UNIVERSIDAD DE LAS PALMAS DE GRAN CANARIA
Departamento de Biología

Institut de Ciències del Mar

Patterns in marine microbial community structure

(Patrones de estructura de las comunidades de microorganismos marinos)

Thomas Lefort

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Director: Dr. Josep M. Gasol

Universidad de Las Palmas de Gran Canaria
Institut de Ciències del Mar (ICM-CSIC)
En Barcelona, a de de

El Doctorando
Thomas Lefort

El Director
Josep M. Gasol

à mes parents
à Juliette

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List of the most common abbreviations and acronyms used in this thesis:

Alpha: *Alphaproteobacteria*
BBMO: Blanes Bay Microbial Observatory
Beta: *Betaproteobacteria*
BHP: Bacterial Heterotrophic Production
BCS: Bacterial Community Structure
CARD-FISH: Catalyzed Reporter Deposition- Fluorescence In Situ Hybridization
CF: Conversion Factor
CHLA: chlorophyll *a*
CTC: 5-cyano-2,3-ditolyl tetrazolium chloride
CTD: Conductivity, Temperature and Depth sensors
CV: Coefficient of Variation
C/V: Ratio of carbon to volum
DCM: Deep Chlorophyll Maximum
DOC: Dissolved Organic Carbon
EUB: *Eubacteria*
FSC: Forward Scatter
Gamma: *Gammaproteobacteria*
HNF: Heterotrophic Nanoflagellates
HPLC: High Performance Liquid Chromatography
OTU: Operational Taxonomic Unit
PCS: Picoplankton Community Structure
pPeuk: photosynthetic Picoeukaryotes
POC: Particulate Organic Carbon
PON: Particulate organic Nitrogen
Pro: *Prochlorococcus*
Ros: *Rhodobacteraceae*
SSC: Side Scatter
Syn: *Synechococcus*
Temp: Temperature

Understanding the distribution of the different picoplankton groups represents a central tenet of marine microbial ecology. Centering our study on the three major groups constituting the bulk picoplankton community (size 0.2-3 μm), we sought to analyze the distribution of autotrophic bacteria (*Synechococcus* and *Prochlorococcus*), photosynthetic Picoeukaryotes pPeuk, and heterotrophic bacteria. For that objective, two different strategies were used, the first one was based on flow cytometry for determining ataxonomic patterns in picoplankton distribution, and the second a comparative analysis approach for identifying broad patterns in bacterial phylogenetic community structure. Given that conversion factors (CFs) were necessary to translate group cell abundance into carbon biomass, but that large discrepancies for CF values of pPeuk had been reported in the literature, we first (re-) evaluated the CF for small phototrophic picoeukaryotes (<5 μm). On one hand, as the set of cultures of Peuk used for that purpose were maintained in non-axenic conditions, we compared two different methods for correcting errors in biomass estimation due to presence of bacteria. Secondly, a relatively higher CF value for pPeuk than those previously reported to date was found, with implications on the role generally attributed to pPeuk in the carbon cycling and other ecosystem processes. Applying this CF, we could identify patterns of variability in picoplankton group distribution at different spatio-temporal scales during winter in a NW Mediterranean coastal station and during a cruise performed in summer from coast to offshore off the Catalan coast. By focusing on the variability at the short time scale, our work showed not only evidences of coupling between picophytoplankton variability and the single-cell bacterial activities but also highlighted how a relatively small variation in meteorology changed considerably the structure of the microbial community. Different trends of variability were observed between the different picoplankton groups, pPeuk cell numbers exhibiting the highest spatio-temporal variability, and bacterial abundance the lowest. Opposite patterns between picoplankton community structure and chlorophyll *a* levels were observed not only spatially, but also at both the short-term and large temporal scale, suggesting that picoplankton group distribution are useful indicators of the ecosystem state. Finally, we assessed the biogeography of the bacterial phylogenetic groups along a continuum of environmental parameters such as chlorophyll *a*, temperature and salinity, and identified different patterns in bacterial community structure as related to phytoplankton biomass among coastal and open ocean ecosystems, suggesting unequal metabolic aptitudes of the different bacterial groups for utilizing algal-derived DOC.

La comprensión de la distribución de los distintos grupos que forman el picoplancton representa una de las preguntas fundamentales de la ecología microbiana marina. Centrando nuestro estudio en los tres grupos principales que constituyen la comunidad de picoplancton (tamaño de 0,2 a 3 μm), hemos tratado de analizar la distribución de bacterias autótrofas (*Synechococcus* y *Prochlorococcus*), picoeucariotas fotosintéticos (pPeuk) y bacterias heterotróficas. Con ese objetivo, se utilizaron dos estrategias distintas: la primera se basa en la citometría de flujo para la determinación de los patrones ataxonómicos de distribución de picoplancton y la segunda, un análisis comparativo para la identificación de patrones generales en la estructura filogenética de la comunidad bacteriana. Teniendo en cuenta que son necesarios factores de conversión para transformar la abundancia de los grupos de células en biomasa, pero que se registran en la literatura grandes discrepancias en los valores de factor de conversión para pPeuk, en primer lugar (re-) evaluamos el factor de conversión para picoeucariotas fototróficos pequeños (menores de 5 μm) . Por un lado, como el conjunto de los cultivos de Peuk utilizados para este fin se mantienen en condiciones no axénicas, se compararon dos métodos diferentes para la corrección de errores en la estimación de la biomasa debido a la presencia de bacterias. Encontramos un valor relativamente más alto para el factor de conversión de los pPeuk que los publicados hasta la fecha, lo que implica consecuencias considerables sobre la función generalmente atribuida a los pPeuk en el ciclo del carbono. La aplicación de este factor de conversión, permitió identificar los patrones de variabilidad en la distribución de los grupos de picoplancton a diferentes escalas espacio-temporales durante el invierno en una estación costera y en el Mediterráneo noroccidental durante una campaña realizada en el verano de costa a mar abierto en el Mar Catalano-Balear. Al centrarnos en la variabilidad temporal, nuestro trabajo mostró evidencias de acoplamiento entre la variabilidad del picofitoplancton y la actividad de las bacterias, pero también puso de relieve cómo una variación relativamente pequeña en la meteorología cambió considerablemente la estructura de la comunidad microbiana. Diferentes patrones de variabilidad se detectaron entre los grupos diferentes de picoplancton, las abundancias de pPeuk exhibieron la variabilidad espacio-temporal más alta y la abundancia bacteriana, la más baja. Patrones opuestos entre la estructura de la comunidad de picoplancton y los niveles de clorofila *a* se observaron no sólo espacialmente, sino también en la escala temporal a corto plazo y en la escala a largo plazo, con lo que se sugiere que la composición de la comunidad de picoplancton es un buen indicador del estado del ecosistema. Por último, evaluamos la biogeografía de los grupos filogenéticos bacterianos a lo largo de un continuo de parámetros ambientales, tales como la clorofila *a*, temperatura y salinidad, y se identificaron patrones diferentes en la estructura de la comunidad bacteriana y la cantidad de fitoplancton entre los ecosistemas costeros y de mar abierto, lo que sugiere aptitudes metabólicas desiguales en los diferentes grupos bacterianos a la hora de utilizar materia orgánica disuelta derivada de las algas.

Un des objectius fonamentals de l'ecologia microbiana marina és comprendre la distribució dels diferents grups que composen el picoplàncton. Centrant el nostre estudi en els tres principals grups que constitueixen la comunitat de picoplàncton (unida entre 0.2-3 μm), hem tractat d'analitzar la distribució de bacteris autotròfics (*Synechococcus* i *Prochlorococcus*), de picoeucarionts fotosintètics (pPeuk) i de bacteris heterotròfics. Amb aquest objectiu, es van utilitzar dues estratègies diferents: la primera es basa en la citometria de flux per tal de determinar els patrons ataxonòmics de la distribució de picoplàncton, i la segona, una anàlisi comparativa per a identificar patrons generals en l'estructura filogenètica de la comunitat bacteriana. Tenint en compte que es necessiten factors de conversió per tal de transformar l'abundància dels grups de cèl·lules en biomassa, i que a la bibliografia hi ha registrades grans discrepàncies en el valor del factor de conversió per a pPeuk, en primer lloc vam (re-) evaluar el factor de conversió per a picoeucariotes fototròfics petits (< 5 μm). Per una banda, com que el conjunt dels cultius de Peuk utilitzats amb aquesta finalitat es mantenen en condicions no axèniques, es van comparar dos mètodes diferents per corregir els errors en l'estimació de la biomassa degut a la presència de bacteris. Vam trobar un valor del factor de conversió dels pPeuk relativament més alt que els publicats fins aleshores, fet que implica conseqüències considerables sobre la funció atribuïda generalment als pPeuk en el cicle del carboni. L'aplicació d'aquest factor de conversió va permetre identificar els patrons de variabilitat en la distribució dels grups de picoplàncton a diferents escales espaciotemporals: durant l'hivern en una estació costera del Mediterrani nord-occidental, i durant una campanya realitzada a l'estiu de costa a mar obert en el Mar Catalano-Balear. Al centrar-nos en la variabilitat temporal a curt termini, el nostre treball va mostrar evidències d'acoblament entre la variabilitat del picofitoplàncton i l'activitat dels bacteris, i també va posar de relleu com una variació relativament petita en la meteorologia canviava considerablement l'estructura de la comunitat microbiana. Es van detectar diversos patrons de variabilitat entre els grups diferents de picoplàncton: les abundàncies de pPeuk van exhibir la variabilitat espaciotemporal més alta i en canvi, l'abundància bacteriana va exhibir la variabilitat més baixa. Es van observar patrons oposats entre la estructura de la comunitat de picoplàncton i els nivells de clorofil·la *a*, no solament espacialment, sinó també en escales temporals a curt i llarg termini, la qual cosa suggereix que la composició de la comunitat de picoplàncton és un indicador de l'estat de l'ecosistema. Finalment, vam evaluar la biogeografia dels grups filogenètics bacterians al llarg d'un continu de paràmetres ambientals, com la clorofil·la *a*, la temperatura i la salinitat, i vam identificar patrons diferents en l'estructura de la comunitat bacteriana i la biomassa de fitoplàncton entre els ecosistemes costaner i de mar obert, la qual cosa suggereix aptituds metabòliques desiguals en els diferents grups bacterians a l'hora d'utilitzar la matèria orgànica dissolta produïda per les algues.

La compréhension de la répartition des différents groupes de picoplancton représente un objectif central de la microbiologie marine. En dirigeant notre étude sur les trois principaux groupes de picoplancton (d'une taille comprise entre 0.2 à 3 μm), notre objectif était d'analyser la distribution des cyanobacteries (*Synechococcus* et *Prochlorococcus*), des Picoeucaryotes photosynthétiques (pPeuk), ainsi que celle des bactéries hétérotrophes. Pour mener à bien cet objectif, deux stratégies différentes furent utilisées, la première étant basée sur la cytométrie en flux pour la détermination de profils ataxonomiques de distribution, la seconde basée sur une analyse comparative afin d'identifier des profils de structure phylogénétique des communautés bactériennes. Etant donné que les facteurs de conversion sont nécessaires pour convertir les abondances cellulaires de chaque groupe de picoplankton en équivalent carbone, mais que d'importantes divergences demeurent dans la littérature quant à la quantité de carbone contenue par pPeuk, un de nos objectifs premier était de réévaluer la relation entre la taille et le contenu en carbone de différentes cultures de Picoeucaryotes écologiquement représentatifs des milieux marins (<5 μm). Ainsi, nous avons mesuré une valeur de densité en carbone par pPeuk relativement plus élevée que celle précédemment rapportées à ce jour, suggérant d'importantes conséquences sur le rôle généralement attribué à ce groupe dans le cycle du carbone océanique. De plus, comme les cultures utilisées à cette fin n'étaient pas axéniques, nous avons comparé deux méthodologies pour corriger le biais dans l'estimation du contenu en carbone. Ensuite et par l'application de ce facteur de conversion, nous avons analysé la variabilité de distribution des differents groupes du picoplancton à différentes échelles spatiales et temporelles: en hiver, dans une station côtière au Nord Ouest de la Méditerranée et en été, lors d'un transect effectué au large de la côte catalane. En mettant l'accent sur la variabilité à court terme, notre étude démontre non seulement l'existence d'un couplage entre la structure de la communauté picophytoplanctonique et l'activité bactérienne, mais souligne aussi l'importance des variations météorologiques à court termes sur la structure de la communauté microbienne. Différents profils de variabilité furent observés entre les différents groupes du picoplancton, l'abondance cellulaire des pPeuks présentant la variabilité spatio-temporelle la plus élevée et l'abondance bactérienne, la plus faible. De plus, des profils opposés entre la structure de la communauté picoplanctonique et la concentration de chlorophylle *a* furent observés non seulement spatialement, mais aussi à court et à long terme, suggérant que la structure de la communauté picoplanctonique est un indicateur utile de l'état des écosystèmes. Enfin, le dernier objectif de cette thèse était d'étudier la biogéographie des groupes phylogénétiques bactériens le long de gradients de paramètres environnementaux tels que la concentration en chlorophylle *a*, la température et la salinité. Nous avons identifié différents profils de structure de communautés bactériennes variant avec la biomasse du phytoplancton entre les écosystèmes côtiers et océaniques, suggérant que les différents groupes bactériens presentaient des aptitudes métaboliques inégales quant à l'utilisation de carbone organique d'origine algale.

