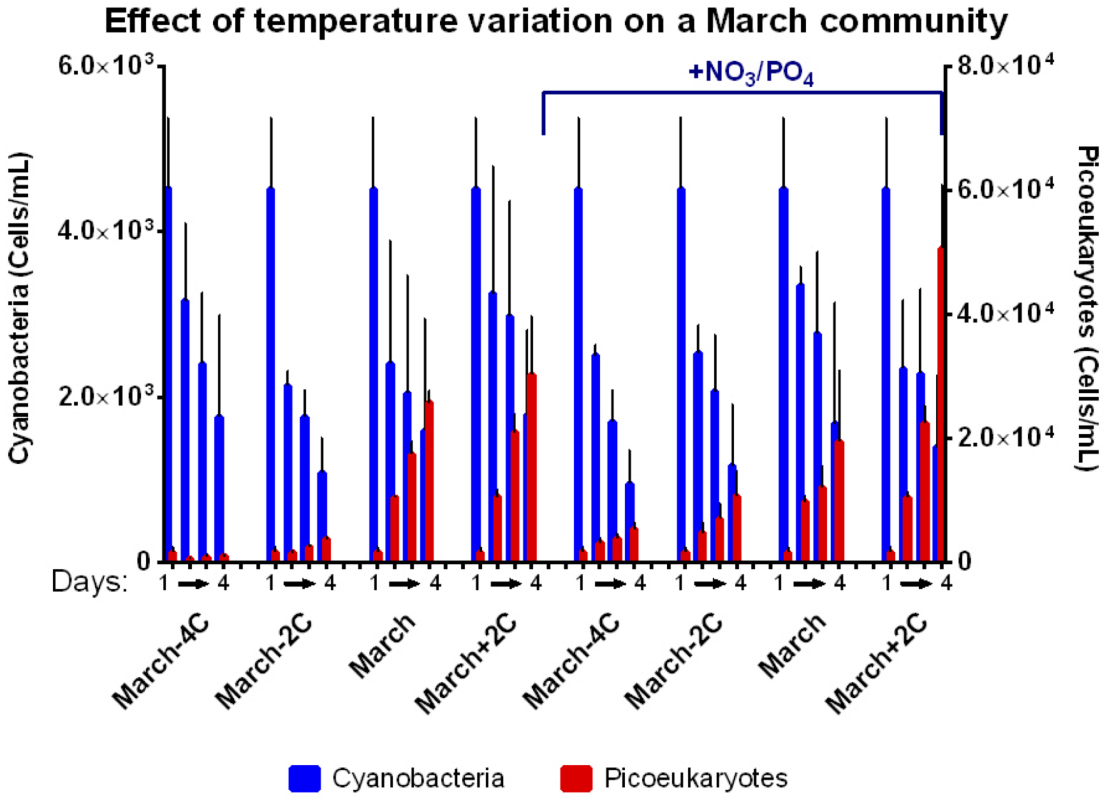

Figure 1: Cell density for picoeukaryotes and cyanobacteria during a 4 day incubation in a light and temperature controlled microcosm. Temperature variations were tested on natural communities in normal or enriched conditions.

Table 2: Microcosm conditions and corresponding temperatures

Condition	Temperature
March -4C	7°C
March -2C	9°C
March	11°C
March +2C	13°C

Concerning the prokaryotes, there is only sequencing data available. Here we can

observe a rather stable trend in the different conditions tested. At higher temperatures, namely March 11°C non-enriched as well as 11°C and 13°C enriched, there is an increase in sequence abundance, but the proportions between the different groups seem to be conserved. Once again, there are not many differences between the enriched and the non-enriched conditions (Fig. 2B).

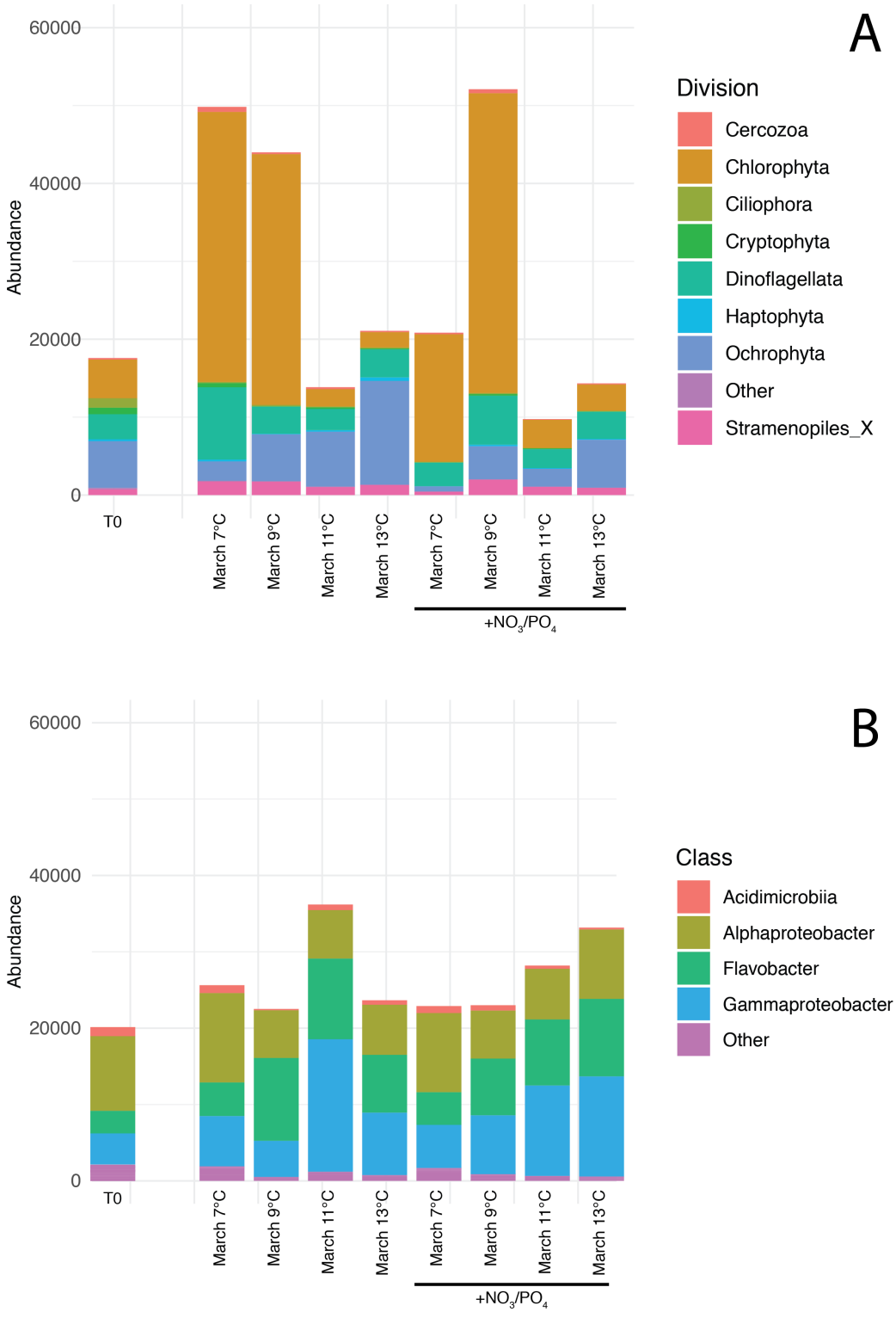

Figure 2: Sequence abundance of eukaryotes (A) and prokaryotes (B) in a microcosm experiments. T0 corresponds to starting moment of the experiment, sampled at SOLA. The legend is the taxonomy of eukaryotes at the division level and prokaryotes at the class level

Chapter 5

Discussion and perspectives

Discussion

It is now well established that marine microbial communities display seasonal reoccurrences in large areas of the world ocean (*59*, *88*, *89*). Here, we wanted to investigate the seasonality of the microbial community in a coastal site, characterized by fluctuating environmental parameters linked to meteorological events. Thanks to a decade long sampling effort, we were able to demonstrate that some marine microbes display a yearly rhythm despite sporadic freshwater and nutrient supply by nearby rivers (Chapter II). Furthermore, improving sampling frequency from 2015 to 2017 to twice a week, allowed us to demonstrate that microbes were capable of switching to novel co-occurring neighbors when environmentally challenged (Chapter III). Going to an even finer scale, microcosms experiments showed that temperature affected community composition by promoting or limiting growth of specific groups. This could potentially account for the bloom dynamics of *Bathycoccus prasinos*, a dominant seasonal microalga, found at our study site, which blooms around the minimum of temperature every year (Chapter IV).

5.1 Reviewing ten years of results

Roughly a decade of sampling data allowed us to show that several marine microbes demonstrated yearly rhythmicity. Taking a step back from the data, we can ask ourselves: where does this rhythmicity come from and what is its ecological meaning? Furthermore, what can physiological studies tell us about these rhythmic species?

5.1.1 Driving forces

Abiotic factors

Identifying yearly rhythms of abundance in a seven-year time series has led us to ask what the underlying physical and chemical drivers of this rhythmicity are. Firstly, abiotic factors such as light and temperature had the strongest effect on community structure and dynamics. Considering the light requirements that autotrophs have to produce organic matter through photosynthesis, it is not surprising to find light, and therefore photoperiodism (day length), as a main structuring factor. Day length is a highly reproducible factor driving seasonality. Additionally, although inter-annual variations were observed, the temperature trend did not increase throughout the seven-year time series (Fig. 5.1).

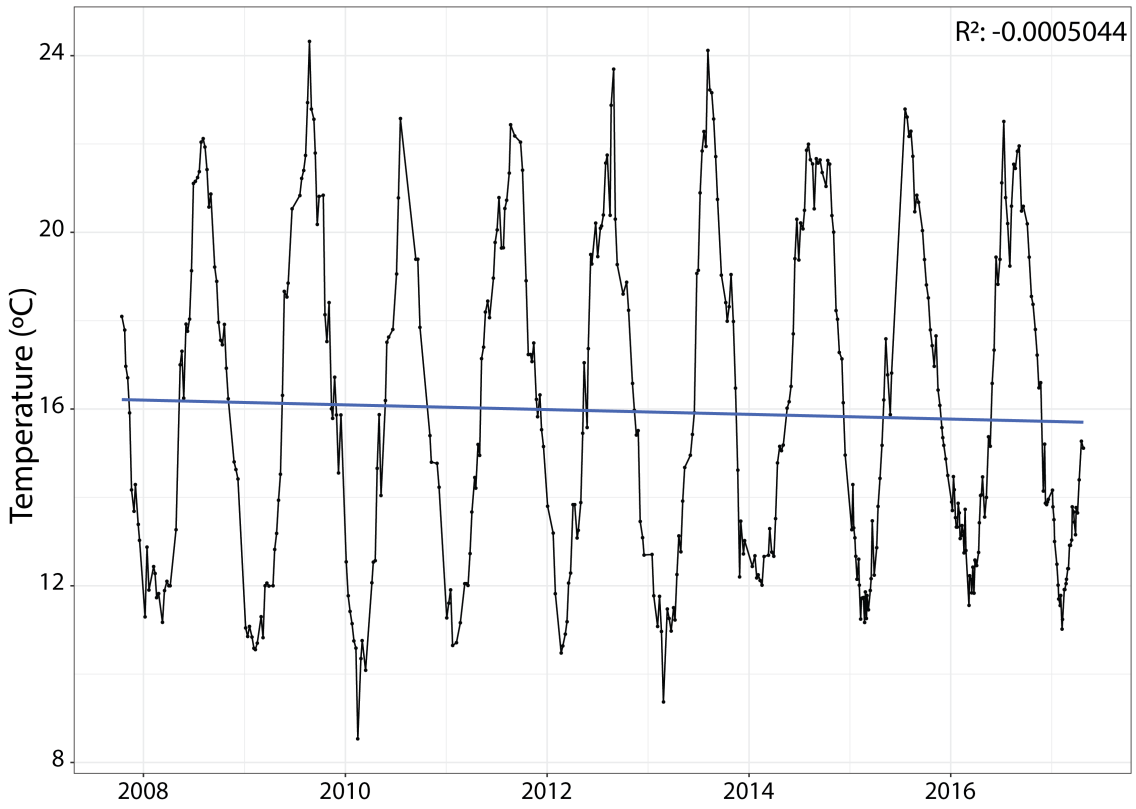

Figure 5.1: **Seasonality at SOLA.** The sea surface temperature is stable throughout the time series.

It is therefore conceivable that light and temperature cycles play an important role in driving biological rhythms. These biological rhythms most probably involve circadian clocks, which are autonomous timekeeping molecular mechanisms that orchestrate bio-

logical and physiological processes along the 24-hour day/night cycle. This enables living organisms to anticipate the predictable night/day and day/night transitions along the year. Despite the fact that the involvement of clock components for all marine microbial species remains to be formally demonstrated, it is plausible that they play a role in photoperiodism. For example, a minimal clock, involving two master clock genes, was found in the mamiellophyceae *Ostreococcus tauri* (*90*). And with respect to prokaryotes, the presence of a functional circadian clock has been shown in the cyanobacterium *Synechococcus* (*91*).