GENERAL INTRODUCTION

BIOGEOGRAPHY AND MICROBIAL ECOLOGY: INITIAL APPROACHES

Understanding the patterns and processes involved in the distribution of life forms constitutes the central tenet of biogeography. Rooting its origins with Carl Linnaeus's (1707-1778) taxonomical classification that was based on both the differences and similarities shared among types of plants and animals (Species Plantarum 1753), biogeography began with the Comte de Buffon's theory (1707-1788) who stated that similar but geographically separated environments presented distinct plants and animals (Histoire naturelle, générale et particulière, 1749-1788). In the late 1800's, contradictory theories emerged, the earth-history explanations of the endemism of species distributions proposed by Charles Lyell's (1797-1875) contrasted with the global dispersal theory proposed by the botanist Alphonse de Condolle (1806-1893) who formulated that "*the lower the organization of the body is, the more generally it is distributed*" implying that microbial life was everywhere and would proliferate under appropriate conditions. This latter theory was quickly considered as one "fundamental law" of biogeography, fueled by the emergence of microbiology.

The study of patterns in marine microbe's distribution has been constrained by the technological limits in observing and identifying tiny organisms. At first, measurement of microbial abundance was based on pure culture isolates and optical microscopy (Certes 1884; Zobell 1946) that greatly underestimated bacterial abundance by several orders of magnitude (Jannash and Jones 1959). The developments of epifluorescence microscopy during the 70´s (Hobbie et al. 1977; Zimmerman 1977; Porter and Feig 1980), as well as the introduction of automated cell counting by Coulter Counter and Flow Cytometry (Sheldon and Parsons 1967; Sheldon 1978) enabled the detection and enumeration of different microbial groups in field samples (Olson et al. 1985). Whether distinct distribution patterns in microbes can be identified depends on the criteria used for the definition and classification of the different microbial groups. These criteria range from size categories, function and activity categories to more phylogenetic-based categorization.

IDENTIFICATION OF MICROBIAL GROUPS BY FLOW CYTOMETRY

Based on the detection by scatter and fluorescence sensors of fluorescently pigmented cells passing through a laser beam, flow cytometry can be used routinely (Marie and Partensky 2006) to identify and quantify cells within at least three ecological distinct groups: eukaryotic algae, cyanobacteria (*Synechococcus* and *Prochlorococcus*) and heterotrophic bacteria (Olson et al. 1993; Gasol and del Giorgio 2000). Chlorophyll *a* pigment concentration constitutes the principal factor that is used to discriminate phytoplankton and photosynthetic picoeukaryotic cells from other particles (Yentsch and Yentsch 1979; Li et al. 1995). Other photo-pigments,

such as phycoerythrin (emitting from 550 to 590 nm once excited by blue light), are used to distinguish between most *Synechococcus* (Johnson and Sieburth 1979; Waterbury et al. 1979; Wood et al. 1985) and *Prochlorococcus* species (Chisholm et al. 1988) (e.g. Figure 1). Non-photosynthetic bacteria are too small for being detected by size alone. And even *Prochlorococcus* autofluorescence in oligotrophic ecosystems is often too low for being accurately detected by the cytometer sensors. Then, DNA-based fluorescent dyes can be used to stain the sample and this allows enumeration of heterotrophic bacteria and viruses (Li et al. 1995; Marie et al. 1999) and to distinguish *Prochlorococcus* from nonphotosynthetic bacteria (Monger and Landry 1993).

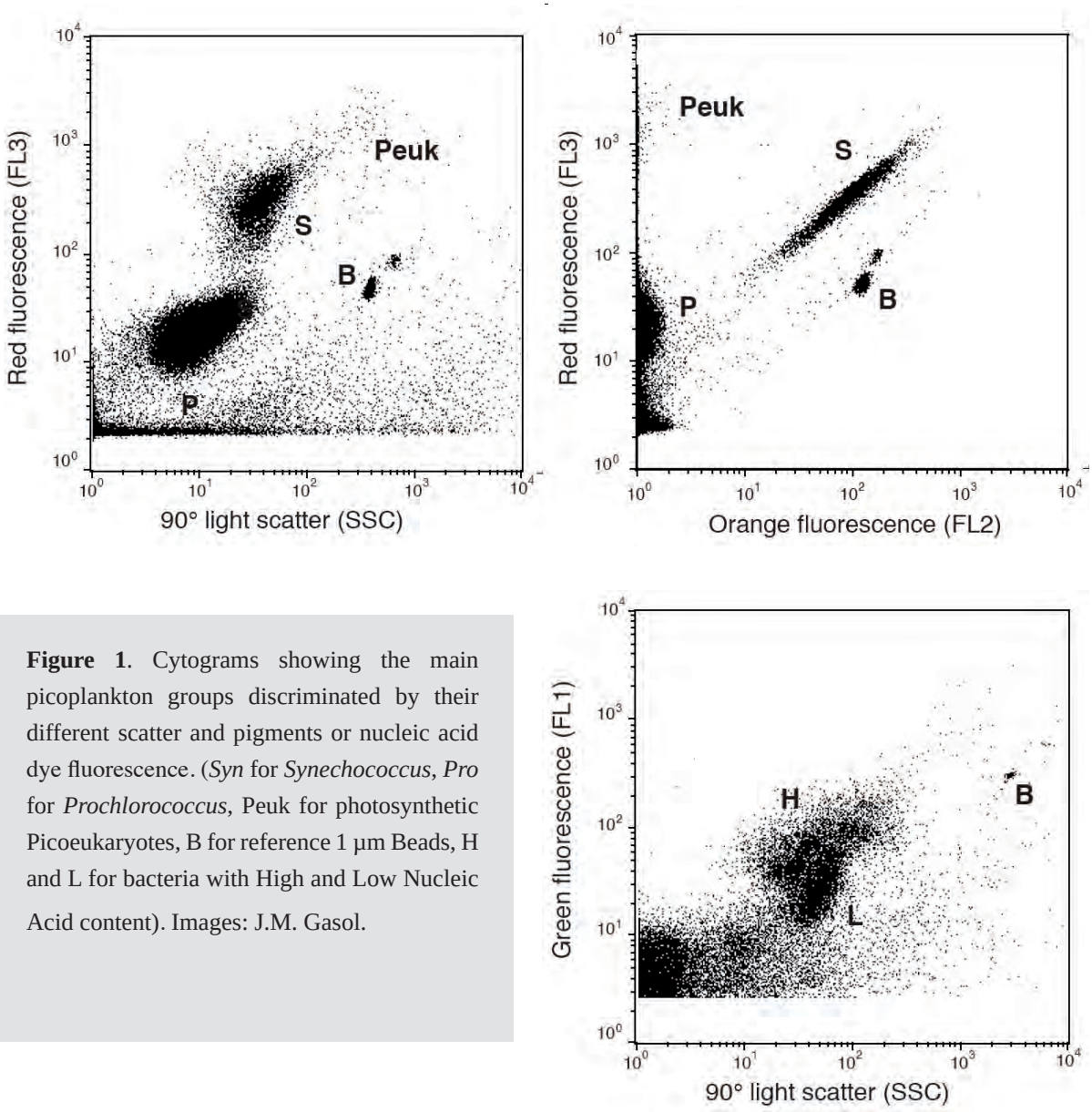


Figure 1. Cytograms showing the main picoplankton groups discriminated by their different scatter and pigments or nucleic acid dye fluorescence. (*Syn* for *Synechococcus*, *Pro* for *Prochlorococcus*, Peuk for photosynthetic Picoeukaryotes, B for reference 1 µm Beads, H and L for bacteria with High and Low Nucleic Acid content). Images: J.M. Gasol.

HETEROTROPHIC BACTERIA IN THE MICROBIAL FOOD WEB

Accounting for a total of 10^{29} cells at the global ocean scale, heterotrophic bacteria are now considered as the most abundant living organisms on Earth (typically found at around 10^{9} cells l^{-1}) (Whitman et al. 1998) and play a key role in the oceanic carbon cycling through the microbial loop (Azam et al., 1983). Heterotrophic bacteria in aquatic ecosystems were soon recognized for their role in the decomposition of organic material and the remineralization of inorganic nutrients, but Pomeroy in 1974 showed that their functions could be more diverse than it was previously thought. Indeed, Hagström et al. (1979) and Fuhrman and Azam (1980) showed that an important proportion of bacteria were not dormant but actively growing through the utilization of oceanic organic matter, bacterial heterotrophic production later estimated as accounting between 20-30% of primary production on average (Cole et al. 1988). The microbial food web, summarized in Figure 2, assumes that heterotrophic bacteria are loosely coupled to phytoplankton (Bird and Kalff 1984; Cole et al. 1988; Fuhrman and Azam 1980) and balanced not only by grazing from ciliates and flagellates (Fenchel 1982; Sherr and Sherr 1984; 1987) but also by crustacean and rotifers (Pedrós-Alió and Brock 1983).

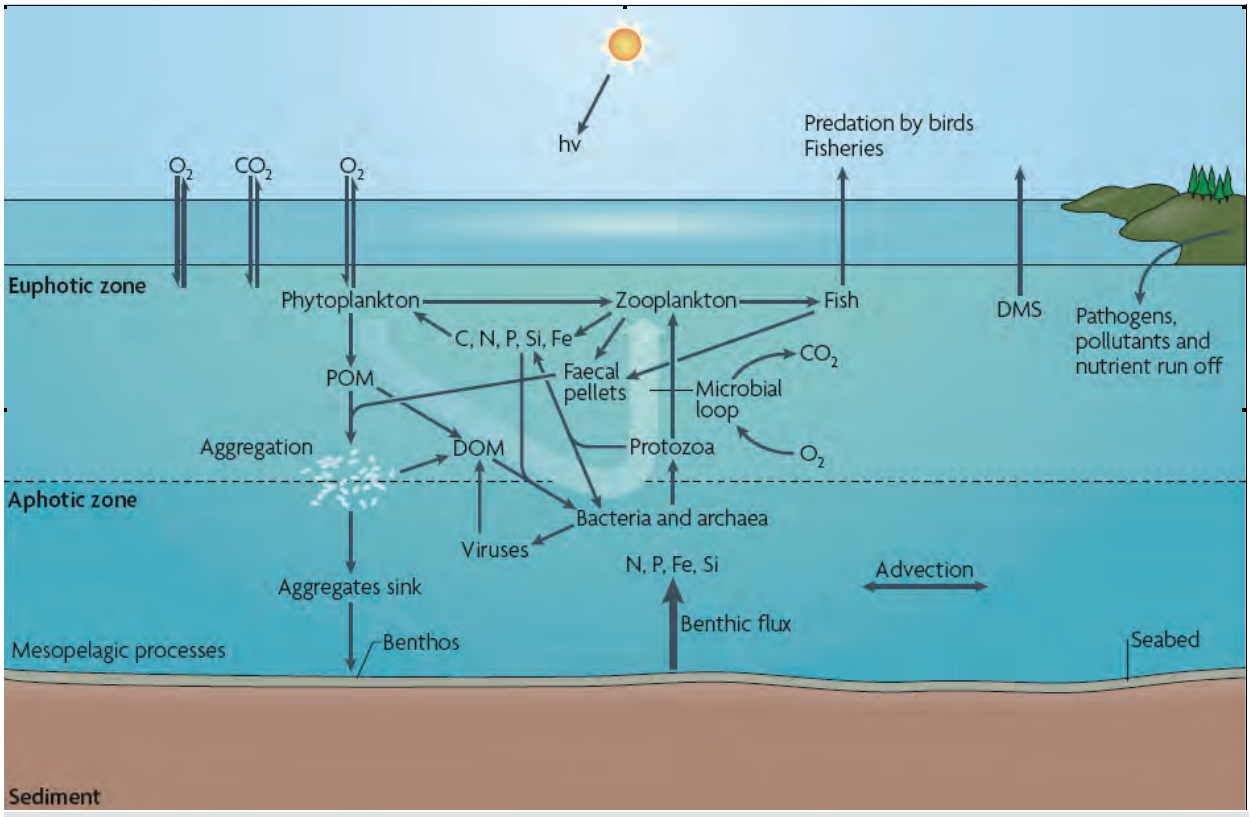


Figure 2. Schematic Illustration of the marine microbial food web and some of the biogeochemical fluxes involved (from Azam and Malfatti 2007)

The link between autotrophs and heterotrophs assumes that bacteria use the dissolved organic matter produced by phytoplankton and grazers to support growth as bacterial secondary production (Nagata et al. 2000; Morán et al. 2002). Studies based on large data set comparisons have demonstrated such a link and described a positive relationship with a log-log slope <1 between heterotrophic and autotrophic biomass (for instance: Gasol et al. 1997), showing that bacterial biomass vary less than chlorophyll *a* along a gradient of trophy and suggesting pronounced heterotrophy in low production ecosystems and a less relevant role of the microbial food web in the most eutrophic sites.

SINGLE CELL LEVEL ACTIVITY

Whether all bacterial cells contribute equally to the bulk metabolic activity of bacterioplankton or only a few key players are involved, has long been a central question of microbial ecology.

Complex aquatic bacterial assemblages present a variety of different metabolic states. Allocating a wide range of different activities to distinct groups of cells by the use of single cell techniques, bulk bacterial activity can be represented as a continuum of different physiological states (Smith and del Giorgio 2003; del Giorgio and Gasol 2008). Microscopy was first used for measuring the division rate of bacteria (Hagström et al. 1979) and microautoradiography is still extensively applied for measuring the substrate uptake activity in aquatic ecosystems (Parsons and Strickland 1961; Wright and Hobbie 1965; Hoppe 1976). To date, the application of specific molecular probes in combination with flow cytometry appears as a suitable set of tools for characterizing rapidly and with statistical significance the physiological community structure (del Giorgio and Gasol 2008). Physiological probes can be used for detecting a variety of cellular states, either measuring cellular death by screening the membrane-damaged cells (e.g. Grégori et al. 2001, **Chapter II**), or quantifying different metabolic processes such as the percentage of actively respiring cells estimated by the quantification of CTC positive cells (e.g. Gasol and Arístegui 2007) (e.g. Figure 3). If the availability of organic carbon represents perhaps the most important factor influencing bulk heterotrophic bacterial activity in marine ecosystems (Azam 1998; del Giorgio and Gasol 2008; Church 2008), the factors regulating the different single cell activities at different spatio-temporal scales still remain poorly studied.

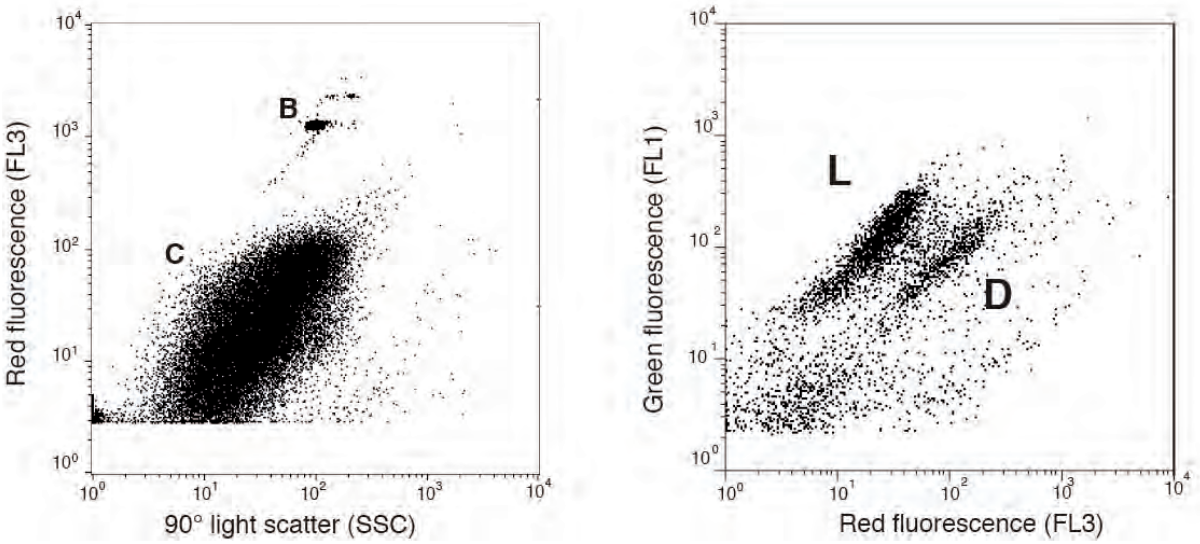


Figure 3. Cytograms showing different physiological groups of bacteria discriminated by the presence of the fluorogenic tetrazolium dye CTC indicator of actively respiring cells (A) and by the action of the cell-permanent nucleic acid stain SybrGreen I and the cell-impermeant propidium iodine (B), C for CTC positive cells, B for reference 1 µm Beads, L for Live cells and D for Dead cells as labelled by the NADS protocol. Image: J.M. Gasol.

PICOPHYTOPLANKTON IN THE MICROBIAL FOOD WEB

While bacterioplankton community processes have been modeled as being solely heterotrophic, and early oceanographic models understated the importance of oxygenic photoautotrophic picoplankton, Richardson and Jackson (2007) noted that picophytoplankton constitutes also an important source of organic carbon for large zooplankton and are also contributing to the flux of particles sinking to the deep ocean. By being of a similar size range than heterotrophic bacteria, are subject to similar (but not identical) loss and growth processes. They are consumed by protists, particularly nano-sized protists (Caron et al. 1991; Dolan and Simek 1998; Guillou et al. 2001), are subject to viral lyses (Proctor and Fuhrman 1991) and compete for nutrients (Hall and Vincent 1990; Li 1994; Vaulot et al. 1996). Quantitative cell counts and flow cytometry have revealed autotrophic picoplankton as ubiquitous players dominating photosynthetic activities in open-ocean gyres (e.g. Partensky et al. 1999). Ubiquitously found at around 10^5 - 10^6 cells ml^{-1} in a variety of ecosystems, *Synechococcus* most likely dominate picophytoplankton in nutrient rich well mixed waters, (Partensky et al. 1999) while *Prochlorococcus* prevalence is observed between 40ºN and 40ºS latitude, peaking in well stratified and nutrient poor deep waters as well as present

in deeply mixed and nutrient rich spring or winter waters (Campbell et al. 1997; Durand et al. 2001; Partensky et al. 1999) where they appear to contribute to up to 30% of the biomass in the oligotrophic North Pacific ecosystem (Campbell et al. 1994). Photosynthetic Picoeukaryotes and *Synechococcus* abundances have been shown to covary in a variety of ecosystems (Campbell et al. 1998; Shalapyonok et al. 2001; Durand et al. 2001; Worden et al. 2004).

In comparison with *Cyanobacteria*, less importance had been given to Picoeukaryotes due to their lower abundances. However, the calculation of their contribution in terms of biomass and primary production reveal a much higher importance in the oceanic carbon cycling that it was expected before (Li et al. 1995; Worden et al. 2004).

Carbon conversion factors for biomass estimation

Underestimation of the Picoeukaryotes' relevance compared to other picoplankton members might have stemmed from the lack of well-defined carbon conversion factors. To convert the different microbial group abundances (as estimated for example from flow cytometry) into carbon biomass, cell size and cellular carbon content are two necessary parameters.

To date, estimations of picoeukaryote carbon content have been mostly made by converting cell size or cell volume into carbon using empirically derived linear relationships (Mullin et al. 1966; Strathmann 1967) established from the study of cultures of larger algal species. However, in comparison with large phytoplankton cells, small eukaryotes (e.g. Figure 4) have relatively smaller vacuoles (similarly than small bacterial cells contain less cellular water (Simon and Azam 1989) and relatively higher cellular carbon content per unit of volume, as revealed by negative relationships between the cellular carbon content per unit of volume (fgC μm^{-3}) with increasing cell volume (Verity et al. 1992).

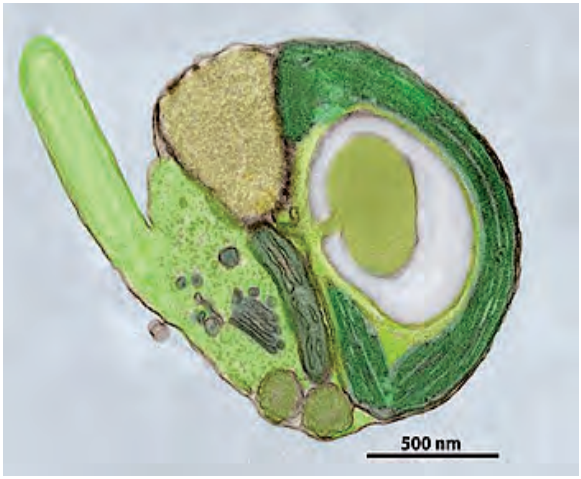


Figure 4. Image of *Micromonas*: T. Deerinck, M. Terada, J. Obiyashi, M. Ellisman (all National Center for Microscopy and Imaging Research) and A. Z. Worden (MBARI).

When no direct cell size measurements are available, the only possible course of action for estimating the cellular carbon content is to assume an average cell size. Within the picoplanktonic size category (0.2 - 3 μm), the cell size averages of *Synechococcus* and *Prochlorococcus* appear as relatively stable when compared to Picoeukaryotes (Durand et al. 2001; Worden et al. 2004). *Prochlorococcus* are the smallest photosynthetic prokaryotes with a cell diameter estimated at 0.7 μm (Shalapyonok et al. 2001; Worden et al. 2004; Durand et al. 2001), closely followed by *Synechococcus* with a slightly larger size average of 0.87 μm (Worden et al. 2004) and ranging from 0.5 to 2 μm (Murphy and Haugen 1985).

When converted in terms of carbon, the variations found over time in *Synechococcus* and *Prochlorococcus* total biomass are mainly determined by changes in cell abundance (Durand et al. 2001), consequence of this average size stability. In comparison, Picoeukaryotes fluctuations in biomass appear as a function of both, changes in cell abundance, and changes in mean size, which more likely reflects changes in species composition of the picoeukaryotic fraction (Worden et al. 2004). The determination of carbon conversion factors based on the knowledge of the taxonomical biogeography of ecologically relevant Picoeukaryotes was still a necessary but unaccomplished goal.

MICROBIAL PHYLOGENY

The observed distribution patterns of the micro organisms are strongly dependent on the method by which organisms are classified. If clear boundaries have been identified between macroscopic species such as in mammals or plants, the categorization of bulk bacteria into distinct phylogenetic bacterial groups has been established only recently.