In the second chapter, we also showed that some heterotrophic prokaryotes, including the dominant order of SAR11, exhibited robust annual rhythms. Additionally, evidence for the presence of circadian rhythms and circadian clocks in heterotrophic bacteria in culture remains scarce. It is therefore tempting to speculate that annual rhythms of microbial heterotrophs could be indirectly controlled by their possible interactions with autotrophs through trophic interactions (see below). The annual patterns of phototroph occurrences would be compatible with this hypothesis, since picoeukaryotes are dominant from autumn to spring, and cyanobacteria during the summer. However, experimental evidence for both circadian rhythms of heterotrophic bacteria in the sea, and of a possible entrainment of heterotrophs by autotrophs, is still lacking.

Biotic factors

Biotic interactions, on the other hand, are less obvious and more complex to study, since they are rarely directly observable (*59*). Positive and negative correlations can reflect not only possible interactions, but also changes in shared environmental niches. Intricate trophic interactions and marine microbial metabolisms are not well understood and have been observed to be dynamic, as the organisms adapt to changing environmental factors (*92*). Thankfully, with the increased taxonomical and temporal resolution of time series, the inferred co-occurrences are becoming more and more robust, but the type of interactions remain to be deciphered.

Among possible inferred interactions, vitamin auxotrophy and vitamin exchanges be-

tween heterotrophs and autotrophs provided an interesting case study. Metagenome-assembled genomes originating from estuaries, marine and freshwater samples revealed that many bacterioplankton are vitamin B_1 auxotrophs (*93*). Auxotrophy for vitamin B_1 was also demonstrated in phytoplankton (*47*). This would suggest that certain marine microbes rely on exogenous vitamin B_1 (or its precursors). With this knowledge, we hypothesized that the co-occurrences observed between SAR11 and *Micromonas* could result from a mutual need for vitamin B_1, rather than an ecological interaction (Chapter II). However, we cannot rule out that, in natural conditions, the observed co-occurrences between *Micromonas* and SAR11 could also stem from several other factors.

Besides, trophic interactions remain a complex subject and most probably involve multiple exchanges and partners, as illustrated in a recent study that demonstrated a mutualistic interaction between *Ostreococcus tauri* and *Dinoroseobacter shibae* (*94*). The microalgae and the bacteria traded vitamins B_1 and B_{12}, provided by the bacteria to the microalga, and in return vitamins B_3 and B_7 were supplied by *Ostreococcus* to *Dinoroseobacter* to alleviate their mutual auxotrophy (*94*).

Grazing is another biotic interaction that has a massive impact on community dynamics. This top-down control is mainly visible towards the end of a winter bloom, since that is the moment when predators have large amounts of available prey (*82*). That being said, a study showed that predation did not have an impact on community composition (*95*). Clearly, there is still much to learn concerning trophic interactions in marine microbes.

In the third chapter, the radar plots showed a co-occurrence between mamiellophyceae and ciliates, the latter being known predators of phytoplankton (*96*). It is very likely that this observed co-occurrence is in reality a predator/prey relationship. Interestingly, the predator/prey interactions (observed as first neighbor in networks) were highly dependent on environmental conditions, highlighting the complexity of interactions between biotic and abiotic parameters.

To expand our understanding of microbial interaction in our coastal system, it would be useful to integrate a time-lagged aspect during the network analysis. For example, at the San Pedro sampling site they use the local similarity analysis (*97*) that allows

to identify significant time-delayed co-occurrences happening in their microbial networks (*54*). Moreover, with this method, they have investigated top-down control on microbial communities (*98*), species succession (*69*) as well as high resolution microbial interactions (*71*, *99*).

5.1.2 Physiological feedback

In order to obtain physiological responses, microcosms with simplified communities are powerful tools at our disposal. This has lead us to investigate the complex interaction between light and temperature and how these factors impact different groups of organisms present in the Bay of Banyuls (Chapter IV). One strain of *Bathycoccus prasinos*, two strains of *Ostreococcus mediterraneus* and four strains of *Ostreococcus tauri* were used to study physiological responses to different light and temperature conditions. The strains behaved differently and demonstrated preferential light and temperature niches. Temperature was the main factor that influenced growth and photosynthetic capabilities of the strains. Indeed, at low temperature, responses to light were strain-dependent, but when the temperature was increased, all strains responded similarly to light. The temperature increase seemed to alleviated a potential photoinhibition under long photoperiods at low temperature.

By simplifying the complexity of the system, researchers can obtain better insight into what is happening in the field. For example, it would seem that we observed a salinity niche for *Flavobacteria* in the third chapter. However, field data and network analysis alone cannot verify this. Furthermore, variations in salinity are a complex subject since this generally involves the mixing of the water column, which is dependent on multiple factors, such as wind direction and strength (*100*). That is why recreating these conditions in the laboratory and testing different salinity levels in microcosm would be a realistic investigation that could demonstrate preferred interactions between species. Then, isolating these species and carrying out molecular studies would allow for a high-resolution answer, or at least a better understanding of the ongoing molecular processes.

5.1.3 Ecological interpretations

Comparing the findings from the seven-year low resolution and the three-year high resolution studies has been very informative. While the long time series revealed robust annual rhythms for specific ASVs, suggesting a partial resilience of microbial communities, the short time series, in contrast, highlighted that the community networks were highly affected by salinity and temperature variations. Both findings may appear contradictory. However, after investigating the co-occurrences of the rhythmic species found during the high-resolution sampling, such as *Micromonas*, *Bathycoccus* and SAR11, we observed that these species were switching to novel neighbors when faced with environmental challenges.

Even though irregular abiotic factors, such as nutrient or salinity, do not impact the overall rhythmicity of specific species (Chapter II), they forced these same species to switch their first neighbors when environmental perturbations arose (Chapter III). Why was there a switch? Could it be possible that co-occurrences were altered because of changes in shared niches (*i.e.* modification of vitamin or nutrient levels)? Or instead, is it possible that these co-occurrences were random and did not have any ecological meaning and that they did not involve any possible biological interactions? Or, moreover, was it because other species could fulfill similar functions? Different species could produce the same compound but be present one year and not the other, due to variations in environmental factors. In other words, autotroph/heterotroph consortia would not rely on specific interactions between defined microorganisms but rather on functional interactions between equivalent microorganisms.

This hints towards functional redundancy, a highly controversial subject in microbial ecology (*101*). Does an ecosystem need specific species to function? Or just specific functions to be carried out, regardless of who is undertaking that role? Is this what we could be showing since specific interactions change yearly, but overall dynamics of rhythmic species is conserved? Unfortunately, there is limited knowledge concerning this point, as coastal sites have either acknowledged the importance of the question (*40*) or just assumed that OTUs demonstrated functional redundancy, but displayed temporal preferences according to their ecological niches (*58*). Metagenomic and metatranscriptomic

studies could help answer these questions concerning functional redundancy in the future.

5.1.4 Microbial seasonality

Seasonality could help maintain diversity

Species that carry out the same function could be maintained because they do not fulfill that function at the same moment of the year (*102*). Photosynthesis, for example, is carried out mainly by mamiellophyceae in the winter, but during the summer, it is carried out by cyanobacteria. This oscillation of abundance can be observed in the flow cytometry data in chapter 2. Despite this very broad example, as mamiellophyceae are not going to replace cyanobacteria any time soon (or vice versa), we could hypothesize, at a finer level, competing organisms carrying similar functions but that thrive at different moments of the year, or that are not suceptible to the same predators. Thus the seasonality of these organisms, or at least the factors driving their seasonality, would allow for a greater diveristy of marine microbes.

Seasonality at the gene level

Another aspect of seasonality that would deserve a deeper investigation is the seasonality of gene transcription. Diel variations were observed for eukaryotes, bacteria and archaea (*103*). Furthermore, they speculated that since short time scale variations lead to changes in microbial communities, they could also lead to seasonal or monthly variations (*104*). Indeed, understanding these short time scale variations would allow for a better understanding of broader and seasonal variations.

Why are some species not rhythmic?

Another point to ponder is why are some species not rhythmic? Indeed, in the second chapter we showed a finite number of rhythmic microbes, consequently, that implies that some microbes are not rhythmic. If we put aside the possible methodological biases (PCR primers, sequence analysis or the LSP), what does it mean to be "not rhythmic" ?

One possibility is that these species could be opportunists that only arise when conditions are favorable. For example, some species could display a bloom or bust behaviour and take advantage of sudden yet irregular influxes of nutrients during flooding events of the Baillaury river. Or resting stages produced by phytoplankton could come out of dormancy during these high nutrient moments as well (*105*). Additionally, the growth of these "non rhythmic" organisms could hinge on a delicate balance between nutrient and predator levels. Perhaps, for these species, the moment when growth overcomes loss only happens sporadically.

Furthermore, a study offers that it is not necessarily the environmental factors that make the organisms seasonal. Predator/prey models with multiple species suggest that interannual variability could arise without variations in external conditions (*106*). This means that seasonality could result from a fine balance between intrinsic parameters such as the biological clock, even though it is entrained by robust drivers (e.g. photoperiod) and less rhythmic drivers (e.g. nutrients supply from the rivers or sediment resuspension). This implies that for some species, which growth depends on both day length and nutrient supply, the annual rhythms may be skewed by the irregular nutrient supply driver. An example of this are diatoms that are strongly influenced by nutrient supply and they did not appear to be rhythmic in our analysis. On the other hand, Mamiellophyceae are an ideal system to study annual rhythms as they have a strong seasonal pattern. In the Bay of Banyuls, a nutrient influx usually occurs in April/May, just after the main blooms of *Micromonas* and *Bathycoccus*. Some years there is a double peak of *Bathycoccus*, the second usually happening when there is a flood event. This could be an example of a rhythmic species taking advantage of a high nutrient moment, but it could be affecting its seasonality, as well as the seasonality of its predators.

We should also mention that another possible reason we do not observe annual rhythms for a large number of species is because we were very stringent on the way we quantified their rhythmicity. We set out to identify rhythmic species in a mathematical sense, which lead us to use harsh cut-offs. In contrast, the Bray Curtis analyses revealed a clear seasonality in our microbial communities.

For the reasons mentioned above, maybe another way of addressing the seasonality of marine microbes would be to survey the seasonality of gene transcription with metatranscriptomic studies as well as to increase the focus on microbial trophic interactions.

5.2 Bathycoccus: A case study for the seasonality of phytoplankton

Going over the results presented in this manuscript, it transpired that one strain, *Bathycoccus prasinos* was found in all three chapters, as well as the study found in annex. By concentrating on this strain and reviewing the results from the different chapters, it is possible to paint a broader picture of the effect of temperature, photoperiod and biotic interactions at different scales of time and resolution (Fig.5.2).

Seven years of sampling at low resolution (I). Thanks to a sampling effort started in 2007, *Bathycoccus* sequence dynamics displayed seasonal reoccurrences. With the use of the Lomb-Scargle periodogram, it was determined that *Bathycoccus* was actually rhythmic since it bloomed every year during the same week. Furthermore, it was observed that these reoccurrences coincided with the yearly sea surface temperature minimum (Chapter II).

Three winters of sampling at high resolution (II). By sampling twice a week during the most productive months, we demonstrated high resolution co-occurrences between marine microbial species. This lead us to determine that even though some species, such as *Bathycoccus prasinos*, are rhythmic, other species that co-occurred with them can change. Among the environmental factors measured, salinity and temperature had the most effect on microbial community composition (Chapter III). It would be interesting to functionally analyze the species co-occurring with *Bathycoccus* under changing environmental conditions through metagenomic and metatranscriptomic approaches. Such analyses may provide cues towards a better understanding of *Bathycoccus* preferred niches, in particular with respect to vitamins and growth factors.

High resolution culture work (III). Via a collaboration with a post-doc in the team, microcosms and culture work showed that different strains had specific responses to light

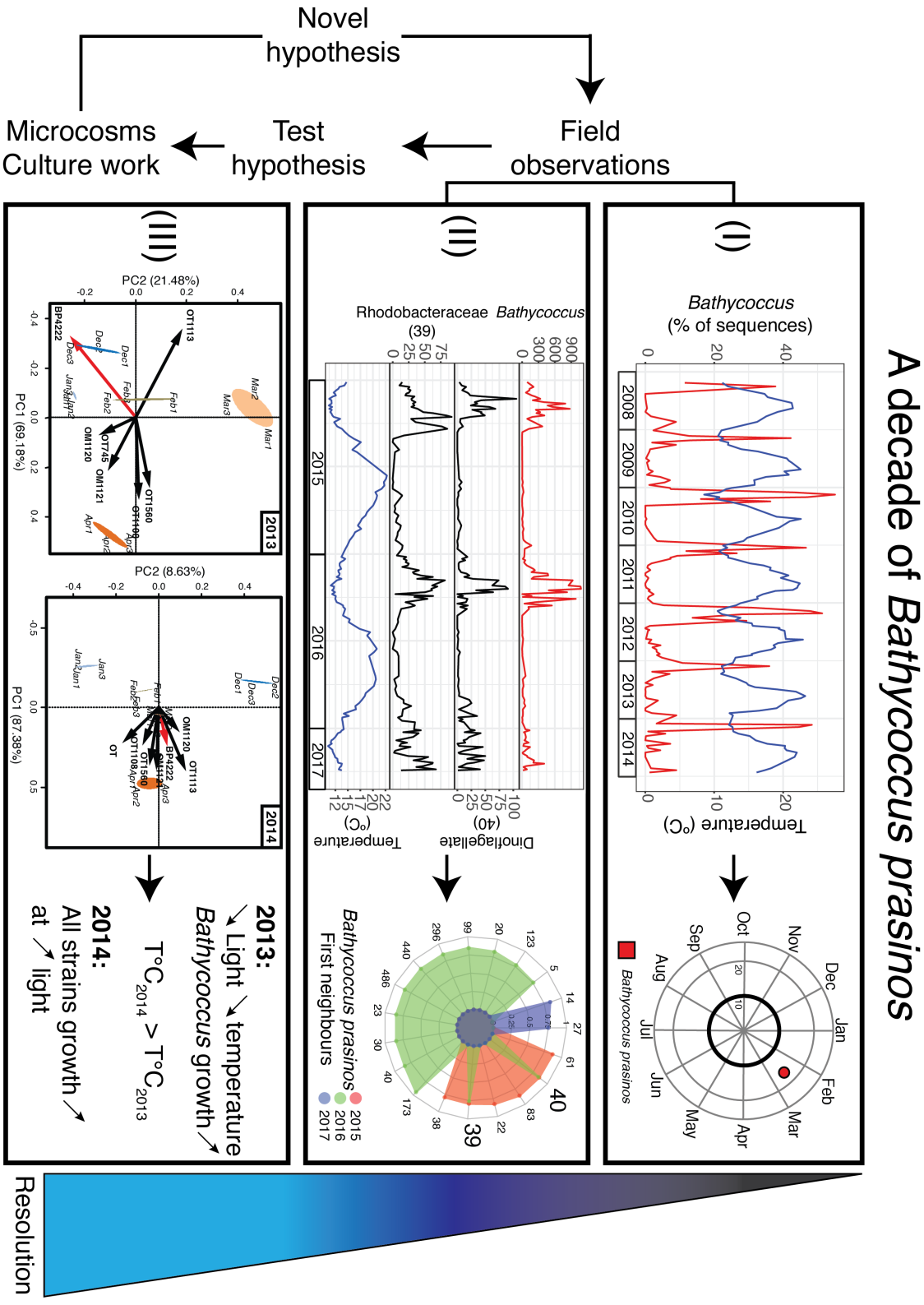

Figure 5.2: **A decade of *Bathycoccus prasinos***. An integrated summary of *Bathycoccus* dynamics.

and temperature conditions. These experiments have allowed us to demonstrate that *Bathycoccus* was the best suited, in average temperature conditions, to shorter photoperiods compared to other strains. However, once the temperature was increased, *Bathycoccus* showed similar preferences as other strains to longer photoperiods. These finding give a possible explanation as to why *Bathycoccus* blooms at temperature minimums every year (Annex).

Perspectives

Considering the involvement of marine microbial communities in biogeochemical cycles, that have an impact on global climate, it seems essential to continue to investigate microbial dynamics. Furthermore, given the context of climate change and how it has been shown to impact phytoplankton communities (*107*), there is a need for continued monitoring. Indeed, the marine food web and half of the primary production depends directly on marine phototrophs. Altering the balance found at such a primary trophic level could have unpredictable impacts on predators and the rest of the food chain.

An additional point to decipher would be determining the precise origin of the salinity decreases and influxes of nutrients (*108*). This would help explain how abiotic factors impact community composition. Particular matter dispersal studies done in the region could help elucidate this point (*109*).

5.3 Enhancing time series

Reaping the benefits of initiatives started over a decade or two ago, publications concerning time series studies have been increasing over the past couple years. However, improvements are still possible. In order to capture high resolution variations in community dynamics, two aspects need to be increased: the sampling frequency and the taxonomic resolution and depth. Augmenting the sampling frequency can be done in two different ways, either by sampling more frequently, for example going from monthly to weekly sampling (*69*, *110*), or by implementing an automated sampler at the sampling

point (*71*, *72*). Increasing sampling frequency seems rather straightforward, but multiplying the number of samples per year directly increases all the steps involved in acquiring and processing the samples, which can lead to a massive increase in work load. On the other hand, automating the sampling would give the best temporal resolution, but is far more expensive, and thus not available for all laboratories. Concerning the taxonomic resolution, implementing metagenomic and metatranscriptomic analyses, instead of limited metabarcoding sequencing, would yield far greater results. However, without powerful bioinformatic capabilities, many laboratories are left stranded, unable to investigate at such a high resolution.

5.4 Potential applications of time series observations

With the increasing number of time series studies being published, and considering the fact that multiple ecosystems have been investigated (*59*) there is a tremendous potential for meta-analyses of time series data (*59*, *111*).

Moreover, specific observations done in the field, either during cruises or with autonomous measurements (buoys, gliders), could be verified with a better integration with microcosm and/or culture studies (Fig.5.3). This could help explain, at a minute level, how different factors can influence community dynamics and help researchers formulate novel hypotheses to be investigated in the field.

By accumulating large amounts of data, for a long amount of time, time series permit the establishment of a baseline that future variations can be compared to. Given the context of climate change, this is particularly useful since long term data can demonstrate actual observations of the sea surface temperatures variations within the last couple of decades. Moreover, the baselines determined by time series data allow for predictions to be made. This is particularly useful when researchers want to study reoccurring events such as phytoplanktonic blooms. These blooms can have consequences on health and the economy. For example, the early detection, or even prediction, of harmful algal blooms would prevent people from getting sick by consuming contaminated shellfish (*113*).

Additionally, given the findings in chapter II, we should be able to predict the re-

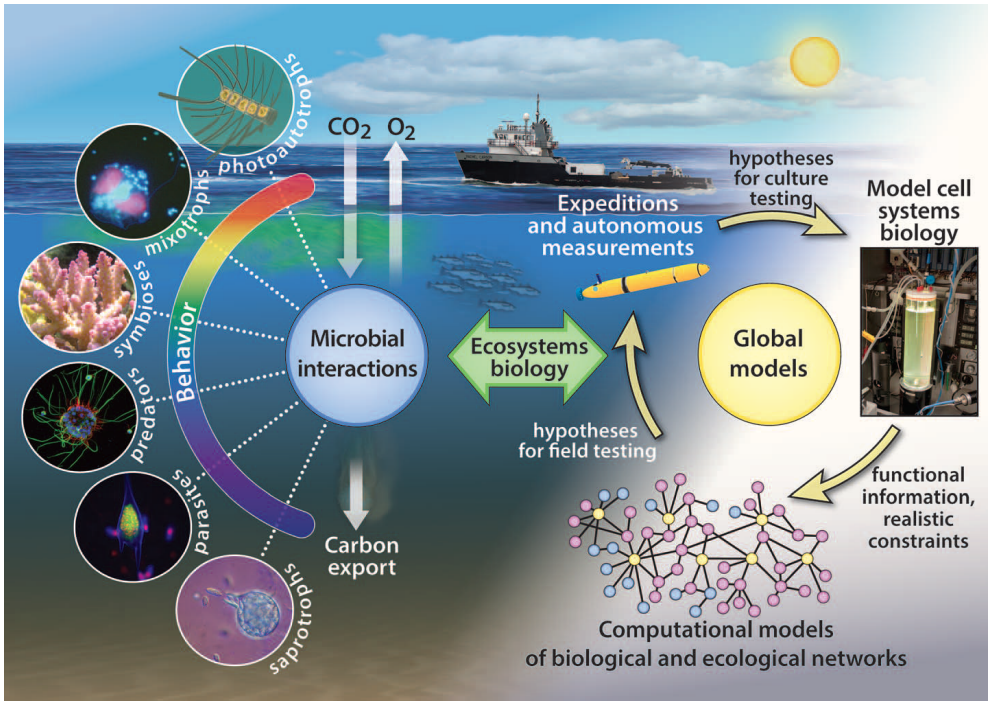

Figure 5.3: **Holistic investigation.** To achieve a better understanding of community behavior, integration between field observations and laboratory experiments is required (*112*).

occurrence of rhythmic species every year. The argument could be made that if these rhythmic species suddenly lose their rhythmicity, or if they disappear entirely, then there must be a significant impact on the ecosystem. Consequently, the rhythmic species found in chapter II could be used as sentinel species to monitor the marine ecosystem of the Bay of Banyuls. Even though the use of specific species as proxies to the health of an ecosystem is a concept that has already been used for macroscopic species, the far greater diversity of microorganisms could yield more fine-tuned results. For example, the *micromonas* genus has a ubiquitous distribution in the world's oceans, with strain specific thermal preferences. Thus, the diversity of the *micromonas* genus makes it an ideal proxy to monitor sea surface temperature changes at a global level (*62*).

All things considered, with the amount of time series, microcosms and physiological data available around the globe, researchers are acquiring better context to build global climate models. These models are used to predict the effects of climate change and help governing bodies decide on the changes that are necessary to limit the anthropological impact on Earth.

Annex

Contribution of light and temperature niches to seasonal patterns of photosynthetic picoeukaryotes

Prologue

The observations made during the time series analysis brought on two main conclusions, *(i)* light and temperature are the main drivers of seasonality and *(ii)* microbial marine species co-exist in intricate ways. They can co-occur (mutualism, predation etc...) or follow variations in shared ecological niches. To be able to elucidate both these points at a higher level of resolution, seven strains of mamiellophyceae, that had been previously isolated from the Bay of Banyuls, were exposed to different light and temperature conditions. For each strain, a corresponding light and temperature preferendum was established. This demonstrated that, even within species, there were different physiological responses. Furthermore, microcosm experiments on natural microbial communities confirmed the importance of temperature in community dynamics. It was also observed that *Bathycoccus prasinos* dominated the incubated communities at low temperature. The results found in this chapter help understand why *Bathycoccus prasinos* is found to bloom every year at the temperature minimum at SOLA. Furthermore, as seen in the microcosms, a mild temperature increase could drastically impact the community structure and potentially the seasonal dynamics of marine microbial communities.

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Contribution of light and temperature niches to seasonal patterns of photosynthetic picoeukaryotes

Jean-Baptiste Guyon[a], Stefan Lambert[a], Mathieu Quentel[a], Jean-Luc Aucouturier[b], Michel Groc[b], Valérie Vergé[a], Jean-Claude Lozano[a], and François-Yves Bouget[a]

[a]CNRS, UMR 7621, Laboratoire d'Océanographie Microbienne (LOMIC), Observatoire Océanologique de Banyuls, Sorbonne Université, Banyuls sur Mer, France; [b]CNRS, Fédération de Recherche (FR3724), Observatoire Océanologique de Banyuls, Sorbonne Université, Banyuls sur Mer, France

In temperate regions of the ocean, seasonal blooms of phytoplankton usually occur in winter and spring and are major contributors to primary production. A recent seven-year metabarcoding analysis of diversity in a North Western Mediterranean coastal site (SOLA station, Banyuls sur Mer, France) revealed robust annual rhythms of abundance of photosynthetic picoeukaryotes belonging to mamiellophyceae (*Bathycoccus*, *Micromonas* and *Ostreococcus*) genera which represent up to 80% of rhythmic ASV sequences in winter. *Bathycoccus*, in particular, showed yearly reoccurring peaks at the time of seawater temperature minima. The abundance of phytoplanktonic species results from both biotic interactions with heterotrophs (bacteria, grazers) and from the influence of environmental factors such as light and temperature, which have a strong impact on the physiology and growth of microalgal photosynthetic cells. In this study, we report the growth response of 7 strains belonging to the *Ostreococcus* and *Bathycoccus*, (including 6 strains previously isolated from the Bay of Banyuls) exposed to realistic day light and temperature conditions in homemade dedicated incubators. Our results reveal the existence of temperature and light *preferenda* at the genus, species but also infra species level, which could contribute to defining ecotypes and temporal niches of occurrence. Raising the temperature by 2℃, corresponding to a warm winter, resulted in a higher influence of the light parameter on the growth response. Finally, microcosm experiments on February and March natural communities revealed that temperature modulations of +/- 2℃ had a great impact shifting initial communities toward either mamiellophyceae or diatoms.

Keywords: Phytoplankton | Seasonality | Environmental niches | Ecotypes | Light | Temperature

Introduction

Eukaryotic and prokaryotic picophytoplankton contribute significantly to primary production in large areas of the oceans, from polar to tropical regions and from oligotrophic open ocean to eutrophic coastal regions (1). In recent years, phylogeographic studies, based on massive metabarcoding of marine microbial communities, have revealed the occurrence of ribotypes of eukaryotic and prokaryotic autotrophs associated to contrasted environmental niches in terms of temperature and photoperiod regimes (2–4). Only few studies have addressed the physiological basis of strain adaptation to light, temperature or nutrients bioavailabilty. Laboratory studies of *Synechococcus* isolates, suggested the existence of thermal ecotypes that are adapted to latitudinal gradients of temperature (5).

In temperate and polar regions, temperature and light vary not only with latitude but also along the year. Seasonal rhythms of phytoplankton diversity and abundance rise from interplays between biotic (light, temperature, nutrients) and abiotic (interaction with bacteria, grazing, viral lysis) drivers (6). In winter, low light limits photosynthesis and growth of phytoplanktonic cells (7). Temperature, as well, differentially affects the growth of phytoplanktonic cells and their interaction with grazers and viruses (8). Therefore, the overall impact of temperature variations on phytoplanktonic blooms is difficult to predict (9, 10). Mamiellophyceae, including the genera *Ostreococcus*, *Bathycoccus* and *Micromonas*, provide a case study to understand how light and temperature interact to regulate spatial and temporal patterns of phytoplankton abundance (11–13). Mamiellophyceae have a worldwide distribution in the ocean, representing the 4th group in 18S barcode in the TARA ocean cruise (14). This order often dominates coastal and mesotrophic transition zones (15–17), however phylogeographic studies revealed an *Ostreococcus* clade OII infeodated to open ocean as opposed to OI, which is predominantly in coastal ecosystems (2). Studies of comparative physiology identified open ocean adapted strains occupying low light (RCC809) or low iron (RCC802) environmental niches in *Ostreococcus* (18, 19). Different responses in growth rates and carotenoid contents were also observed in response to light, temperature and salinity between the RC802, RCC809 and *Ostreococcus*

tauri lagoon strain (20). Using a metabarcoding approach on a seven-year time series in the Bay of Banyuls we recently showed that Mamiellophyceae dominated the eukaryotic picophytoplankton in winter, exhibiting strong annual rhythms (17).