The first approaches for understanding bacterial phylogeny arose at the end of the 19th century and were based on the study of the metabolic and morphological similarities between bacteria isolated on agar plates. However, this method soon was seen as biased by the unrealistic concentration levels of organic matter and nutrient used in culture media, falsely stimulating particular traits of bacterial metabolism. From the 70´s to the 90´s, the developments of culture independent techniques emerged with improvements made in nucleic acid extraction and sequencing methods. In parallel, the identification of genetic markers universally shared by organisms allowed to conceptualize a new representation of life, not any more based on morphological and physiological criteria but on genetic comparison of the conserved small subunit ribosomal RNA sequences which organized all live beings into three-domains composed of Archaea, Bacteria and Eukarya (Woese et al. 1977, 1987). Developments in the late 1980's of the Fluorescence in situ hybridization (FISH) method combined to epifluorescence microscopy, based on the targeting

of rRNA by fluorescently labeled oligonucleotide probes, allowed the in situ identification and quantification of different phylogenetic bacterial groups, with a specificity of identification spanning from the species level to the level of phyla and domain (see review by Amann and Fuchs 2008). A Biogeography of microbial populations at some phylogenetic level was then possible (Alfreider et al. 1996; Llobet-Brossa et al. 1998; Murray et al. 1998; Simon et al. 1999; Kirchman et al. 2005). *Alphaproteobacteria* was shown to dominate in marine coastal waters of Delaware Bay (Kirchman et al. 2005), or in the northwestern Mediterranean Sea (Alonso-Sáez et al. 2007), contrasting with *Betaproteobacteria* found more abundantly in freshwaters (Glöckner et al. 1999). The SAR11 cluster, a distinct branch within the *Alphaproteobacteria* phylum and probably the most abundant bacterial group in the surface ocean, dominates particularly in nutrient-depleted areas such as oligotrophic waters of the Sargasso Sea (Morris et al. 2002) and in coastal Mediterranean waters (Alonso-Sáez et al. 2007). The *Rhodobacteraceae* group of marine *Alphaproteobacteria* has been identified in most marine environments (Buchan et al. 2005), and is generally more abundant in bacterial communities associated with marine algae (Buchan et al. 2005). *Bacteroidetes* (previously known as *Cytophaga-Flavobacteria-Bacteroidetes*) constitute one of the major groups of picoplankton (Glockner et al. 1999; Kirchman 2002), abundantly represented in a variety of ecosystems such as cold waters (Simon et al. 1999; Abell and Bowman 2005), coastal waters (Eilers et al. 2001; O'Sullivan et al. 2004; Sáez-Alonso et al. 2007), accounting for as much as half all bacterial cells counted by FISH in California coastal seawater samples (Cottrell and Kirchman 2000), in offshore conditions (Simon et al. 1999; Abell and Bowman 2005; Schattenhofer et al, 2009) and generally associated to phytoplankton blooms (Simon et al. 1999).

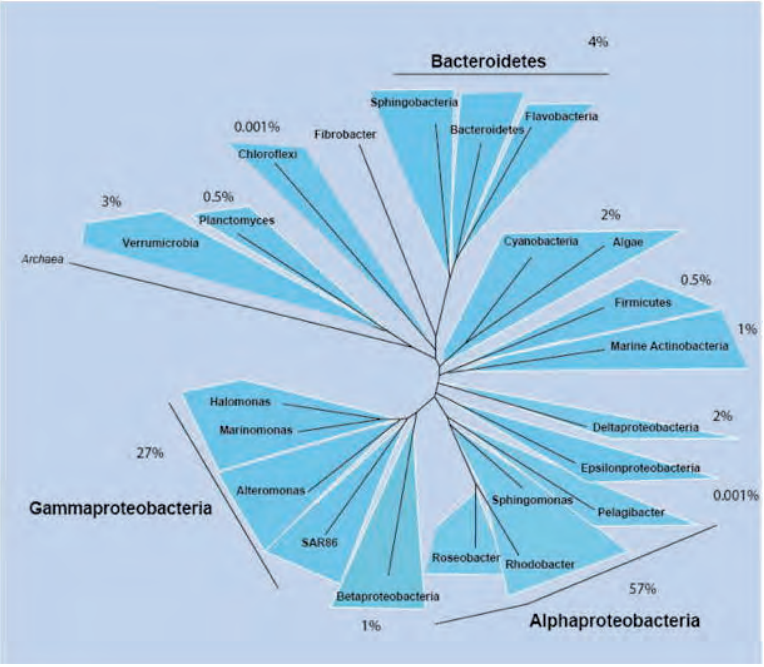


Figure 5. A phylogenetic tree of bacteria showing the major groups identified in Blanes Bay and contribution to total cells as determined by 454 analysis of 16SrRNA (Data of C. Pedrós-Alió and T. Pommier, drawing by J.M. Gasol).

Biome-related patterns versus continuum hypothesis

We have seen above how we can divide the picoplankton community either in functional groups (determined by flow cytometry), in activity groups (determined by fluorescent activity probes) or in phylogenetic groups using certain oligonucleotide probes. The patterns in microbial group distribution (flow cytometrically, activity-based, or phylogenetically determined) can be predicted by taking two different approaches, from either the characterization of different marine ecosystems with specific biogeochemical properties (Longhurst 1995; 1998) or assuming that the relative contributions of the different groups vary along a continuum of physical parameters such as sea surface temperature or chlorophyll *a* (Gasol et al. 1997; Li 1998). If the first strategy assumes the existence of distinct boundaries by the division of oceans into different marine provinces (Longhurst 1995), in contrast, "the continuum hypothesis" assumes the study of microbial community structures over a large range of parameters, smoothing out most of the variability found at smaller scales. For example, heterotrophic bacteria are known to increase following chlorophyll *a* concentration and temperature at large scales (e.g. Li et al. 2004).

Scales of variability: coastal vs open-ocean ecosystems

The ecological function of the different picoplankton groups can be inferred from the study of their distributions at different spatial and temporal scales. Seasonality in picophytoplankton groups has been often observed (Campbell et al. 1997; Jacquet et al. 1998; Li 1998; Grégori et al. 2001; Li and Dickie 2001). Large spatial scale studies have shown that the relative contribution to picoplankton community structure varies not only with ecosystem trophic level (Zhang et al. 2008), but also with temperature and stratification of the water column (Bouman et al. 2011), suggesting that microbial community structure does not vary at random but might represent ecological indicators of water mass properties. Similarly, general distribution patterns of heterotrophic bacterial abundance and bacterial activity have been identified across a range of trophic levels (as estimated from chlorophyll *a* concentration) (Cole et al. 1988; Billen et al. 1990; Ducklow and Carlson 1992; Bird and Kalff 1984). Similarly, the biomass ratio of heterotrophic bacteria to autotrophic phytoplankton (which can be traced back to Odum 1971) was shown to decrease over a large continuum of chlorophyll *a* concentration, reaching or even exceeding unity in waters of low chlorophyll *a* levels (Fuhrman et al. 1989; Cho and Azam 1990; Li et al. 1993; Buck et al. 1996), indicating dominance of heterotrophy in the more oligotrophic environments. Different relationships were later described among different types of ecosystem, the slope varying from freshwaters, coastal to open-ocean waters (Simon et al. 1992; del Giorgio and Gasol 1995; Gasol et al. 1997) (Figure 6).

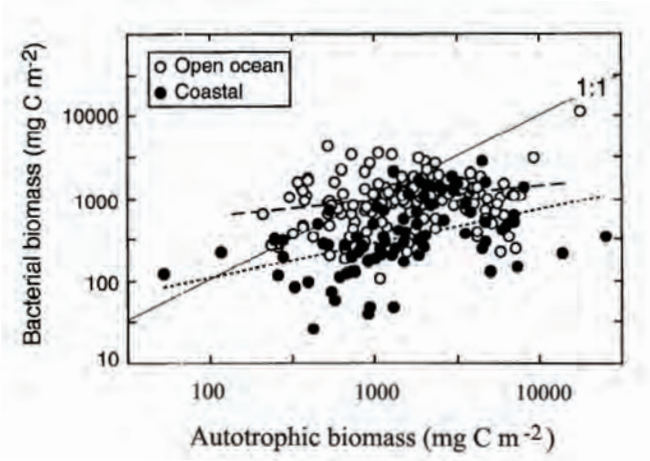


Figure 6. Relationship between bacterial biomass and autotrophic biomass for surface-integrated samples in open-ocean or coastal communities. The line of equal autotrophic and heterotrophic biomass is shown. From Gasol et al. 1997

The distinction between coastal and open-ocean ecosystems is not arbitrary but relies on pronounced physical differences, which act as source of variability in microbial distribution patterns. Among these differences, Cloern (1996) defined coastal areas as complex ecosystems corresponding to transition zones at the land-sea interface, whose shallowness allows for the rapid exchange between the sediment surface and the water column by sedimentation and resuspension. In such ecosystem type, salinity stratification due to riverine input of freshwater enhances the stability of the water column. The vertical mixing in the open-ocean is strongly influenced by seasonality: thermal stratification of the water column occurs in summer. Several studies have shown that shifts in the bacterial community succession in freshwater to saltwater transition zones were accompanied by metabolic shifts at both single-cell and community level in particular habitats (e.g. Bouvier and del Giorgio 2002). How different ecosystems exhibit predictable patterns in microbial community structures still remain to be ascertained.

LARGE VS SHORT SCALES OF VARIABILITY

The identification of widely observable patterns represents a central tenet of biogeography. Meta-analysis and secondary analysis summarize effects and relationships, obscure to other approaches, to produce effect estimates with much more statistical power than individual studies (Lipsey and Wilson 2001). However, picoplanktonic group abundance can fluctuate drastically over short time scales and large seasonal measurement of picoplankton abundance reduces variance and smoothes out most of the variability measured at shorter temporal scales (Li 2007) (Figure 7).

The relevance of diel variability has been commonly disregarded as compared to monthly or annual variability. Several studies have shown that *Synechococcus, Prochlorococcus* and Picoeukaryotes abundances follow diel variations, (e.g. Jacquet et al. 1998 and 2002; Vaulot et

Marie 1999; **Chapter II**). Similarly, bacterial abundance and activity can vary over short periods likely due, for example, to variations in phytoplankton extracellular release of DOC (Gasol et al. 1998; Ruiz-González et al. 2012; **Chapter II**). Similarly, picoplanktonic group abundance can fluctuate drastically over short distances as in the Celtic Sea (<1 km), indicating that the magnitude of variation in picoplankton community structure (PCS) patterns at the short spatial scale might be greatly underestimated in comparison to larger spatio-temporal patterns (Martin et al. 2005).

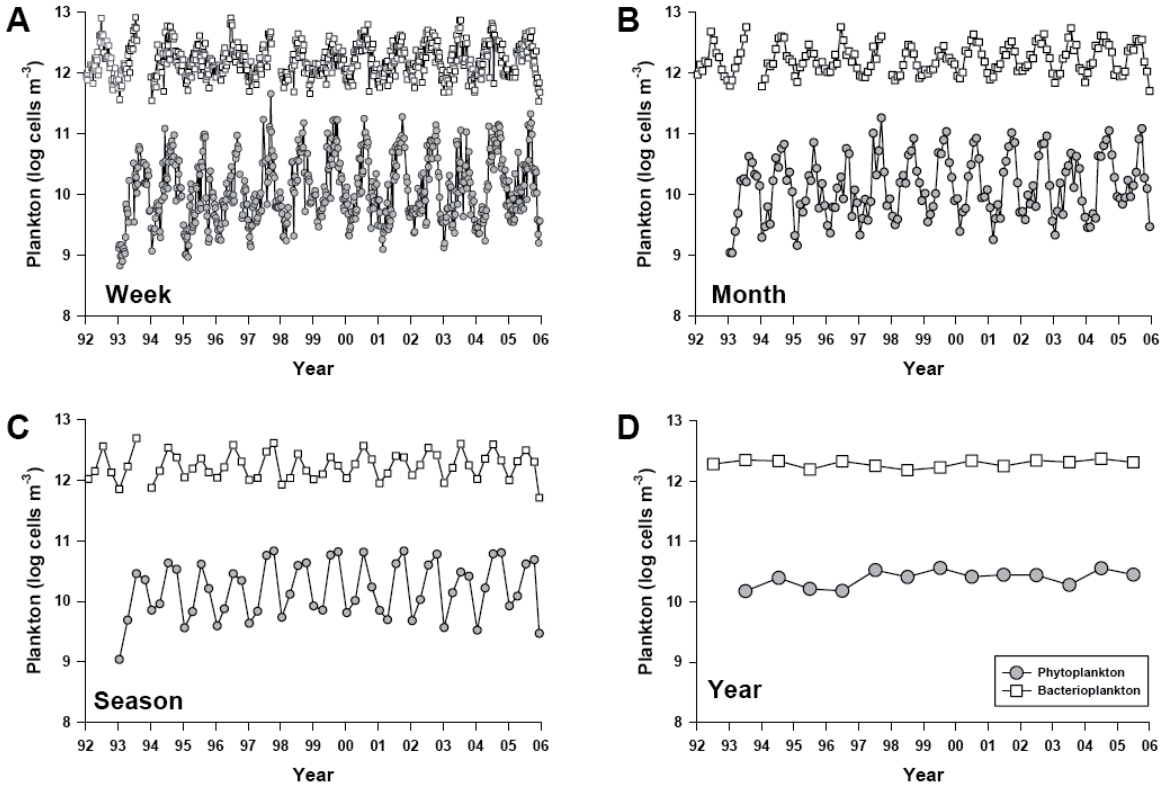


Figure 7. Multi-scale analysis by coarse graining. Time series of phytoplankton and heterotrophic bacterial abundance in Bedford Basin, Canada from 1992 to 2005. (A) Weekly measurements, (B) Monthly averages, (C) Seasonal averages, (D) Annual averages. (From Li 2007)

AIMS AND OUTLINE OF THIS THESIS

This thesis is centered in the identification of **ataxonomic** patterns in picoplankton distribution by means of **flow cytometry** and broad **phylogenetic** patterns in bacterial community structure using a **comparative analysis approach**. To study the patterns in picoplankton community structure, we first (re-) evaluated the carbon conversion factor for small phototrophic Picoeukaryotes in order to compare and estimate their relative contribution to picoplankton biomass in the different studies of this thesis organized into different chapters. We sought to identify patterns of variability in picoplankton group distribution at different spatio-temporal scales during winter and summer in a NW Mediterranean coastal station, focusing more particularly on the variability at the short time scale and the coupling between picoplankton groups and heterotrophic activities. Finally, we assessed the biogeography of the bacterial phylogenetic groups along a continuum of environmental parameters such as chlorophyll *a*, temperature and salinity, by using a secondary analysis approach, seeking to identify patterns in bacterial community structure among coastal and open-ocean ecosystems.