In this study, we explored the effect of light and temperature on the physiological responses of 7 strains of Mamiellophyceae and natural picoplanktonic communities from the Bay of Banyuls by simulating light and temperature conditions corresponding to "low" and "warm" years in microcosms. For each strain, we could define a distinct light and temperature month preferendum, corresponding to a potential environmental niche. Inter- and intraspecific comparisons of light and temperature responses in *Ostreococcus tauri* and *Ostreococcus mediterraneus* revealed that the species taxonomic level resolution does not account for differences in physiological responses between strains. Growth appeared to result from complex interactions between light and temperature. Metabarcoding of natural microbial communities exposed to various temperatures in microcosms further supported the role of temperature in shaping microbial community and the fitness of *Bathycoccus prasinos* at low temperatures.

Materials and Methods

Description of strains and cell culture conditions. *Ostreococcus tauri* (OT745, OT1108 OT1560 and OT1113), *Ostreococcus mediterraneus* (OM1120 and OM1121) and *Bathycoccus prasinos* (BP4222) were isolated from the Bay of Banyuls in the Northwestern Mediterranean Sea. The isolation of these strains was carried out over several years, but always between the months of October and May (Table 1). Since then, these strains have been maintained in the Roscoff Culture Collection (RCC). For maintenance, all strains were cultured in 50mL flasks containing 20mL of artificial sea water with 36 $g.L^{-1}$ salt (20). The strains were cultivated in 96 deepwell plates (Nuc, Perki Elmer) during experimentation phases. Cells were incubated under five different light conditions corresponding to realistic sunlight measurement done in the Bay of Banyuls. The photoperiods applied were of 299, 318, 461, 640 and 799 $\mu mol.quanta.m^{-1}.s^{-1}$ maximal irradiance at midday corresponding to December, January, February, March and April, respectively. For each treatment, sun light curves of $8.29h.d^{-1}$(December), $8.53h.d^{-1}$(January), $10.02h.d^{-1}$(February), $11.22h.d^{-1}$(March) and

12.46h.d^{-1}(April) of light irradiance were applied in temperature controlled incubators (Panasomic MIR-154-PE). The light intensity that was applied for each condition corresponded to measurements done at three meters of depth in the water column at the SOLA sampling station present in Bay of Banyuls.

Microalgae cells were inoculated in triplicate at 1 million cell.mL^{-1} during the initial acclimation period and during kinetics experiments. The acclimation period corresponded to 7 days of culture in the same conditions as the growth rate experiment. To quantify cells, the BD accury C6 flow cytometer was used. Samples from biological triplicate (20µl) were fixed with 0.25% glutaraldehyde (Sigma-Aldright, St Louis, MO) for 15 minutes at room temperature. Thereafter, fixed sample were frozen and stored at -20°C until cell counting.

Table 1. Description of Mamiellophyceae strains used in this study

Species	Strain (RCC #)	Isolation date	Clade
Bathycoccus prasinos[1]	BP4222 (RCC 4222)	2006.01.01	NA
Ostreococcus mediterraneus[1]	OM1121 (RCC 1121)	2007.02.19	D
Ostreococcus mediterraneus[1]	OM1120 (RCC 1120)	2007.02.19	D
Ostreococcus tauri[1]	OT1113 (RCC 1113)	2006.10.02	C
Ostreococcus tauri[1]	OT1560 (RCC 1560)	2007.02.19	C
Ostreococcus tauri[1]	OT1108 (RCC 1108)	2006.01.01	C
Ostreococcus tauri[2]	OT745 (RCC 745)	1995.05.03	C

Isolated at sea surface from the Bay of Banyuls[1] or the Thau Lagoon[2]

Acquiring kinetic values and specific growth rates. After inoculating at 1 million cells.mL^{-1} of acclimate cells, samples were monitored in batch culture for seven to ten days until the end of the exponential phase. Daily measurements were carried out with the BD accury flow cytometer. At the end of each experiment growth curves and specific growth rates were determined.

In batch culture, the specific growth rate (µ) was calculated according the formula: $\mu=(\ln N_{t2}-\ln N_{t1})/(t2-t1)$, where N is the number of cell during the exponential growth phase and t is the time in days.

Microcosms data processing. Seawater sampled from SOLA in the Bay of Banyuls was filtered on 3µm pore-size polycarbonate filters (Merck-Millipore, Darmstadt, Germany), to remove large cells, into 400mL flasks. These flasks were then incubated in light and temperature controlled

incubators for 4 days under gentle agitation. Each condition was carried out in triplicate. As described previously, realistic light and temperature conditions were applied. Alongside testing natural conditions, these incubators allow for modifications in light and temperature that would not be feasible *in situ*. At the end of the incubation period, the triplicates were pooled together and filtered onto 0.22µm pore-size GV Sterivex cartridges (Merck-Millipore) to collect the microbial biomass. These filters were then stored at -80°C awaiting extraction.

The DNA samples analyzed in this study consisted of time series data (59 samples) and microcosms data (23 samples), which were extracted and analyzed independently.

Firstly, the time series samples were obtained and extracted as previously described (17). Total DNA was extracted and purified with the Qiagen AllPrep kit (Qiagen, Hilden, Germany). The primers used for the amplification phase were 515F (5'-GTGYCAGCMGCCGCGGTA-3') and NSR591 (5'- TTGGYRAATGCTTTCGC-3') (21, 22). Sequencing was carried out with illumina Miseq 2x300 bp kits by Research and testing Laboratory (Lubbock, Texas). Sequence analysis was done with the DADA2 package (23) (https://benjjneb.github.io/dada2/index.html, version 1.6) in "R" (https://cran.r-project.org)). Details concerning the sequence analysis have been published previously (17).

Secondly, the microcosm samples were extracted with the Nucleospin plant II kit (Macherey-Nagel, Düren, Germany). The primers used for this amplification phase were TAReukF1 (5'-CCAGCASCYGCGGTAATTCC-3') and TAReukR (5'-ACTTTCGTTCTTGATYRATGA-3'). These are updated primers that have a better eukaryotic coverage (24). Similarly, the standard pipeline of the DADA2 (version 1.6) was used to do the analysis of the raw microcosm sequences. The parameters used were: trimLeft=c(20, 21) ,truncLen=c(280,230), maxN=0, maxEE=c(2,2), truncQ=2. The taxonomy assignments were done with PR2 v.4.10.0 database (https://github.com/vaulot/pr2database/releases).

RDA, Heatmap and statistical analysis. Heatmap, boxplot and Redundancy analyses (RDAs) were generated using the statistical software "R". All statistical analyses were performed in "R" at the significant level of 0.05. Environmental parameters were analyzed using Redundancy analysis (RDA). RDAs were used to explain the variability in phytoplankton growth rates.

The vegan package, and more particularly the adonis and vegdist function using the Bray-Curtis permutation tests (999 permutations), was applied to compute the distance matrices. The Simper function established the significance of hypothetical relationships between treatments on the different strains. Heatmaps illustrated the abundance of different ASVs and diversity from microcosms. In order to test robustness of the diversity response, Shannon tests were used, excluding all species with a relative abundance <5%. The results from the "2013 and 2014 years" were compared using the one-way analysis of variance method. The results are summarized using boxplot diagram. Each boxplot shows the growth rate values of Mamiellophyceae species compared to environmental factor.

Student t-test was used to compare growth rates (Fig. 2B).

Results

Mamiellophyceae temporal occurrences in the 2007-2014 metabarcoding dataset. We have previously observed, in a seven-year time series at SOLA, that *Bathycoccus* and three clades of *Micromonas* were among the most abundant ASVs (amplicon sequence variant) (17). For these two genera, maximal numbers of ASV were found in winter between December and April (Fig. 1 and Fig. S1). *Bathycoccus* occurred every year around the time of temperature minima (Fig. 1). A temporal succession was observed for the three clades of *Micromonas*. *Micromonas bravo* and *Micromonas sp.* were usually preceding *Micromonas commoda*. Although less abundant, the 4 clades of *Ostreococcus* (A, B, C and D) were present in the metabarcoding dataset. *Ostreococcus tauri* showed irregular occurrences, usually in the fall but it was occasionally detected in January (2014), summer (2008 and 2010) or absent (2007 and 2009). *Ostreococcus mediterraneus* had a more even distribution from year to year with maximal abundances between January and March (Fig. 1C). Similarly, *Ostreococcus sp* and *Ostreococcus lucimarinus* were usually detected in fall and winter, reaching up to 25% and 15% of photosynthetic picoeukaryote ASVs in fall or late winter, respectively (Fig. S1 D and E). The climatology of the 2007-2014 time series was marked by important interannual variations in temperature in particular for the months of December or June.

Effect of temperature on selected strains of Mamiellophyceae. We first tested the effect of temperature on the growth of 6 mamiellophyceae isolated from the Bay of Banyuls, including one

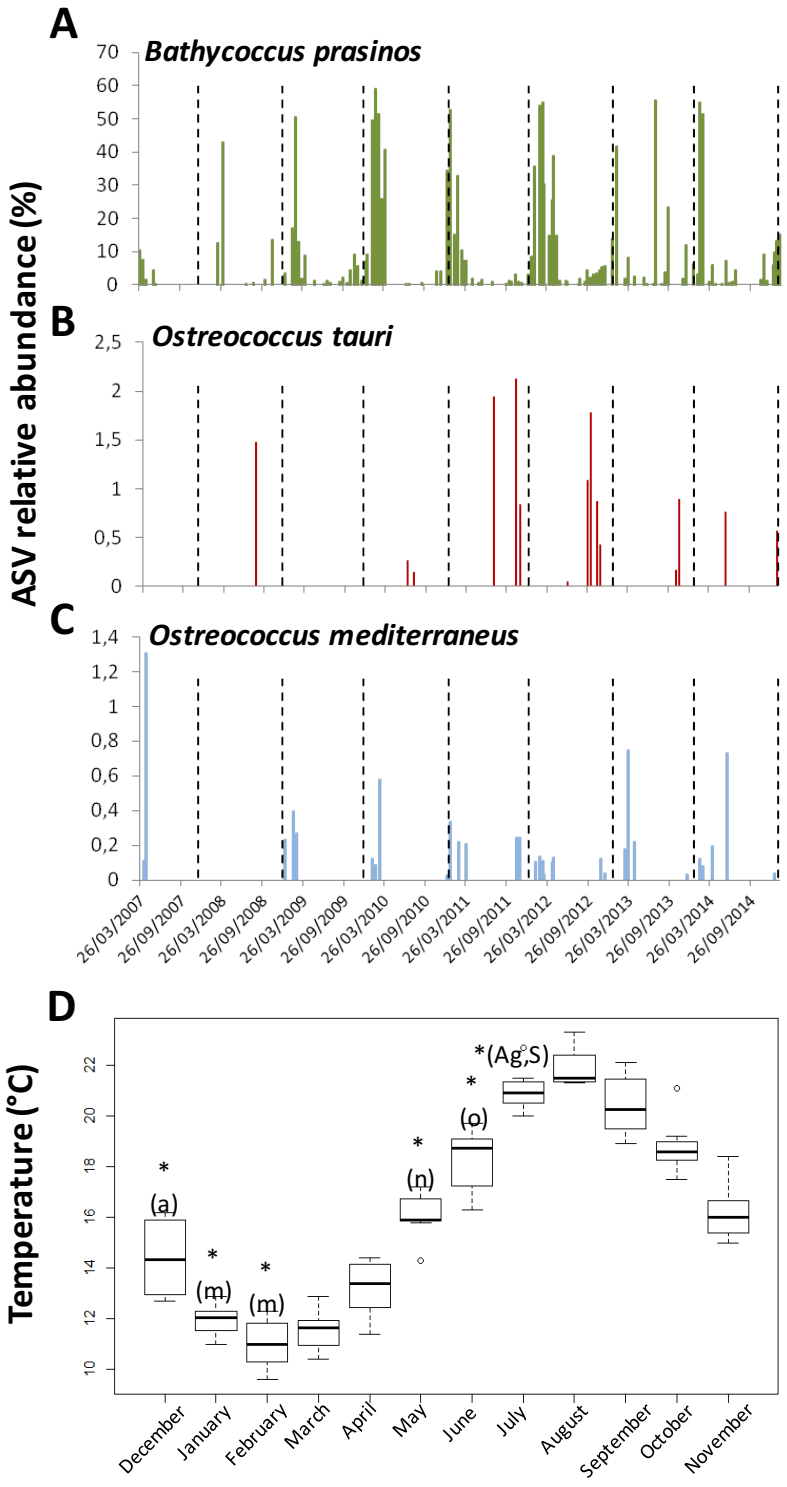

Fig. 1. : Distribution of *Bathycoccus prasinos* (A). *Ostreococcus tauri* (B). *Ostreococcus mediterraneus* (C) at SOLA sampling station (Northwestrern Mediterranean Sea) from 2007 to 2015, plotted alongside average monthly sea surface temperatures in the Bay of Banyuls for the same period (D). Significant differences between the months of the year are represented by letters; December (d). January (j). February (f), March (m), April (a), May (my), June (jn), July (jl), August (ag), September (s), October (o), November (n) and all (*) ($p<0.05$ t-test).

strain of *Bathycoccus prasinos* (BP4222), two strains of *Ostreococcus mediterraneus* (OM1120 and OM1121), three strains of *Ostreococcus tauri* (OT1113, OT1560 and OT1108) as well as the reference strain of *O. tauri* (OT745) isolated from the Thau lagoon (Table 1).

Dedicated incubators, that were developed in our laboratory, were used to simulate realistic environmental conditions of light at 3m of depth on February 15th at the latitude corresponding to the Bay of Banyuls (day length: 10H02, 0.461 mmol at solar noon). This corresponded to an average photoperiod during the period of presence of Mamiellophyceae in the metabarcoding dataset (Fig. 2A). Four temperatures were tested (9.2°C, 11.2°C, 13.2°C and 15.2°C) to cover the range of temperature observed during the course of the Mamiellophyceae bloom (17). Growth rates were measured on cultures that had been acclimated for a week at the different temperatures. A redundancy analysis (RDA) was conducted to compare the 7 strains under the different temperature conditions (Fig. 2A). The first component accounted for 84.28% of the observed variability. Overall the highest temperature 15.2°C and to some extent 13.2°C accounted for the most observed differences in growth rates, with a more pronounced effect on two *O.tauri* strains OT745 and OT1108. Comparing growth rates at different temperatures revealed striking differences between the strains. Lowering the temperature had little effect on the growth rate of *O.tauri* (OT1113), *O.mediterraneus* (OM1121) with values of 0.48 and 0.52 d^{-1} at 15.2°C compared to 0.45 and 0.45 d-1 at 9.2°C, respectively. In contrast, lowering temperature from 15.2°C to 9.2°C resulted in a 2-fold decrease in *O.tauri* strains OT745 and OT1108. *Bathycoccus prasinos* 4222, *O. tauri* OT1560 and *O. mediterraneus* OM1120 showed an intermediate response with a 1.5 to 1.2 fold reduction in growth rate between 15.2°C and 9.2°C, respectively. Noteworthily, marked temperature-dependent variations in growth rates between strains belonging to the same genus (eg. OT1108 vs OT1113 in *O. tauri*) were observed, but strains belonging to different genera had similar responses (e.g. *Bathycoccus* BP4222 and *O. tauri* OT1113 or *O. mediterrenaeus* OM1121).

Effect of photoperiod on growth rates. The Mamiellophyceae strains were exposed to various photoperiod lengths corresponding to the months of December, January, February, March and April at the latitude of the Bay of Banyuls. Day lengths varied between 8h29 and 12h46 (December and April respectively). Light intensities ranged from 0.299 to 0.799 mmol.quanta.m^{-1}.s^{-1} between December

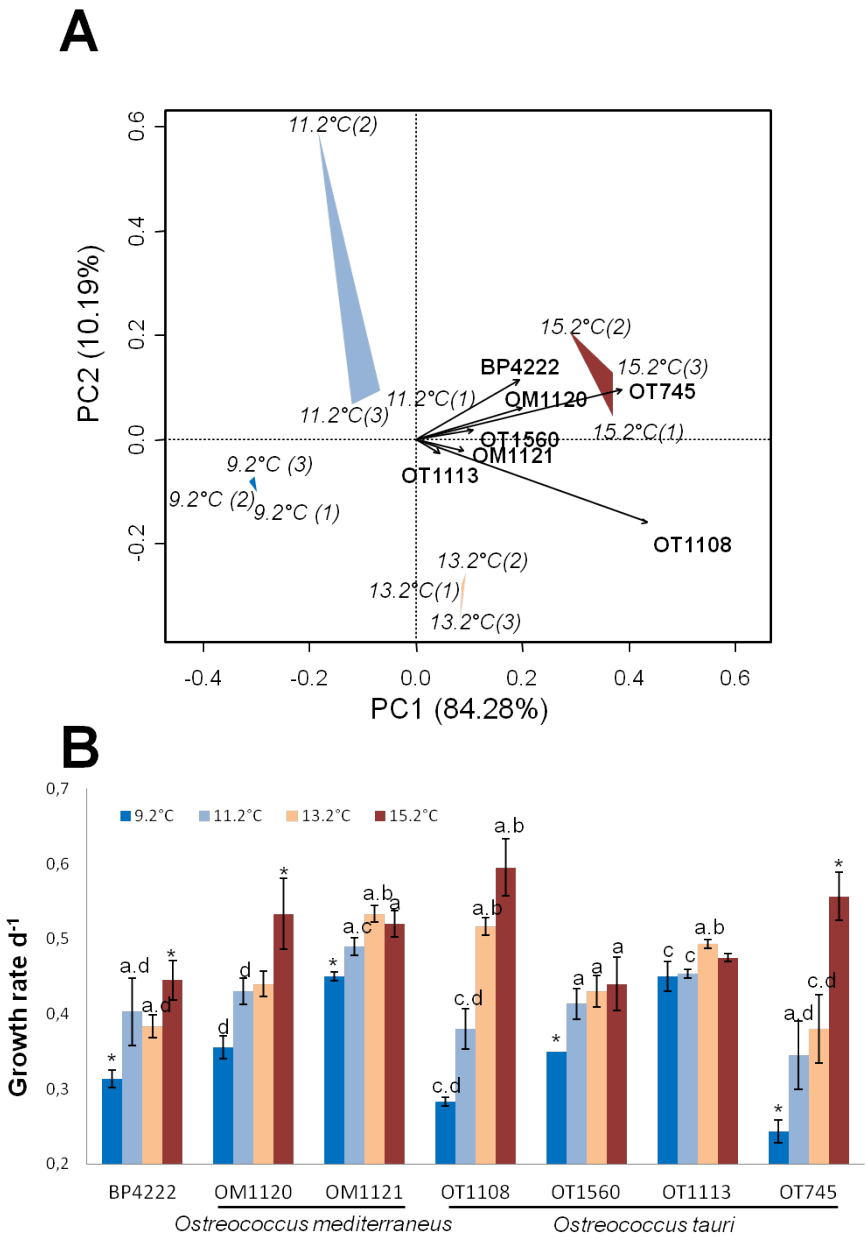

Fig. 2. : Redundancy analysis (RDA) illustrating the growth rates of the seven strains incubated in different temperature conditions(A). Phytoplankton strains are represented by solid black arrows, whereas growth rate triplicates are grouped according to temperature (9.2℃, 11.2℃, 13.2℃ and 15.2℃) as colored polygons (blue, light blue, orange and red, respectively). Temperature dependent growth rates of seven strains are plotted as bar charts (B) with SD (n=3). Significant differences between temperature conditions 9.2℃, 11.2℃, 13.2℃ and 15.2℃ are represented by letters a, b, c and d, respectively. Significant differences with all other treatments is symbolyzed by * ($p<0.05$ t-test).

and April. Photoperiod experiments were performed at two temperatures, which corresponded to the mean temperature observed in February (11.2°C) and December/April (13.2°C) in the 2007-2014 time series. RDAs performed on growth rates revealed contrasted responses to photoperiod between the two temperature conditions. The first component accounted for most of the variability observed under the different photoperiod conditions (50.05% (PC1) vs 26.76% for the second component at 11.2°C and 73.75% (PC1) vs 13.91% for the second component at 13.2°C). At 11.2°C OT745, OM1121 and, to some extent, OT1560 were associated to the February photoperiod. OT1108 and BP4222, in contrast, were the main contributors to the March and December photoperiod responses. At 13.2°C, photoperiod responses were much less influenced by specific strain responses except for February and April which were associated with OT1560 and OM1120, respectively.

Comparing the combined effect of photoperiod and temperature on growth rates has revealed that, in general, high temperature and long photoperiod stimulated cell growth (Fig. 3B). However, contrasted responses were observed between the strains. Under short photoperiods (December and January), little or no differences in growth rates were observed between 11.2°C and 13.2°C for BP4222, OM1121, OT1108 and OT1113 (Fig. 3). In contrast, OT1560 and OM1120 grew better at 11.2°C. When comparing the overall responses of strains to photoperiod, the growth rate profiles fell into 4 mains categories. BP4222 and OT1108 showed little variations in growth rates in all conditions. The growth of OM1121 and OT1113 increased under long photoperiods at high temperature but not at low temperature. The strains OT1560 and OM1120 had similar responses, with higher growth rates under short photoperiod at low temperature and under long photoperiod at high temperature. Finally, *O. tauri* differed from the other strains in that temperature promoted growth regardless of photoperiod length. RDAs performed on each individual strain further confirmed the differences in growth rates in responses to light and temperature (Fig. S3).

Growth under simulated light and temperature conditions. The selected Mamiellophyceae strains were exposed to realistic photoperiods (December to April) and temperature conditions, corresponding to two contrasted winter/spring periods. Between December and April, the mean monthly temperature in 2013 was low (11.8°C) compared to the average monthly temperature of 2007-2014 (12.4°C). In contrast, 2014 could be considered a warmer winter since the mean monthly temperature

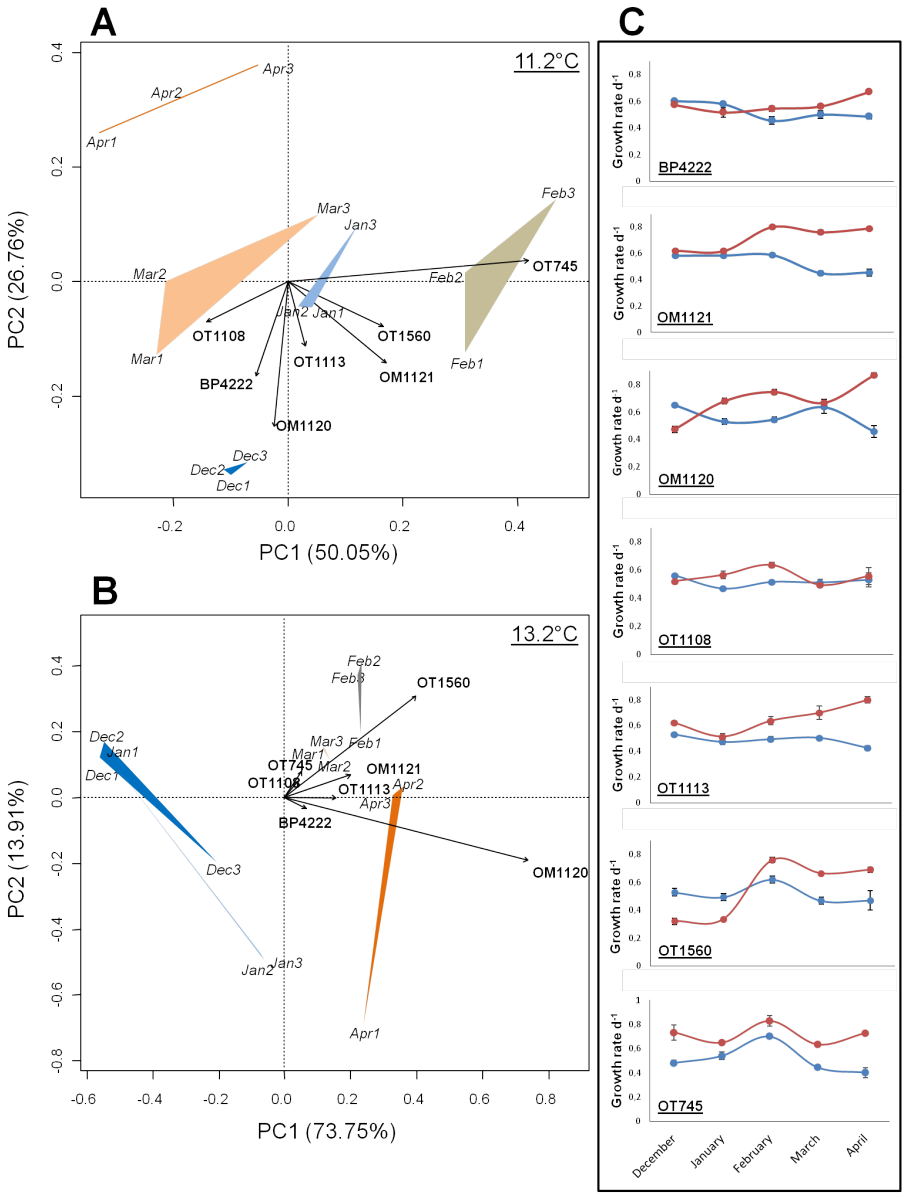

Fig. 3. : Redundancy analysis (A & B) and line charts (C) depict growth rates of seven strains incubated under several photoperiod conditions, separated into two temperature conditions (11.2℃ and 13.2℃; represented, respectively, as blue and red lines in (C)). These light and temperature conditions mimic naturally occurring conditions found in the Bay of Banyuls. Monthly photoperiod conditions, corresponding to the months of December (blue), January (light blue), February (brown), March (light orange), and April (orange) are represented by colored polygons. Each corner of the polygon represents a growth rate measured during that specific irradiance condition. The percentage of variation in growth rates explained by each axis is indicated on the side of each biplot. Photoperiod variables used in the RDA are represented by polygon. Each point represents the growth rate measure at a specific month.

was 12.9 °C between December and April (Fig. S4). RDAs performed on growth rates revealed that the first component accounted for most of variability in 2014 (87.38%) and to some extent in 2013 (69.18%) (Fig. 4). Contrasted responses were observed between 2013 and 2014. In 2013, BP4222, and OT1113 were associated to December and February/March photoperiods, respectively. All other strains showed a preference for April, the longest tested photoperiod condition. In contrast, in 2014 all strains showed a preference for the April photoperiod. Correlation plots confirmed that, compared to other strains, BP4222 and OT1113 had different responses in 2013 but not in 2014 (Fig. 4).