This thesis is organized in five main chapters; all contain new data and results, the last chapter is an overall discussion including analyses that have not made it into specific papers because of time constraints. Each chapter is based on several background hypotheses and aims to answer specific questions:

Chapter I. Direct determination of carbon conversion factors for ecologically relevant small photosynthetic eukaryotes

Theoretical Background: Conversion factors are necessary to translate picophytoplankton cell abundance into carbon biomass. While such factors are relatively well constrained for *Synechococcus* and *Prochlorococcus*, large discrepancies are still observed concerning the small photosynthetic picoeukaryotes (<3 μm). Three major reasons of such uncertainty can be cited: 1) Few cultures of ecologically relevant Picoeukaryotes are available, 2) pPeuk cultures are generally maintained in non-axenic conditions, causing a bias in cellular carbon estimation, 3) most relationships between cell size and cellular carbon content have been estimated/calculated using cultures of relatively large phytoplankton containing large vacuoles, not representative of the organization generally found in smaller cells.

The objectives of this first chapter are i) to compare the use of flow cell sorting with image analysis, two different methods for correcting the bias generated by the presence of bacteria in cultures maintained in non axenic conditions; ii) to establish a new relationship between cell size

and cellular carbon content for small photosynthetic eukaryotes using a set of 16 different cultures of ecologically relevant pPeuk; and iii) to see whether it is possible to distinguish conversion factors specific of coastal and of open-ocean ecosystems.

Chapter II. Short-time scale coupling of picoplankton community structure and heterotrophic activity in winter coastal NW Mediterranean Sea

The distribution of *Synechococcus, Prochlorococcus* and pPeuk has mostly been studied at relatively large time scales and only a few times at the diel scale. Given that events of major ecological relevance often result from transient environmental perturbation (e.g. wind stress, turbulence, high irradiance), and that the microbial life history most likely operates at short time frames, it is necessary to determine the significance of the short time scale as structuring factor of the large scale patterns in microbial communities. *Synechococcus, Prochlorococcus* and Picoeukaryotes have been shown to follow diel patterns with different growth synchronization and phasing. Tight coupling between phytoplankton and bacteria should result in bacteria also following circadian cycles. Evidences for diel patterns in bacterial abundance and activity have been reported from the coastal NW Mediterranean but how picophytoplankton variability is coupled with bacterial single cell activities has not yet been analyzed.

The objectives of this second chapter are i) to follow the diel variations of picoplankton abundance by flow cytometry with a high frequency sampling in a NW Mediterranean coastal station in winter 2007; ii) to determine to what extent picophytoplankton was coupled with heterotrophic bacteria, bacterial activity, and heterotrophic nanoflagellate abundance: and iii) to study how this coupling could be altered at the short time scale by physical forcing, such as that provoked by shifts in wind direction and strength.

Chapter III. Patterns in picoplankton community structure: Multi-scale spatial and temporal variability in the NW Mediterranean Sea during late summer

Several studies have shown that *Synechococcus, Prochlorococcus* and Picoeukaryotes are differentially distributed in space and time. The factors contributing to the variability in PCS and heterotrophic activity at short spatial scales have been less studied and the relevance of diel variability is commonly disregarded as compared to monthly or annual variability. Some ecological parameters, such as abundance or activity of one or all picoplanktonic groups, can be used to estimate the changes occurring in ecosystem conditions, and then microbial community structure or activity variables might capture the complexity of the ecosystem.

The objectives of this third chapter are i) to identify patterns in picoplankton group distribution and activity at different spatio-temporal scales; ii) to quantify and compare variability of each parameter at each spatial or temporal scale; and iii) to determine the links between the variability of the parameters with the different environmental and ecological factors, such as stratification and trophic level (as estimated from chlorophyll *a* concentration).

Chapter IV. Patterns in marine bacterial group distribution, as measured by FISH, in relation to chlorophyll *a*, temperature and salinity

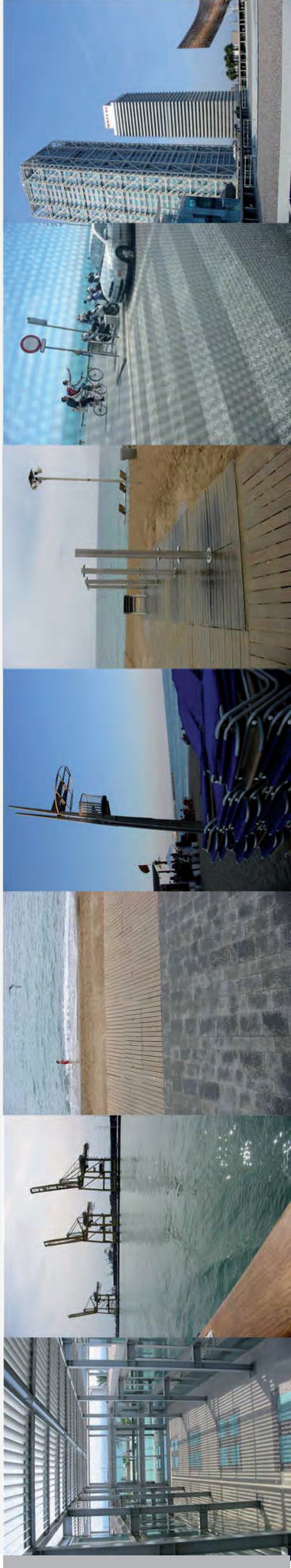
Evidences of a trophic coupling between phytoplankton and bacteria in most marine ecosystems have been found empirically in analyses of large data sets, by observing significant correlations and strong relationships between bacterial and phytoplankton biomass with log-log slopes <1, indicating that bacterial biomass vary proportionally less than chlorophyll *a*. This particular relationship was shown to differ among habitat type such as open-ocean, which had more heterotrophic biomass per unit autotrophic biomass than coastal ecosystems. However, the existence of similarly well-constrained relationships at the level of phylogenetic groups remains to be established.

How these relationships are similar, or different, from those existing at the bulk community level was studied in this chapter by collecting most published FISH data and combining them to environmental variables such as chlorophyll *a* concentration, temperature and salinity.

Chapter V. Synthesis of results and general discussion

The objective of the last chapter was to apply our conversion factor determined for Picoeukaryotes to a set of abundance data as obtained by flow cytometry in a wide range of different oceanic provinces in order to i) re-evaluate the relative contribution of this group to the picoplankton biomass ii) to identify macroecological patterns between the picoplankton community structure, the bacterial community structure and environmental parameters such as chlorophyll *a* concentration and temperature**.**

Direct determination of carbon conversion factors for ecologically-relevant photosynthetic picoeukaryotes



Thomas Lefort, Fabrice Not, Ian Probert,
Dominique Marie and Josep M. Gasol

2010

ABSTRACT

Discrepancies in conversion factor (CF) values used to translate abundance to biomass limit determination of the ecological importance of photosynthetic Picoeukaryotes (pPeuk, < 3 μm). In order to constrain these conversion factors, we determined the cell size and the C and N content of 16 different monospecific pPeuk cultures. Since the cultures were not axenic, two different protocols were used to correct for the presence of bacteria: 1) estimation of bacterial C and N content in each culture by flow cytometry, image analysis and standard bacterial conversion factors; 2) flow cytometric sorting of cells to remove bacteria prior to analysis. Cellular C and N contents varied from 230 fgC $cell^{-1}$ (±1.21%) and 38.8 fgN $cell^{-1}$ (±2.73%) for *Ostreococcus* to 21800 fgC $cell^{-1}$ (±23.61%) and 4920 fgN $cell^{-1}$ (±14.11%) for *Pycnococcus*. Correcting for bacterial carbon resulted in decreases of pPeuk cellular C content values by 7 to 33%. The efficiency of bacterial removal by cell sorting was always superior to 74%. We describe new relationships between cell volume and C and N content for the range of cell sizes considered (1.38-5.06 μm), and an average cellular carbon per unit volume (C/V) ratio for global unspecific pPeuk communities of 467 fgC μm^{-3} (±4%). An average CF of 1540 fgC $cell^{-1}$ (±12.01%) for a cell volume of 2.14 μm^3 was estimated from a mixture of pPeuk cultures. We also suggest that more specific CFs can be chosen for certain ecosystem types based on the known composition of the pPeuk communities.

INTRODUCTION

In many oceanic regions tiny unicellular photosynthetic organisms (i.e. picophytoplankton, cell diameter ≤ 2-3 μm) contribute significantly to carbon fluxes (Agawin et al. 2000; Bell and Kalff 2001). Despite relatively low abundance compared to marine cyanobacteria *(Synechococcus* and *Prochlorococcus),* photosynthetic Picoeukaryotes (pPeuks) have been shown to dominate in various marine settings in terms of contribution to biomass (Partensky et al. 1996; Blanchot et al. 2001; Worden et al. 2004) and bulk primary production (Li 1994). The biomass of a phytoplankton population can be estimated by converting cell abundance to overall quantity of carbon by means of a C-per-cell conversion factor (CF). In this context, different values have been used for the relationship between cell volume and cellular C content (C-per-unit-volume CF). Initially, diatoms were used to empirically determine such CFs (Mullin et al. 1966), but because of the presence of vacuoles, large phytoplankton contain less C and N per unit volume than smaller phytoplankton. More appropriate C-per-unit-volume CFs for smaller organisms have been obtained by applying non-linear regression models to data obtained from measurement of unialgal cultures, mostly from the nanoplankton size range (3-20 μm), by Verity et al. (1992) and others. Variations among phytoplankton taxa in chemical composition, and consequently in cellular C and N content, have long been highlighted (e.g. Strathmann 1967; Moal et al. 1987).

Patterns of pPeuk biomass have been described via large-scale surveys of pPeuk cell abundances from of a wide range of ecosystems (Li et al. 1992; 1995). However, discrepancies in the CF used limit inter-comparisons between such studies. Estimation of pPeuk biomass in samples from an Atlantic Meridional Transect cruise was performed using a CF of 1.5 pgC per algal cell (Zubkov et al. 1998, 2000) obtained from the C-to-volume ratio of 0.22 pgC μm^{-3} described for organisms <4 μm (Mullin et al, 1966; Strathmann 1967, Booth 1988) applied to an average cell volume estimated by microscopy and image analysis to be 6.8 ± 6.0 μm^3 (Zubkov et al. 1998). Analyses of Peuk community structure and biomass distribution in the central north Pacific Ocean (Campbell et al. 1994) and in subarctic to subtropical oceans (Zhang et al. 2008) used 2.1 pgC $cell^{-1}$ as the abundance-to-biomass CF for Peuks derived from the carbon-to-volume ratio of 0.36 pgC μm^{-3} from Verity et al. (1992) for an average cell volume of 6.22 μm^3 evaluated by microscope analysis. Other studies have used a carbon-to-volume value of 0.24 pgC μm^{-3} directly measured from Peuk culture isolates (Worden et al. 2004).

Peuk community composition has been shown to vary according to oceanic region and nutrient characteristics of the water masses (coastal or open-ocean, eutrophic or oligotrophic, see review by Worden and Not 2008). Prasinophyceae (Archaeplastida) typically dominate Peuk communities in coastal waters (Not et al. 2004), while Prymnesiophyceae (Haptophyta), and

to a lesser extent Chrysophyceae and Pelagophyceae (both Heterokontophyta), appear to make up a large fraction of pPeuk communities in more open ocean ecosystems (Mackey et al. 2002; Fuller et al. 2006; Liu et al. 2009), being present but less abundant in oligotrophic Pacific regions (Vaillancourt et al. 2003) and in the Sargasso Sea (Goericke 1998). PPeuk community composition should be taken into account when determining biomass.

Algal cultures can be used to determine CFs, one of the conditions being that the cultures should be taxonomically and physiologically representative of the species that dominate in the ocean. Cultures of the prasinophyte *Micromonas pusilla* have been used for CF calibration (Durand et al. 2002; Grob et al. 2007), this species being one of the most abundant and cosmopolitan of all pPeuks described to date (Thomsen and Buck 1998), dominating pPeuk communities all year long in coastal systems such as the English Channel (Not et al. 2004) as well as in the Norwegian and Barents seas and near the polar front (Not et al. 2005). *Ostreococcus,* another prasinophyte, has been shown to be abundant in coastal Pacific surface waters with a maximum at the DCM (Countway and Caron 2006) using Q-PCR. Considered as the smallest eukaryote described to date, *Ostreococcus* isolates have also been used to determine C-to-biovolume CFs with reported values ranging between 0.24 pgC μm^{-3} (Worden et al. 2004) and 0.422 pgC μm^{-3} (Grob et al. 2007). For other ecologically important pPeuk groups such as the Chrysophyceae and Prymnesiophyceae, cultures of the picoplanktonic size range have never been used to constrain CFs.

In this study, unialgal cultures were used to investigate whether a range of pPeuks exhibit C-to-biovolume relationships similar to those previously described for larger phytoplankton organisms. We used 16 pPeuk culture strains, as representative as possible of the three ecologically important divisions (Archaeplastida, Haptophyta, Heterokontophyta) that mainly compose natural pPeuk communities. Cultures were harvested in exponential growth to determine cell size and cellular C and N content. Since axenic cultures are difficult to obtain, we used a flow cytometric cell sorting methodology to minimize biases in the determination of pPeuk C content due to the presence of bacteria in the non-axenic cultures. We describe specific empirical relationships between cellular C content and cell biovolume for different members of the Picoeukaryote phytoplankton community.

MATERIALS AND METHODS

Selection of Picoeukaryotes and culture conditions- Sixteen unialgal, non-axenic pPeuk culture strains were selected from the Roscoff Culture Collection (www.sb-roscoff.fr/Phyto/RCC) based on their representativeness in terms of diversity and abundance in natural marine ecosystems (Table 1). Cells were grown under different light conditions (Table 1) in 75 cm^2 tissue culture flasks in either K medium (Keller and Guillard 1985; Keller et al. 1987) or f/2 medium (Guillard and Ryther 1962). Two successive growth cycles were monitored daily using a FACSCanto II flow cytometer (Becton Dickinson, San Jose, CA) to enumerate cell abundance, following the protocol described in Marie et al. (1999). The first cycle was used to determine the timing of the mid-exponential growth phase for each of the 16 cultures. The second growth cycle was performed in triplicate for each of the 16 cultures. Average growth rates for each culture strain are presented in Table 1.

Cell size and volume determination. The triplicate cultures of the 16 different strains were harvested midway through the exponential phase of growth. Mean cell diameter was determined with a Cell-Lab-Quanta SC Flow Cytometer (Beckman Coulter) calibrated before each size determination with 3 μm beads (Polysciences) diluted in MilliQ water. Cell counts and diameter analyses were performed after plotting FL3 (red fluorescence) against EV (electronic volume) parameters. Cell size was assumed to be normally distributed and the peak of distribution was taken as representative of the arithmetic mean cell diameter. Biovolumes were subsequently calculated assuming cells of all strains to be spherical.

C and N determination. Duplicate samples for C and N measurement from each of the triplicate cultures of the 16 strains were collected by gentle filtration onto 25 mm glass fiber filters (Whatman GFF, previously ashed for 4 hours at 450ºC and kept in the dark under axenic conditions). The volumes filtered ranged from 10 to 100 ml according to the cell density in each culture. The filters were placed into sterile cryovials and frozen at –20°C until analysis with an Elmer 2400 CHN analyzer.

Methods for correcting C estimations

(1) Flow cytometric cell sorting of Peuk cultures. Flow cytometric cell sorting was used to correct for errors in biomass estimation due to presence of bacteria in 11 of the cultures (RCC245, RCC287, RCC480, RCC239, RCC927, RCC 361, RCC 299, RCC 419, RCC 422, RCC 497, RCC 504). In the other 5 strains either cell density was not high enough or the bacteria/

STRAIN	DIVISION	CLASS	Genus species	Growth Medium	Temperature °C	Light regime µEm^{-2}.s^{-1}	Growth rate d^{-1}	Area of isolation	Ecosystem assignment
RCC 245	**Archeaplastida**	Prasinophyceae	*Pycnococcus sp.*	K	20 °C	4	0.89	Mediterranean Sea	C
RCC 287			Clade VIIA	K	20 °C	100	0.86	Pacific ocean	O-O
RCC 419			*Bathycoccus prasinos*	K	15 °C	150	0.93	English channel	C
RCC 422			*Ostreococcus sp.*	K	15 °C	150	0.83	English channel	C
RCC 299			*Micromonas pusilla* Clade A	K	20 °C	100	0.85	Pacific ocean	C
RCC 497			Micromonas *pusilla* Clade C	F/2	20 °C	100	0.94	Mediterranean Sea	C
RCC 927			*Prasinoderma singularis*	K	20 °C	N.A	1.00	Pacific ocean	C
RCC 656	**Haptophyta**	Prymnesiophyceae	*Chrysochromulina sp.*	K	20 °C	100	0.95	Atlantic ocean	O-O
RCC 361			*Imantonia rotunda*	K	15 °C	150	1.00	English channel	C
RCC 703	**Heterokontophyta**	Bacillariophyceae	*Minutocellus sp.*	K	20 °C	4	0.93	Indian Ocean	C
RCC 101		Pelagophyceae	*Pelagomonas calceolata*	K	20 °C	4	0.86	Atlantic ocean	O-O
RCC 480		Chrysophyseae	*Ochromonas sp.*	K	25 °C	100	0.83	Indian Ocean	O-O
RCC 446		Dictyophyceae	*Florenciella parvula*	K	15 °C	150	0.85	English channel	C
RCC 239		Bolidophyceae	*Bolidomonas mediterranea*	K	20 °C	100	0.88	Mediterranean Sea	O-O
RCC 503		Pinguiophyceae	*Phaeomonas* sp.	F/2	20 °C	100	0.90	Mediterranean Sea	C
RCC 504		Eustigmatophyceae	*Nannochloropsis gatidana*	F/2	20 °C	100	0.94	Mediterranean Sea	C

Table 1. Phototrophic picoeukaryote cultures used in this study and geographical origins of each isolate. All are from the Roscoff Culture Collection. Also shown are the parameters of culture media, temperature, and light conditions used to obtain the exponential growth.
N.A: information not available
Ecosystem assignment: C for Coastal and OO for Open-Ocean were chosen based on the current knowledge (see text).
More detailed information about the Peuk cultures on www.sb-**roscoff**.fr/Phyto/RCC

pPeuk ratio was very high, hence a mathematical correction for the contaminating bacteria was applied (see below). Using a FACSAria flow cytometer (Becton Dickinson) with freshly prepared 0.2 μm filter-sterilized seawater as sheath fluid, algal cells were discriminated from bacterial cells in the SSC (side scatter) versus FL1 (green fluorescence) plot after DNA staining with SYTO13 (Molecular Probes, Eugene, USA; 5 μM) and then sorted in purity mode. Analysis and cell sorting were made using a 70 μm nozzle, with a sheath pressure of 70 psi and the sample flow was adapted to maintain the particle rate below 1,000. The 11 sorted strains were subsequently filtered for CHN analysis as described above. A 1 ml aliquot from each of the sorted samples was preserved with glutaraldehyde (0.25% final concentration) and stored in liquid nitrogen for subsequent determination of algal and bacterial abundance by flow cytometry.

(2) Estimation of bacterial C and N by image analysis. 1 ml samples from culture triplicates were preserved with glutaraldehyde (0.25% final concentration), flash frozen in liquid nitrogen, and then stored at -80ºC until analysis. In order to enumerate bacteria, 0.2 ml of the preserved samples was stained for 10 min in the dark with SYBRGreen I (Molecular Probes, Eugene, USA) (dilution x10,000). Bacterial abundance was then measured with a FACSCalibur flow cytometer (Becton Dickinson) equipped with a laser emitting at 488 nm and the standard filters setup. Data were acquired in log mode and analysis was performed using the Cell-Quest software (Becton Dickinson) using the SSC versus FL1 plots (Marie et al. 1997; Gasol and del Giorgio 2000). 0.5 ml of the samples stored for bacterial abundance were stained with DAPI (final concentration 5 μg ml^{-1}) for 5 min and filtered through 0.2 μm pore-size black polycarbonate filters (Porter and Feig 1980). Filters were mounted on microscope slides with non-fluorescent oil (R. P. Cargille Lab., Inc.) and stored frozen. Bacteria were counted by epifluorescence microscopy with a Nikon Labophot microscope. About 200 to 400 bacteria per sample were counted and bacterial cell size was determined by image analysis following Massana et al. (1997). To convert bacterial biovolumes to cellular C content, the allometric relationship CCC (pg $cell^{-1}$) = 0.12 x $BB^{0.72}$ proposed by Norland (1993) was used. The calculated bacterial volumes ranged from 0.11 μm^3 to 0.31 μm^3.

Blank corrections. Since only a small volume was filtered for the 11 sorted cultures and the values of C and N content per filter were very low, a blank correction was applied to account for C and N in filters. C and N were measured for triplicate pre-combusted dry GFF filters. To correct for the filter effect, 0.019 μmol N and 0.517 μmol C were respectively subtracted from all PON and POC results. In order to account for contributions of C and N from the growth media, C and N were measured for triplicate pre-combusted GFF filters through which 30 ml of sterile K/2 medium had been filtered. An average value of 1.55 μmol C (± 0.12 SD) per "wet" GFF filter was

determined.

Measurement of cellular C and N content in a mixture of pPeuk cultures. In order to test whether the average cellular C and N value of a mixture of pPeuk cultures corresponded with the average cellular C and N values determined by the different estimation methods, 5 ml samples from 15 different cultures in exponential growth were mixed in 75 cm^2 tissue culture flasks in triplicate. Samples were then taken for mean size determination and PON and POC measurements as described above.

Statistical analyses. Least squares regression analysis was used to determine the relationship between cellular C content and biovolume. The relationships were fitted using natural log transformations. Equations of the regressions are presented as log (Y) = a + b log (X) with Y= fgC per cell; a=intercept; b=slope; X =biovolume (μm^3). In order to test whether the slopes and intercepts of the relationships were significantly different, Student's t-tests were conducted after applying model I regression analyses. All conducted in JMP 7 software (SAS institute Inc).

RESULTS

Culture growth- The algal cell concentration at the time of sampling varied from 6.1 10^4 cells ml^{-1} for *Pelagomonas calceolata* RCC101 to 3.4 10^7 cells ml^{-1} for *Ostreococcus* RCC422 (Table 2). Growth rates varied from 0.83 divisions per day for *Ochromonas* and *Ostreococcus* to 1 division per day for *Imantonia rotunda* and *Prasinoderma singularis* (Table 1). All cultures were grown in non-axenic conditions and bacteria were detected and enumerated by flow cytometry (Table 3). Bacterial cell abundances varied from 1.0 10^6 cells ml^{-1} in the *Ochromonas* culture to 8.2 10^6 cells ml^{-1} for *Minutocellus*. The highest bacterial/pPeuk concentration ratio was observed in the *Pelagophyceae* culture with a value of 108.7 (±13.6) bacteria per algal cell. High values were also found in the *Pycnococcus* strain and *Prasinoderma singularis*, with 71.5 (±15.5) and 45.5 (±10.1) bacteria per algal cell respectively. The lowest ratio was observed in *Ostreococcus* at 0.1 bacteria per algal cell, followed by *Bathycoccus prasinos*, *Micromonas* Clade A, and *Micromonas* Clade C, with 0.2, 0.4, and 0.4 bacteria per algal cell, respectively (Table 3).