Plotting growth rates against temperature confirmed that in the 2014 simulation, growth rates increased in response to photoperiod lengthening in all strains (Fig. 5). In the 2013 simulation, however, marked differences were observed between the different strains. In the April photoperiod condition growth rates were higher for OT1108 and OT1560 but they were lower for BP4222 and OT1113 compared to other months. Several strains, including OT745 and OM1120, showed little variations in growth rates between the different month conditions (Fig. S5).

Effect of light and temperature on phytoplankton growth in microcosms. Together figure 3 and 4 indicate that *(i)* temperature is the main regulator of growth in our study, *(ii)* at lower temperatures, complex interaction between light and temperature regulate growth in a strain dependent manner. Microcosms experiments were designed to estimate the impact of temperature on growth of natural phytoplankton communities sampled at different months of winter and spring (February to June 2015).

The 19 most abundant ASVs in the time series dataset from the Bay of Banyuls samples were selected for comparative analysis (Fig. 6). *Bathycoccus prasinos*, *Micromonas bravo*, Bacillariophyta (Diatoms) and Dinoflagellates were the four dominant groups present in the control and treatments (53-91% of total reads, Fig. 6).

Although the sequence composition of starting communities varied, *Bathycoccus* was found in all of them except for June. *Micromonas bravo* ASVs were more abundant in February (16/02/2015 and 23/02/2015) compare to others initial communities. Natural communities were prefiltered on 3 µm and were subsequently incubated for 5 days. They were submitted to photoperiods corresponding

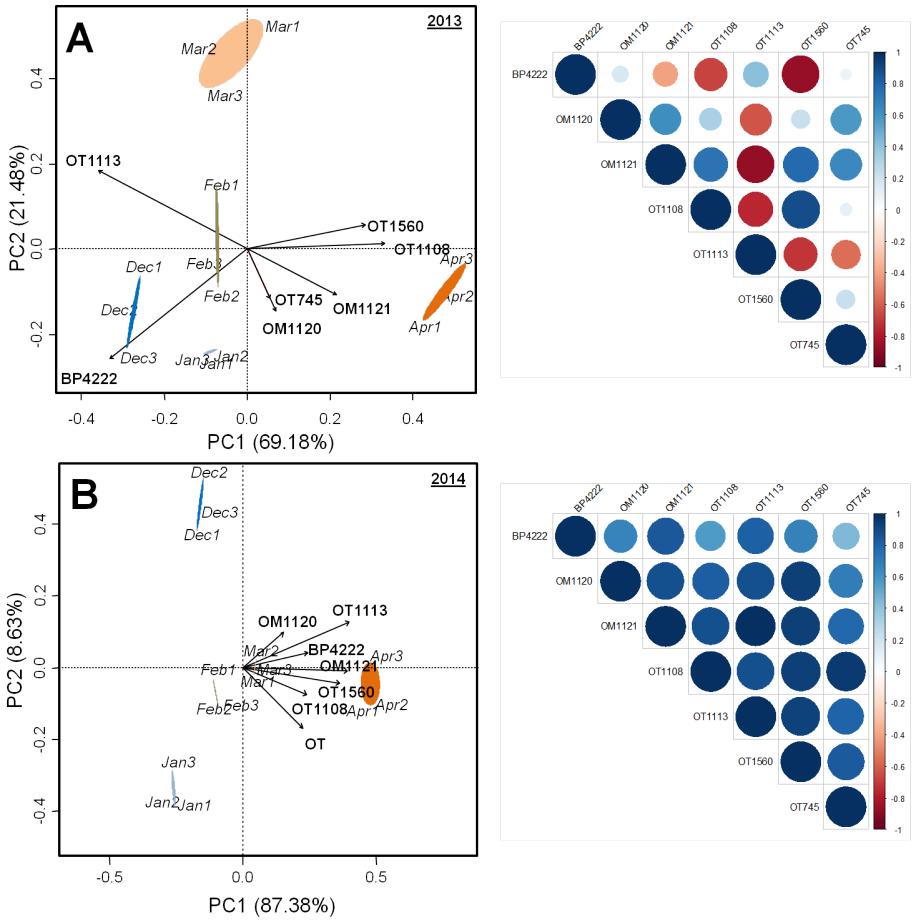

Fig. 4. : Redundancy analysis (RDA) showing growth rates of seven strains under light and temperature conditions emulating the year 2013 (A) and 2014 (B). Phytoplankton strains are represented by solid black arrow and monthly growth rate triplicates as colored ellipses (December (blue), January (light blue), February (brown), March (light orange) and April (orange)). Associated correlations represent positive (Light blue to blue) and negative (Light red to red) affiliation between strains.

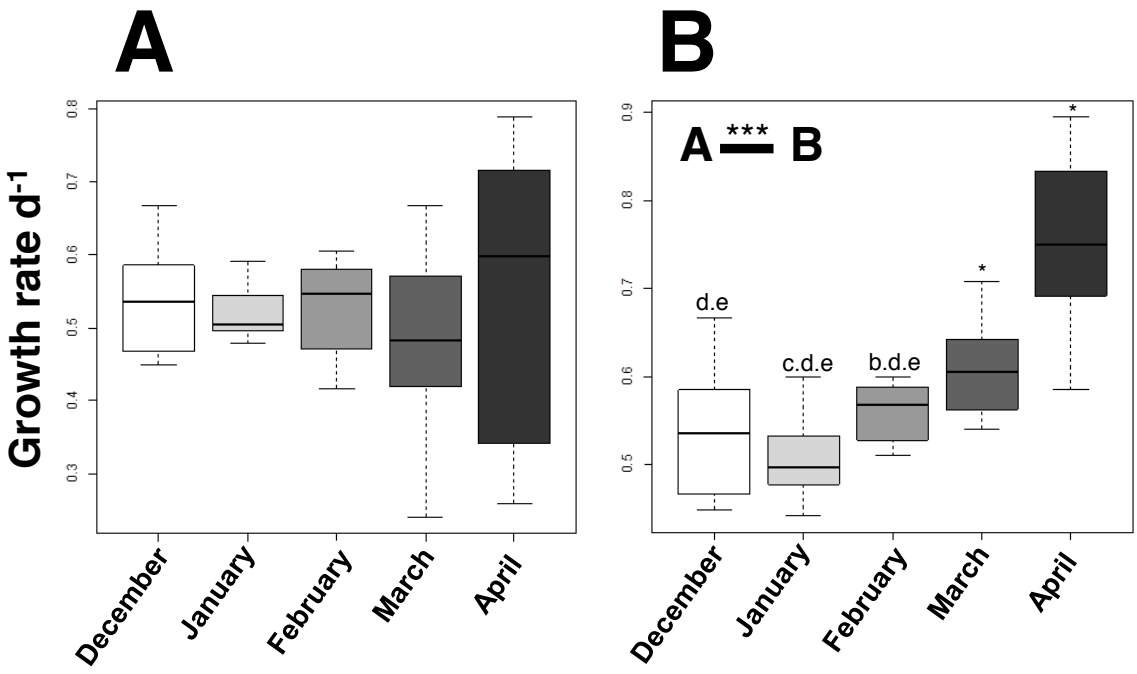

Fig. 5. : Comparison of overall strains growth rates under irradiance and temperature levels corresponding to the winter of 2013 (A) and 2014 (B). The boxplots are displayed with whiskers extending to the highest and lowest values (n=3) per month. Intra-annual differences between the months of December, January, February, March and April are represented by letters a, b, c, d and e, respectively. Differences with all treatments is symbolized by * ($p<0.05$ t-test).

to the month of sampling and different temperatures ranging from -4 °C below to +4°C above the sampling temperature (Fig. 6). At day 5 we observed 19 mains ASVs of phytoplankton (>5% of the dataset).

March communities were dominated by diatoms, and more particularly *Skeletonema*. However, lowering the temperature by 2 to 4°C (i.e from 11°C to 9°C and 7°C, respectively) promoted the growth of *Bathycoccus* to the detriment of *Skeletonema*. Conversely, increasing the temperature in the February photoperiod from 11.2°C to 13.2°C or 15.2°C stimulated the growth of *Skeletonema* and had a negative impact on the growth of *Bathycoccus*.

Discussion

In the Bay of Banyuls, Mamiellophyceae sequences dominated the 18S metabarcode dataset between December and April. Photosynthetic picoeukaryotes ASVs represented up to 80% of sequences in January and February between 2007 and 2014 (Fig. 1, (17)). Highly reproducible annual patterns were observed, with *Micromonas* species preceding *Bathycoccus* in order of appearance every winter

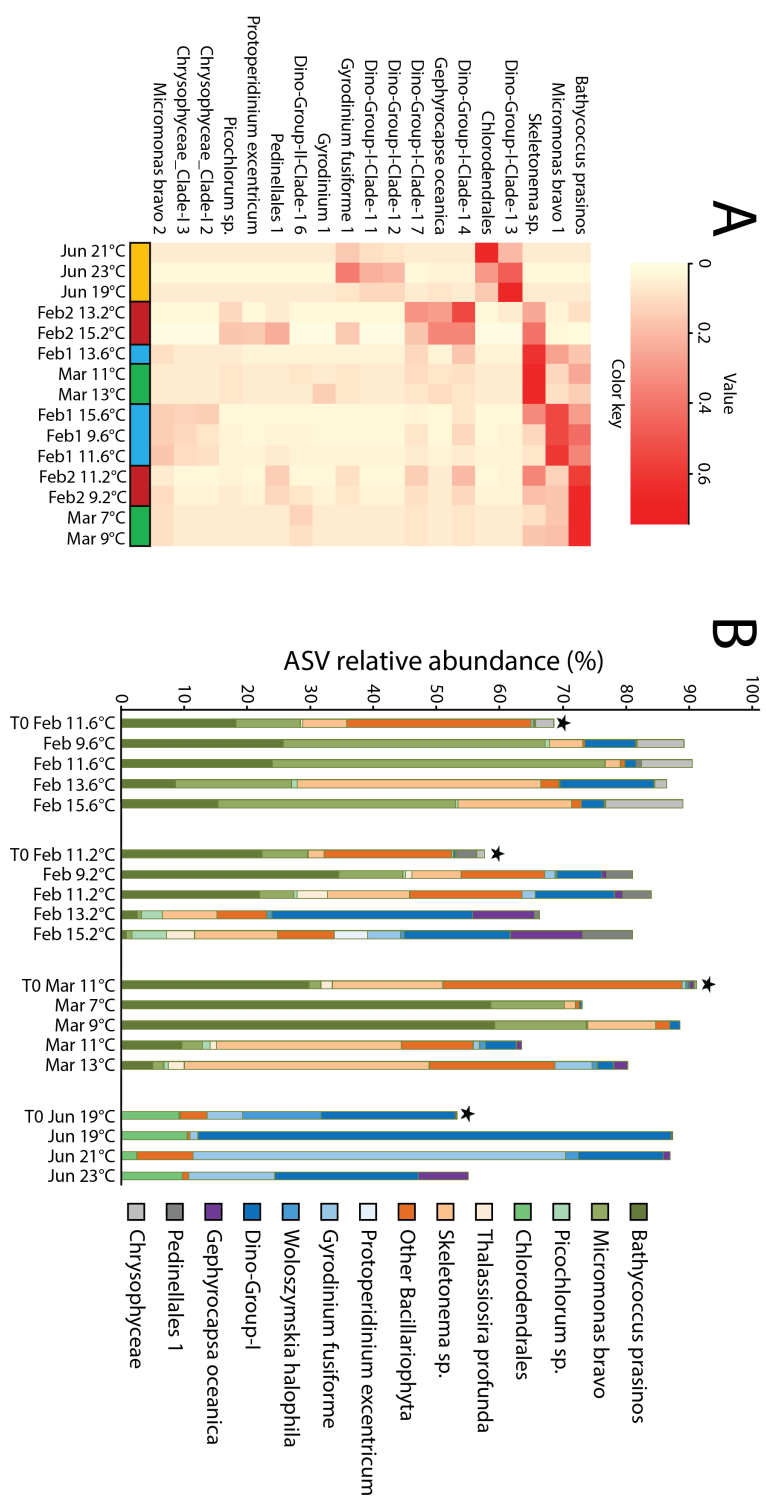

Fig. 6. : Relative abundances of phytoplankton communities sampled from the SOLA are presented as a heatmap (A) and a bar chart (B). The heatmap compares the relative abundance at the end of the incubation period for each microcosm condition. On the other hand, the bar chart shows the relative abundance in percentages of the initial sample (black star) and the final sample for each microcosm condition. Only ASVs that demonstrate at least 5% of relative abundance in at least one sample are shown here. Temperature conditions that microcosm communities were exposed to are reflected in sample names.