Cell sizes and cell diameters- Average cell diameters varied from 1.38 μm for *Bathycoccus prasinos* to 5.06 μm for *Chrysochromulina* sp. (Table 2). The strains with the lowest cell diameters attained the highest cell concentrations (Table 2). Of the 3 sets of cultures representing different taxonomic classes, the Prymnesiophyceae exhibited the highest average diameter (3.78 μm) due mainly to the inclusion of *Chrysochromulina* with a diameter of 5.06 μm. The average diameter for the heterokont cultures was 3.16 μm and for the Prasinophyceae 2.01 μm (Table 2). The arithmetical average diameter of cells of all strains used in this study was 2.73 (±1.13) μm, with a median value of 2.61 μm. The coefficients of variation (CV) for cell diameters measured with the Cell Lab Quanta were always below 4%.

The weighted average cell diameter of the mix of 15 different species, taking into account the respective cell concentrations, was calculated at 1.60 μm (±1.06).

Cellular C and N content in non-axenic conditions (without correction)- The measured pPeuk C content ranged from 230 fgC per cell for *Ostreococcus* to 21800 fgC per cell for *Pycnococcus sp*.. The coefficients of variation (CV) of average values of C content per cell varied from 1.2% for *Ostreococcus* to 32.6% for *Phaeomonas* sp.. The highest CV values (between 15% and 30%) were recorded for *Ochromonas sp.* (28.6%), *Pycnococcus sp.* (23.6%), *Prasinoderma singularis* (22.6%), *Bathycoccus prasinos* (21.4%) and *Minutocellus sp*. (18.3%) (Table 2). The N content per pPeuk cell varied from 38.8 fgN $cell^{-1}$ (±2.7%) for *Ostreococcus sp.* to 4920 fgN $cell^{-1}$ (±14.1%) for *Pelagomonas calceolata,* followed closely by *Pycnococcus sp.* with a N content of 4450 fgN $cell^{-1}$ (±4.6%, Table 2).

Genus species (Strain)	Algal concentrations (cells ml^{-1}) (±SD)	Cell diameter (µm)(±CV%)	Per group cell diameter average (µm) (±CV%)	Per cell Carbon (not corrected) fgC $cell^{-1}$ (±CV%)	Per cell Nitrogen (not corrected) fgN $cell^{-1}$ (±CV%)	Corrected per cell Carbon (2^{nd} method) fgC $cell^{-1}$ (±CV%)	Corrected per cell Nitrogen (2^{nd} method) fgN $cell^{-1}$ (±CV%)	C/N ratio (±CV%)	Carbon per cell after sorting fgC $cell^{-1}$ (±CV%)
Pycnococcus sp. (RCC245)	$9.48 \pm 2.08\ 10^4$	2.61 (±3.31%)		21,800 (±23.61%)	4,450 (±4.65%)	19,500 (±24.66%)	4,170 (±4.85%)	4.67 (±24%)	7,420 (±)
Clade VIIA (RCC287)	$1.31 \pm 0.05\ 10^6$	2.28 (±0.48%)		2,990 (±8.96%)	520 (±16.50%)	2,710 (±9.79%)	477 (±18.00%)	5.78 (±13%)	3,090 (±)
Bathycoccus prasinos (RCC419)	$1.75 \pm 0.09\ 10^7$	1.38 (±0.71%)		349 (±21.43%)	77.2 (±40.06%)	344 (±21.63%)	76.7 (±40.37%)	5.30 (±52%)	999 (±21.28%)
Ostreococcus sp. (RCC422)	$3.39 \pm 0.11\ 10^7$	1.44 (±1.24%)	2.01 (±34.47%)	230 (±1.21%)	38.8 (±2.73%)	226 (±1.20%)	38.2 (±2.75%)	5.91 (±3%)	503 (±)
Micromonas pusilla Clade A (RCC299)	$1.68 \pm 0.08\ 10^7$	1.66 (±16.87%)		811 (±5.27%)	128 (±3.43%)	793 (±5.26%)	125 (±3.41%)	6.33 (±3%)	661 (±54.26%)
Micromonas pusilla Clade C (RCC497)	$1.25 \pm 0.06\ 10^7$	1.51 (±0.66%)		857 (±9.07%)	105 (±9.12%)	843 (±9.27%)	103 (±9.34%)	8.18 (±4%)	2,120 (±38.56%)
Prasinoderma singularis (RCC927)	$2.08 \pm 0.74\ 10^5$	3.17 (±1.69%)		15,300 (±22.56%)	2,670 (±25.28%)	14,100 (±23.55%)	2,530 (±25.57%)	5.64 (±14%)	4,840 (±)
Chrysochromulina sp. (RCC656)	$8.42 \pm 0.09\ 10^5$	5.06 (±2.53%)	3.78 (±47.89%)	14,700 (±1.95%)	1,830 (±1.94%)	14,500 (±1.98%)	1,790 (±1.96%)	8.06 (±3%)	----------
Imantonia rotunda (RCC361)	$1.76 \pm 0.07\ 10^6$	2.5 *		5,180 (±4.32%)	819 (±7.42%)	5,030 (±4.43%)	800 (±7.47%)	6.32 (±7%)	3,310 (±)
Minutocellus sp. (RCC703)	$4.86 \pm 0.57\ 10^5$	3.26 (±0.33%)		8,810 (±18.25%)	1,420 (±13.19%)	8,210 (±18.90%)	1,350 (±14.35%)	6.27 (±28%)	----------
Pelagomonas calceolata (RCC101)	$6.13 \pm 2.40\ 10^4$	2.31 (±3.55%)		20,500 (±14.04%)	4,920 (±14.11%)	16,100 (±7.04%)	4,370 (±9.95%)	3.06 (±1%)	----------
Ochromonas sp. (RCC480)	$1.22 \pm 0.14\ 10^5$	4.38 (±0.53%)		18,200 (±28.64%)	1,050 (±64.78%)	17,900 (±28.99%)	1,010 (±66.92%)	18 (±123%)	20,800 (±)
Florenciella parvula (RCC446)	$4.54 \pm 0.06\ 10^5$	3.56 (±30.14%)*	3.16 (±23.81%)	19,000 (±8.98%)	2,480 (±8.19%)	18,700 (±9.07%)	2,440 (±8.26%)	7.65 (±3%)	----------
Bolidomonas mediterranea (RCC239)	$2.09 \pm 0.06\ 10^5$	2.18 (±0.59%)		8,800 (±5.17%)	1,530 (±4.35%)	7,130 (±6.07%)	1,290 (±5.34%)	5.52 (±4%)	4,390 (±)
Phaeomonas sp. (RCC503)	$7.33 \pm 3.19\ 10^5$	3.38 (±1.61%)		11,000 (±32.59%)	1,660 (±29.94%)	10,700 (±32.54%)	1,630 (±29.86%)	6.52 (±5%)	----------
Nannochloropsis gatidana (RCC504)	$1.94 \pm 0.14\ 10^6$	3.06 (±1.34%)		5,090 (±5.38%)	633 (±8.74%)	5,000 (±5.40%)	622 (±8.83%)	8.07(±4%)	4,810 (±)

Table 2. Carbon and Nitrogen per cell content and average cellular sizes at exponential growth. Algal concentrations and bacterial concentrations at the time of filtration and cell size determination.

*: Coulter estimation missing for RCC 361, diameter estimated by microscopy.

**: Theoretical cell diameter, calculated as the weighted average of the different strain diameter, Mix contains 5 ml of all strains except *Florenciella parvula* RCC446

STRAIN	Genus species	Bacteria ml^{-1} ($x10^6$) ±SD	fgC per bacteria ±SD	Bacteria/Peuk ratio	Bacteria/Peuk ratio after sorting
RCC 245*	*Pycnococcus sp.*	7.11 ± 0.11	28.78 ± 13.09	71.48 ± 15.46	0.34
RCC 287*	clade VIIA	7.26 ± 0.06	47.51 ± 32.46	5.54 ± 0.26	0.01
RCC 419*	*Bathycoccus prasinos*	3.41 ± 0.11	23.62 ± 10.56	0.20 ± 0.02	0.00
RCC 422*	*Ostreococcus sp.*	3.22 ± 0.12	39.40 ± 23.72	0.09 ± 0.00	0.09
RCC 299*	*Micromonas pusilla* Clade A	5.87 ± 0.10	47.85 ± 28.25	0.35 ± 0.02	0.06
RCC 497*	*Micromonas pusilla* Clade C	5.34 ± 0.41	33.92 ± 8.57	0.43 ± 0.02	0.11
RCC 927	*Prasinoderma singularis*	7.93 ± 0.11	25.38 ± 11.79	45.54 ± 10.08	n.d
RCC 656	*Chrysochromulina sp.*	6.85 ± 0.05	32.21 ± 17.07	8.14 ± 0.11	n.d
RCC 361*	*Imantonia rotunda*	6.92 ± 0.16	35.53 ± 15.00	3.93 ± 0.25	0.15
RCC 703	*Minutocellus sp.*	8.22 ± 0.03	33.41 ± 17.84	17.09 ± 1.85	n.d
RCC 101	*Pelagomonas calceolata*	7.57 ± 0.02	30.43 ± 10.59	108.71 ± 13.62	n.d
RCC 480*	*Ochromonas sp.*	1.01 ± 0.03	33.92 ± 8.57	8.37 ± 0.81	0.05
RCC 446	*Florenciella parvula*	6.62 ± 0.28	24.05 ± 14.70	14.58 ± 0.77	n.d
RCC 239*	*Bolidomonas mediterranea*	7.41 ± 0.06	45.28 ± 20.63	35.55 ± 1.24	0.57
RCC 503	*Phaeomonas* sp.	6.39 ± 0.36	24.58 ± 15.15	9.92 ± 3.43	n.d
RCC 504*	*Nannochloropsis gatidana*	4.14 ± 0.12	36.84 ± 21.67	2.14 ± 0.11	0.02

Table 3. Carbon content per bacteria in each culture as determined by image analysis, and the bacteria-to-Picoeukaryotes concentration ratio before and after flow cytometric cell sorting. n.d : not done

First correction method: pPeuk C content after cell sorting- For the 11 pPeuk cultures sorted by flow cytometry (Tables 2 and 3), bacterial abundance was measured before and after cell sorting to quantify the efficiency of the procedure. The efficiency of bacterial removal estimated as:

Efficiency = (1- (ratio Bac:pPeuk before sorting)/(ratio Bac:pPeuk after sorting)) x 100

was always greater than 95%, except for *Micromonas pusilla* Clade A and Clade C, with 83% and 74% efficiency, respectively (Table 3).

After pPeuk cell sorting, the highest C content was recorded for *Ochromonas sp.* with 20800 fgC $cell^{-1}$ followed by *Pycnococcus sp.* with 7420 fgC $cell^{-1}$ (Table 2). The lowest C content after cell sorting was measured for *Ostreococcus sp.*, as with the other approaches (with and without bacterial correction) (Table 2).

After flow cytometric cell sorting, strong increases in the estimation of C content per pPeuk cell, ranging from 150% to 190%, were observed for the smallest species such as RCC419, RCC422, and RCC497 (Table 2). By contrast, important decreases in the estimation of C content per pPeuk cell were obtained for most other organisms, with a maximum decrease of 65.67% for *Prasinoderma singularis* followed by a 62% for *Pycnococcus sp.*. Remarkable decreases (38.5% and 34%) were also recorded for RCC239 and RCC361, respectively, and to a lesser extent (16.5%) for RCC299. No significant differences were observed for *Nannochloropsis gaditana* and RCC287.