(Fig. 1, (17)). Light and temperature were the main factor driving seasonality of picoeukaryotes.

Evidences for thermotypes in Mamiellophyceae. Our present study aimed to evaluate the light and temperature preferences of selected Mamiellophyceae from Banyuls both *(i)* at the species level by comparing the physiological responses of three species *Bathycoccus prasinos*, *Ostreococcus mediterraneus* and *Ostreococcus tauri*; and *(ii)* at the infra-species level by comparing 2 strains of *Ostreococcus mediterraneus* and 4 strains of *Ostreococcus tauri* (including one from the nearby Thau lagoon). As shown in Figure 2, growth rates increased along with temperature for all strains, even though strains like OT1108, OM1120 or OT745 were much more sensitive to temperature variations than OT1560, OT1113, OM1121 and to some extent BP4222, which maintained steady state growth rates between 9.2°C and 15.2°C. Temperature is a key environmental parameter regulating the growth and driving the geographic distribution, of phytoplankton (4). Studies investigating *Micromonas* and *Synechococcus* unveiled the existence of thermotypes adapted to specific latitudes (5, 8). However, to the best of our knowledge, the impact of temperature has not been investigated on the temporal succession of phytoplanktonic species neither at the species nor the infra-species levels.

Temperature dependent effect of photoperiod on growth. While temperature increases had an overall positive impact on the growth of all strains, the effect of light was more complex and temperature dependent. Temperature primarily affects metabolic rates and physiological processes which are involved in light assimilation and conversion into organic compounds (25). Low light supply under short photoperiod may therefore be limiting for photosynthesis under optimal temperature conditions. Under the December/January short photoperiods, all strains were weakly impacted by temperature variations (11.2°C to 13.2°C) but OM1120 and OT1560 were not impacted by temperature increases, suggesting that light, not temperature, is the primary factor limiting cell growth (Fig. 3C). At 13.2°C, photoperiod lengthening enhanced cell growth of all strains except for OT1108 and BP4222 (Fig. 3B). Under long photoperiods, both OT1108 and BP4222 displayed similar growth rates at both temperatures suggesting that light is the main limiting factor to growth. Few examples in the literature have reported the interactions between environmental factors in the regulation of phytoplankton growth. In *Thalassiosira pseudonana* diatom growth is strongly influence

by temperature-nutrient interaction (4) and it was shown that photoperiod and temperature can compensate moderate nutrient limitation from *Stephanodiscus minutulus* and *Nitzschia acicularis* diatoms growth (26).

Connecting light/temperature responses to environmental niches. The ultimate goal of our study was to determine the light/temperature responses of phytoplanktonic strains and to what extent they accounted for temporal patterns of appearance in annual time series. Contrasted physiological responses were observed when exposing selected Mamiellophyceae to various combinations of photoperiod and temperature (Fig. 2, 3 and 4). The *Ostreococcus* strains in our study represented only a few percent of 18S sequences in the metabarcode dataset, making it difficult to correlate their physiological responses to their time of occurrence in the environment (Fig. 1 and Fig. S1). Even though *O. mediterraneus* ASVs represented less than 1% of sequence, the patterns were reproducible from year to year with maximal numbers between February and April. OM1120 and OM1121 had similar responses showing a preference for April conditions of 2013, a low temperature year, compared to the 2007-2014 time series (Fig. 4A). *O tauri* strains had more complex and heterogeneous responses to photoperiod and temperature. While OT1113 was associated to the February 2013 condition, OT1108 and OT1560 were associated to the April 2013 condition (Fig. 4A). The 4 strains of *O. tauri* also exhibited contrasted responses to photoperiods at 11.2°C and 13.2°C (Fig. 3C). While OT1108 maintained stable growth rates under all conditions, the growth of OT1113 increased in response to photoperiod and temperature. Finally, OT1560 displayed an unusual response compared to other strains with higher growth rates at low temperature under short photoperiod (Fig. 3C). The temporal pattern of *O. tauri* ASV abundances in the environment was also irregular from year to year. The contrasted responses of the different strains of *Ostreococcus tauri* suggest that these strains may correspond to different ecotypes of *O. tauri* which may occupy different environmental niches along the year.

Unlike *O. tauri*, *Bathycoccus prasinos* presented robust annual rhythms of abundance in the metabarcoding dataset, with maximal number of sequences in February, corresponding to the yearly minimum of temperature ((17); Fig. 1). Microcosm experiments performed on natural communities, filtered on 3 µm to remove predators, revealed that low temperature promoted the growth of BP4222

in February and March communities. In contrast, an increase in temperature in February shifted the picoeukaryote community towards *Micromonas*. *Bathycoccus* appeared to dominate around 60% of ASVs in March (Fig. 6B). The growth of *Bathycoccus* was stimulated in response to a temperature decrease. Compared to several other Mamiellophyceae, the growth of *Bathycoccus* was neither stimulated by temperature increases above 11.2°C (Fig. 1B), nor by photoperiod lengthening (Fig. 2).

When taken together, environmental observations, culture work and microcosms experiment results, suggest some plasticity in the physiological response of *Bathycoccus* to temperature variations. This could explain the predominance of *Bathycoccus* at low temperatures, while at higher temperatures other microalgae, such as *Skeletonema*, or even *Micromonas bravo*, could outcompete *Bathycoccus* in natural communities. To test this hypothesis, it would be important to determine the physiological response to light and temperature of *Micromonas* strains isolated from the Bay of Banyuls.

Conclusion

Several studies investigating phytoplankton have unveiled the existence of thermotypes in both the cyanobacteria of the genus *Synechoccocus* and Mamiellophycea of the genus *Micromonas*, which are adapted to specific latitudes (5, 8). In contrast, *Ostreococcus* clades are distributed along a coast to ocean gradient (12). Our study demonstrated, for the first time, which Mamiellophyceae strains, isolated at a single location, have distinct temperature/photoperiod preferenda in culture. Differences in physiological responses were observed not only between strains belonging to different genera and species but also between strains of *O.tauri* and of *O.mediterraneus* suggesting the existence of ecotypes in these species. An increase in temperature had an overall positive effect on cell growth in culture. The effect of photoperiod, in contrast, was more complex and strain dependent. Under short photoperiods (*e.g.* December), light was the main limiting factor to growth. In contrast, under longer photoperiod conditions (*e.g.* April) light led to photoinhibition and reduced growth. Microcosms experiments confirmed the predominance of *Bathycoccus* in microbial communities under low temperature conditions. The growth rates of *Bathycoccus* varied little between the different photoperiod conditions at low temperature. The yearly reoccurrence of *Bathycoccus* at the minimum

of temperature in February may result from its improved growth at low temperatures and, conversely, its reduced growth at higher temperatures compared to other strains. Simulation of an unusually warm winter shifted the photoperiod preferences of *Bathycoccus* from short to long, suggesting that, in the long term, a 2°C increase in temperature may affect the seasonality of *Bathycoccus*.

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The authors declare no conflict of interest.

Author Contributions: J-B.G., V.V., J.-C.L., P.S., S.L. and M.Q. performed the experiments; J.-B.G. and S.L. analyzed the data; J-B.G. and F-Y.B. conceived the experiments; J-B.G., S.L. and F-Y.B. wrote the manuscript.

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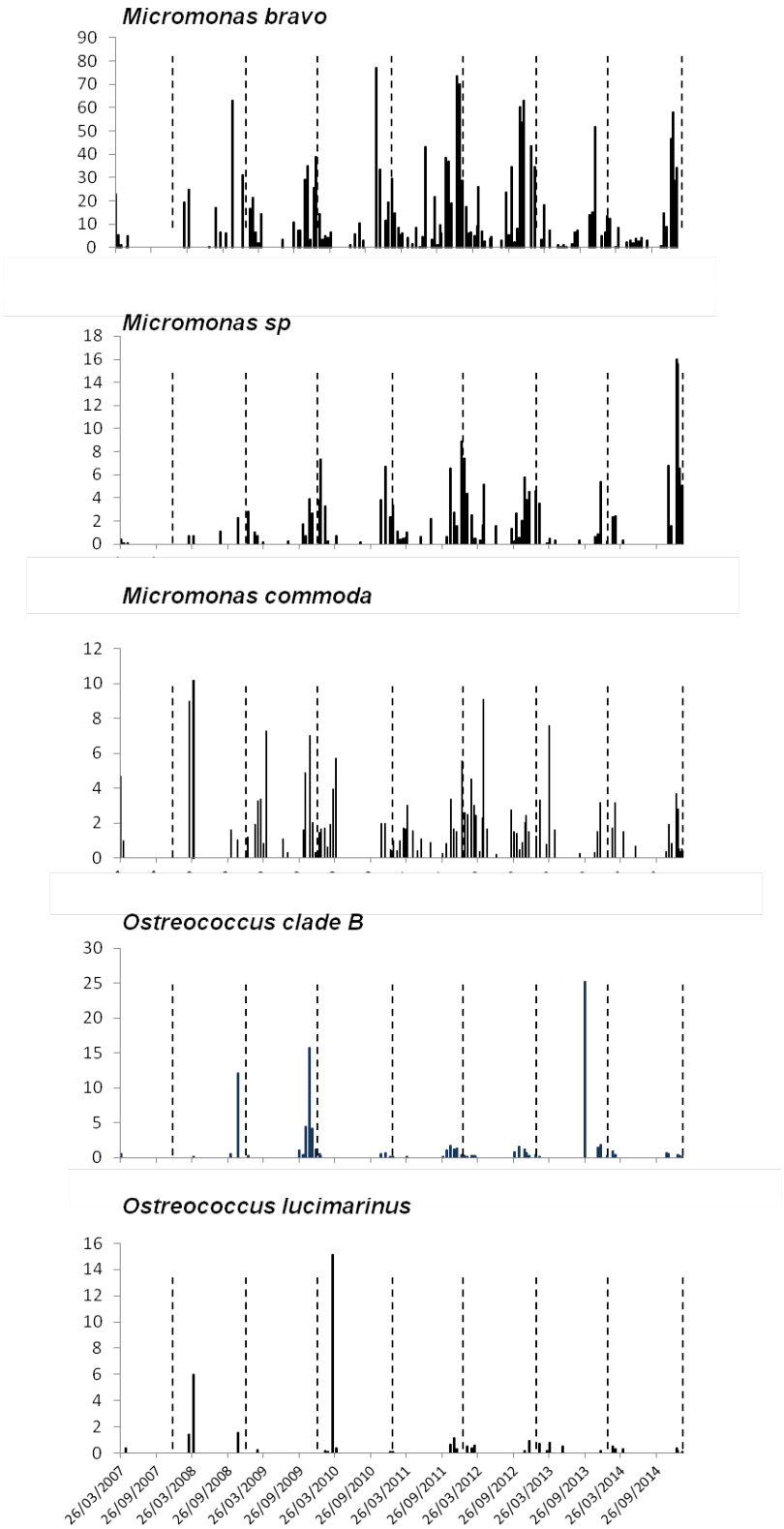
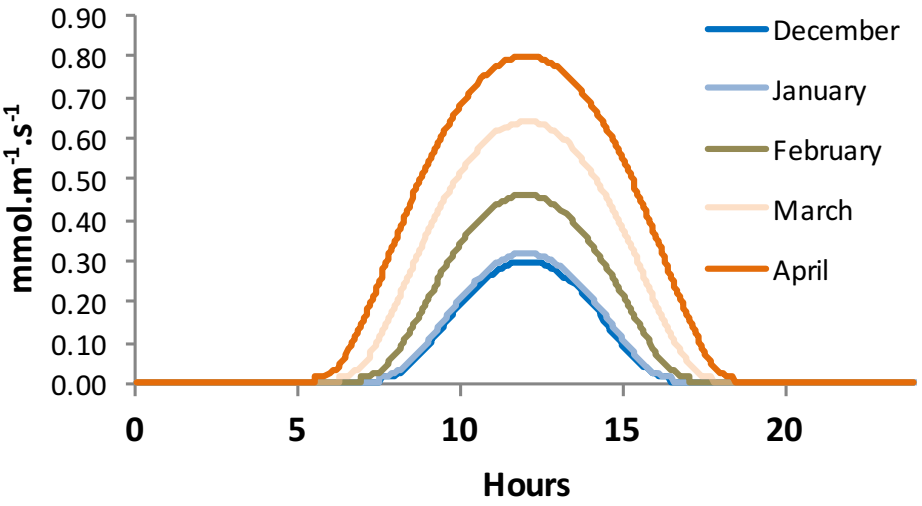
Supplementary information

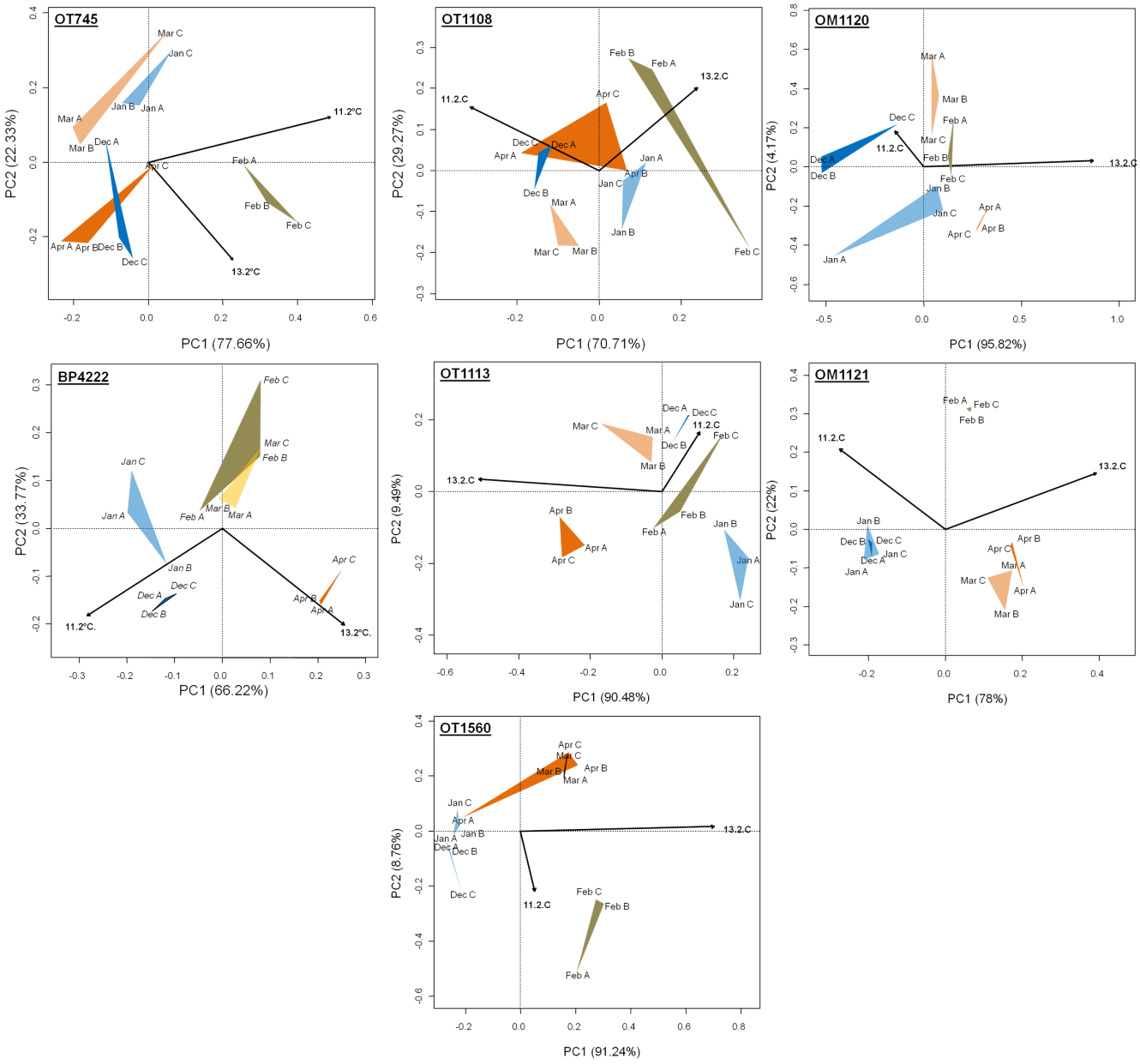
Supplementary Fig 1: Bar charts of the relative abundance, in percentages, of reads corresponding to *Micromonas bravo* (A). *Micromonas sp* (B). *Micromonas commoda* (C). *Ostreococcus clade B* (D) and *Ostreococcus lucimarinus* (E) Taxa were assigned with PR2.



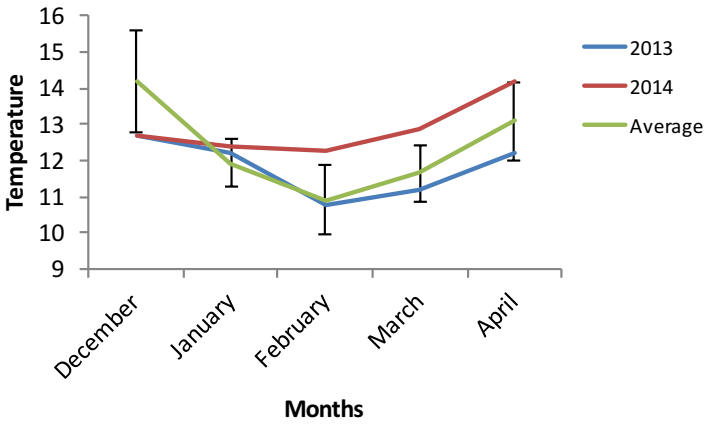
Supplementary Fig 2: Line graph of light intensities that microalgae were exposed to during growth phase. Each curve (blue, light blue, brown, light orange and orange) represents the light intensity measured at a specific month (December, January, February, March and April, respectively) during one day.



Supplementary Fig 3: Redundancy analyses (RDA) relating monthly irradiance to temperature variables for each specific strain. The axes explain the percentage of variation in growth rates for each strain. Temperature variables used in the RDA are represented by vectors (11.2°C and 13.2°C). Each point represents growth rate triplicates and are grouped according to photoperiod (December, January, February, March and April) as colored polygons (blue, light blue, brown, light orange and orange, respectively).

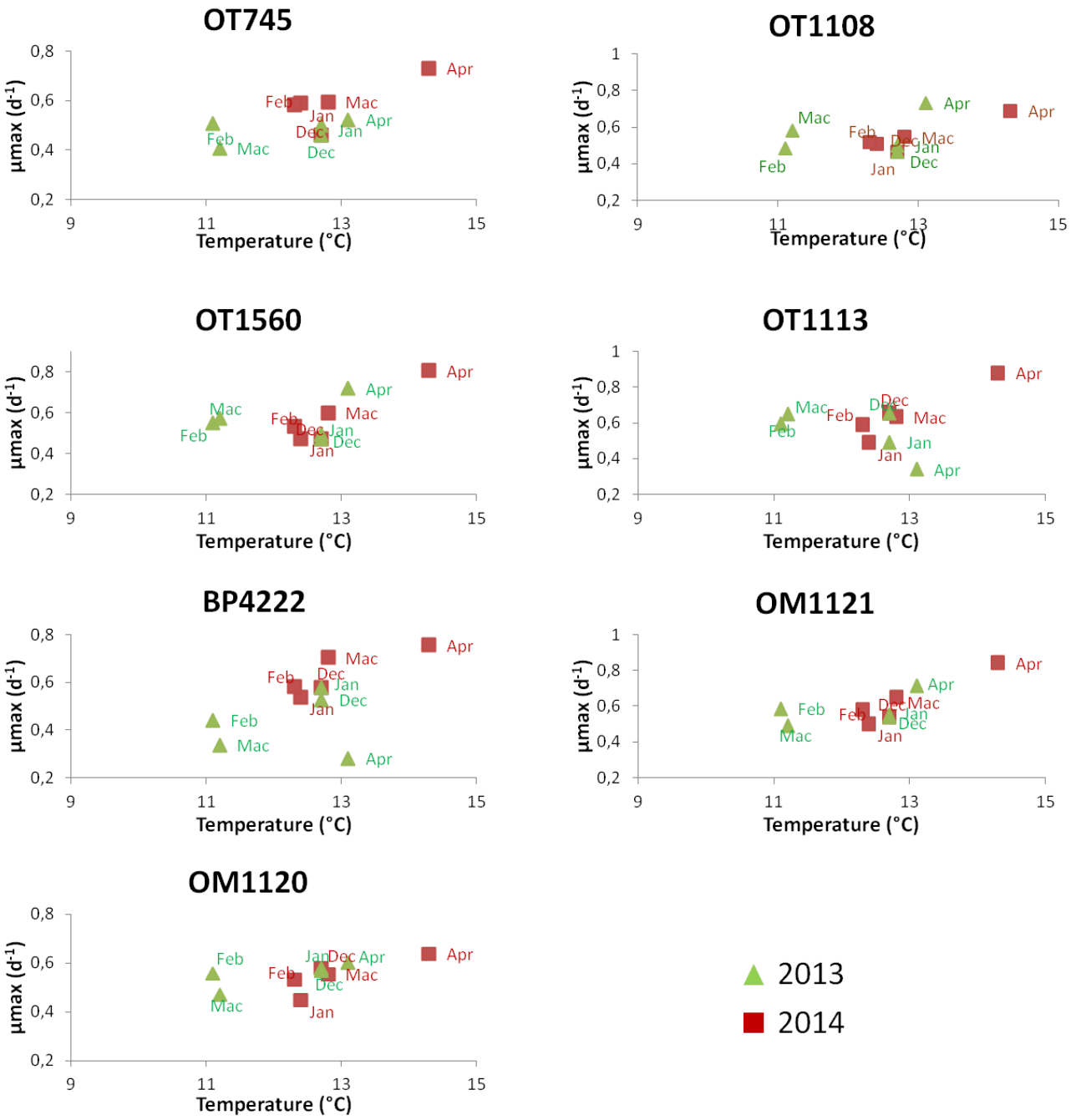


Supplementary Fig 4: Line graph and values of winter sea surface temperature in 2013, 2014 and the 2007-2014 average at 3m of depth in the bay of Banyuls.



Month	Years 2013	2014	Average (2007-2014)
December	12,7	12,7	14,2
January	12,2	12,4	11,9
February	10,8	12,3	10,9
March	11,2	12,9	11,7
April	12,2	14,2	13,1

Supplementary Fig 5: Monthly growth rates for each specific strain when exposed to simulated natural conditions. Green triangles correspond to 2013 and red squares to 2014.



Supplementary Table 1:
Growth rates for each microcosms condition (d^{-1}). Initial sea surface temperatures at SOLA are marked with black star.

Date	T°C	µmax (d^{-1})
16/02/2015	9,6	0,25 ± 0,04
	11,6	0,5 ± 0,02
	13,6	0,63 ± 0,02
	15,6	0,56 ± 0,02
23/02/2015	9,2	0,19 ± 0,01
	11,2	0,35 ± 0,04
	13,2	0,30 ± 0,08
	15,2	0,52 ± 0,08
02/03/2015	7	0,47 ± 0,23
	9	0,49 ± 0,09
	11	0,68 ± 0,22
	13	0,76 ±0,20
15/06/2015	19	0,58 ± 0,16
	21	0,77 ± 0,04
	23	0,92 ± 0,05

Addendum

My contribution: In this chapter my contribution amounted to carrying out the microcosms experiments, extracting and amplifying the DNA from those experiments and doing the sequence analysis. I also provided the time series data and helped write, edit and format the manuscript.

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Abstract

In temperate oceans, yearly transitions from winter to spring are accompanied by a phytoplanktonic bloom. Phytoplankton, at the basis of the food chain in the oceans, plays an essential role in biogeochemical cycles as it generates 50% of the global primary production. A time series established in 2007 at SOLA, a coastal site in the North Western Mediterranean Sea, monitors environmental and biological parameters. In the first chapter, we demonstrated that several microbial amplicon sequence variants (ASVs) displayed yearly rhythmicity, despite stochastic environmental perturbations, inherent to coastal ecosystems. Network analyses, described in the second chapter, revealed that salinity and temperature deeply impacted the microbial community structure. Subnetworks highlighted that persistent ASVs switched their first neighbors depending on environmental perturbations. These observations suggest the existence of functional redundancy in marine microbial communities. In the third chapter, microcosms confirmed that temperature fluctuations strongly affected natural microbial community structure. Picophytoplankton dominated the incubated communities at low temperature, whereas diatoms prevailed at higher temperatures. These results help explain *Bathycoccus prasinos* peak of abundance every year at the temperature minimum at SOLA. By integrating results from a time series, cell culture and microcosms experiments, this manuscript helps unravel the impact of anthropologically driven climate change on marine microbial communities.

Key words: Mediterranean Sea | Time series | Microbial communities | Rhythmicity | Microcosms

Résumé

Dans les océans tempérés, les transitions printanières annuelles sont accompagnées de blooms phytoplanctoniques. Le phytoplancton joue un rôle essentiel dans les cycles biogéochimiques et produit la moitié de la production primaire globale. Une série temporelle établie en 2007 à SOLA, un site côtier dans le Nord-Ouest méditerranéen, surveille les paramètres environnementaux et biologiques. Dans le premier chapitre, plusieurs « amplicon sequence variants » (ASVs) microbiens avaient des motifs annuels récurrents, malgré les perturbations environnementales caractéristiques des zones côtières. L'analyse de réseaux, décrite dans le deuxième chapitre, a révélé que la salinité et la température impactaient la structure des communautés microbiennes. Des sous-réseaux ont montré que des ASVs persistant changeaient de partenaires en fonction des perturbations environnementales. Ces observations suggèrent l'existence de redondance fonctionnelle dans les communautés microbiennes marines. Dans le troisième chapitre, des experiences microcosmes ont confirmé que des variations de température affectaient la structure des communautés microbiennes naturelles. À basse température, les picophytoplanctons étaient dominants, tandis que les diatomées prévalaient aux températures plus fortes. Ces résultats permettent d'expliquer le maximum d'abondance de *Bathycoccus prasinos* tous les ans au minimum de température à SOLA. Ce manuscrit, intégrant à la fois les résultats d'une série temporelle, de cultures cellulaires et de microcosmes, a permis d'éclaircir l'impact anthropologique sur les communautés microbiennes marines.

Mots clefs: Mer méditerranée | Séries temporelles | Communautés microbiennes | Rythmicité | Microcosmes