Second correction method: Estimation of culture-associated bacterial C and N by image analysis- To correct the C and N values of each pPeuk strain, the C and N content of bacterial cells were estimated in each pPeuk culture using image analysis and standard conversion factors. Image analysis of bacteria stained with DAPI revealed that the bacterial biovolumes varied from 0.11 μm^3 to 0.31 μm^3, with an average value of 0.19 μm^3 (± 0.07μm^3) (details not shown). Applying a relationship that relates bacterial biovolume to carbon biomass (Norland 1993) and an assumed value for bacterial N content of 25 fgN μm^{-3} (Nagata et al. 1986), the resulting C and N contents per bacteria were different in each pPeuk culture. Bacterial C content varied from 23.62 fgC $cell^{-1}$ to 47.85 fgC $cell^{-1}$ and N content from 2.74 fgN $cell^{-1}$ to 7.56 fgN $cell^{-1}$ in *Bathycoccus prasinos* and *Micromonas pusilla* Clade A cultures, respectively (Table 3), with an average value of 33.92 fgC per bacterial $cell^{-1}$ (±8.57 fgC $cell^{-1}$). The bacterial cells in the algal cultures were large, much larger than commonly in the ocean.

Multiplying the calculated cellular C and N contents by the total number of bacteria in each culture, the bacterial C and N contents per filter were subtracted from the total C and N contents per filter (corrected for medium and filter effects). The cultures exhibiting highest bacteria/pPeuk ratio and highest C and N content per bacterial cell were the most impacted by this correction. A decrease of 21.5% in C content per cell resulted for *Pelagomonas calceolata,* followed by *Bolidomonas mediterranea* and *Pycnococcus sp*. with decreases of 19% and 10.5%, respectively (Table 2). Between 5 and 10% decrease was observed for Clade VIIa, *Prasinoderma singularis* and *Minutocellus sp.*. Less than 3% decrease was observed for the other strains presenting lower bacteria/algae ratios. The correction for N content followed the same pattern, but also appeared influenced by the C/N ratio differences observed among the pPeuk cultures. For instance, a decrease of 15.5% of N per cell was observed for *Bolidomonas* after bacterial N subtraction, 11% decrease for *Pelagomonas calceolata* and only a 6.5% decrease for *Pycnococcus sp*. (compared with a 10.5% decrease of C content per cell after bacterial C correction). Less than 10% decrease of N per cell after bacterial N content correction was observed in the other cultures (Tables 2 and 3).

Relationships between cellular C and N content and biovolume- The cellular C and N contents estimated with the different methodologies were scaled with the biovolumes measured with the coulter method (Figure 1, Table 4). Carbon contents and biovolumes were log transformed and fitted using the linear model:

Non-axenic relationship:

$$\text{LOG (fgC cell}^{-1}) = 2.68 \pm 0.20 + 1.08 \pm 0.19 \text{ LOG Size } (\mu m^3) \quad \text{(eq. 1)}$$

Corrected by calculation:

$$\text{LOG (fgC cell}^{-1}) = 2.65 \pm 0.19 + 1.08 \pm 0.18 \text{ LOG Size } (\mu m^3) \quad \text{(eq. 2)}$$

After flow cytometric sorting:

$$\text{LOG (fgC cell}^{-1}) = 2.78 \pm 0.13 + 0.88 \pm 0.15 \text{ LOG Size } (\mu m^3) \quad \text{(eq. 3)}$$

All regressions were significant ($P<0.0001$, N=16, and N=11 for eq. 3). A larger R squared value ($R^2=0.8$) and smaller root mean squared error (0.22) for relationship 3 (Table 4) indicate that the fit was improved when C per cell was determined after flow cytometric sorting. No significant differences were observed (Student's t-test) between intercepts and slopes of the different relationships. The Student t-test showed identical slopes and no significant differences between intercepts for relationships 1 and 2, indicating that bacteria in the cultures do not seem to affect the relationships between pPeuk C content and biovolume. However, as a consequence of correction for bacterial C content, the regression line of relationship 2 was below that for relationship 1,

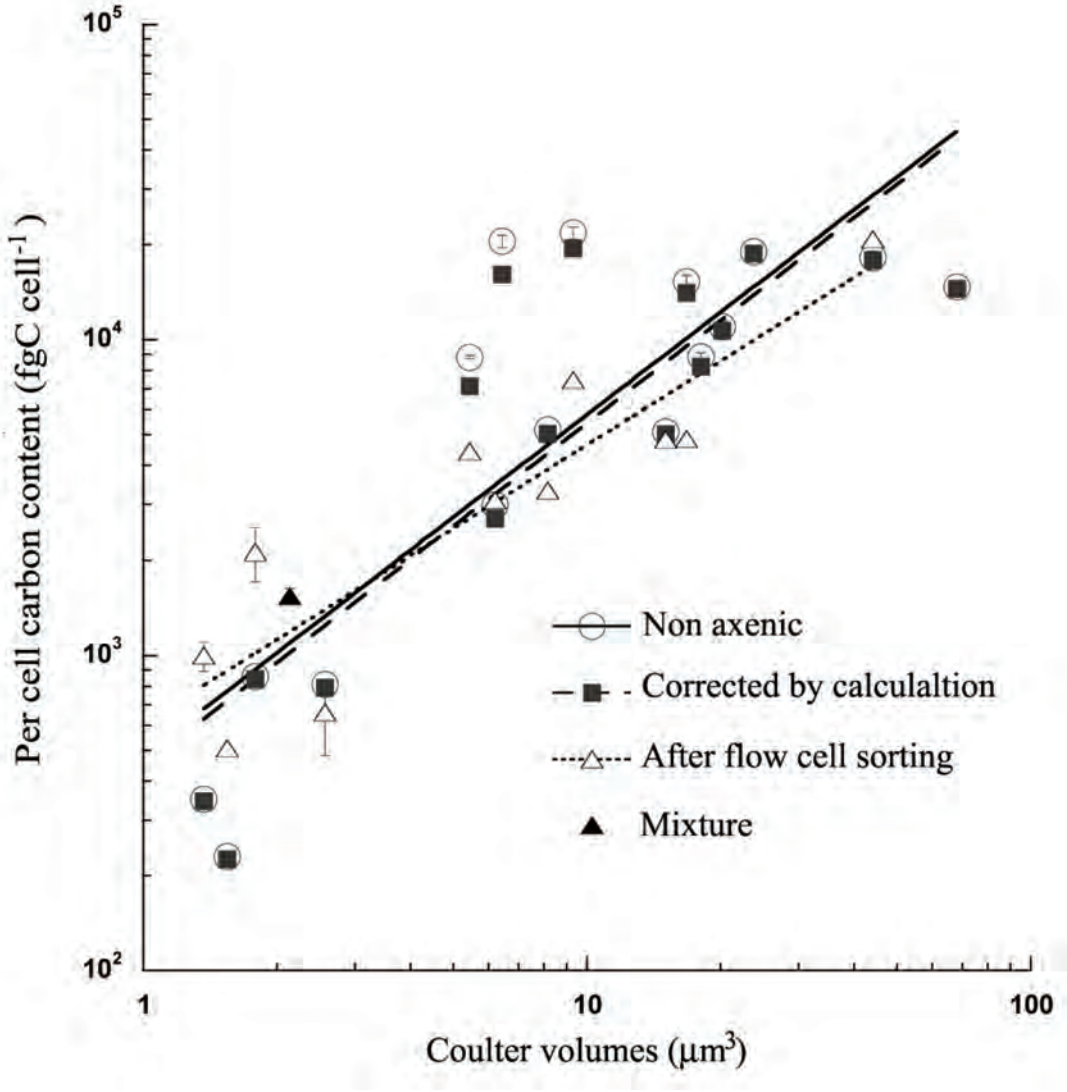


Figure 1. Relationship between Picoeukaryotes cellular carbon content (fgC $cell^{-1}$) and coulter volume (μm^3). X and Y-axes are Log-transformed. The relationship obtained in non-axenic conditions is represented by white circles and a black regression line, the relationship corrected-by-calculation is represented by black squares and a black dashed regression line, and the relationship obtained after Picoeukaryotes flow sorting is represented by white triangles and a black dashed regression line. The black triangle corresponds to the mixture of the 15 cultures used in this study. Error bars correspond with standard errors of the data.

because of a slightly lower intercept (2.68 compared with 2.65) (Table 4). In spite of a notably lower slope of 0.88 in relationship 3, the Student's-t test showed no significant differences between intercepts and slopes of relationships 2 and 3, indicating that flow cell sorting did not significantly

Equations	N observations	R square	Root Mean square error	Intercept (±SE)	Lower 95%	Upper 95%	Slope (±SE)	Lower 95%	Upper 95%
(1) Cellular C non-axenic	16	0.69	0.38	2.68 (±0.2)**	2.25	3.11	1.08 (±0.19)**	0.67	1.49
(2) Cellular C corrected by bacteria	16	0.72	0.35	2.65 (±0.19)**	2.25	3.06	1.08 (±0.18)**	0.7	1.47
(3) Cellular C after bacterial sorting	11	0.8	0.22	2.78 (±0.13)**	2.49	3.08	0.88 (±0.15)**	0.56	1.21
(4) Cellular N non-axenic relationship	16	0.54	0.45	2 (±0.24)**	1.48	2.52	0.94 (±0.23)**	0.44	1.43
(5) Cellular N corrected by bacteria	16	0.56	0.44	1.97 (±0.24)**	1.47	2.48	0.94 (±0.22)**	0.46	1.42
(6) Cellular N after bacterial sorting	10	0.43	0.50	2.03 (±0.31)*	1.31	2.76	0.83 (±0.34)*	0.05	1.61
(7) Cellular C per volume unit (bacterial calculation)	16	0.005	0.35	2.66 (±0.19)	2.26	3.06	0.05 (±0.18)	-0.33	0.42
(8) Cellular C per volume unit (after flow cell sorting)	11	0.007	0.22	2.79 (±0.13)**	2.50	3.07	-0.12 (±0.14)	-0.44	0.20

Table 4. Parameter estimates of log-log bivariate fits of relationships between mean cellular carbon and cellular nitrogen content as related to cell biovolume.
Relationships 1-6 follow the equation: Log C (fgC per cell)= Intercept (±SE) + slope (±SE) x Log (Biovolume μm^3)
Equation 7 and 8 follow the equation: Log C/V (fgC per μm^3)= Intercept (±SE) + slope (±SE) x Log (Biovolume μm^3)
**p<0.0001 *p<0.0008

alter the relationship between pPeuk C content and biovolume.

The relationships between N content per cell (measured in fgN per cell) and biovolume (measured in μm^3) are represented also by linear fits as:

Non-axenic relationship:

$$LOG\ (fgN\ cell^{-1}) = 2.00 \pm 0.24 + 0.94 \pm 0.23\ LOG\ Size\ (\mu m^3) \qquad (eq.\ 4)$$

Corrected by calculation:

$$LOG\ (fgN\ cell^{-1}) = 1.97 \pm 0.24 + 0.94 \pm 0.22\ LOG\ Size\ (\mu m^3) \qquad (eq.\ 5)$$

After flow cytometric sorting:

$$LOG\ (fgN\ cell^{-1}) = 2.03 \pm 0.31 + 0.83 \pm 0.34\ LOG\ Size\ (\mu m^3) \qquad (eq.\ 6)$$

As for cellular C content, bacterial N in the cultures apparently does not affect the relationship between cellular N content and biovolume (eq. 4 and 5). No significant differences were observed (Student's t-test) between intercepts and slopes of the different relationships 4, 5 and 6. The intercept of relationship 5 appeared below that of eq. 6 as a consequence of subtraction of bacterial N. The slope of relationship 6 was also lower than slopes 4 and 5. However, smaller R^2 values and high root mean square errors indicated a worst fit of this relationship (Table 4).

Determination of average cellular C content and C/V in a mixture of pPeuk cultures- The direct determination of cellular C content of a mixture of 15 different cultures and calculation of the average cell diameter was compared to the application of the general relationships previously described. The average C content per cell of the mixture was 1540 fgC $cell^{-1}$ (±12.01%) and fell inside the confidence limits of the different relationships 1, 2 and 3. This C content per cell corresponded to a weighted average diameter of 1.60 μm, equivalent to a biovolume of 2.14 μm^3 (Table 2).

The ratio between cellular C content and cell biovolume (C/V) represents an additional factor needed to convert pPeuk cell abundance (of known size) into biomass. We tested the significance of the relationships between this ratio (C/V) and volume and found slopes not significantly different from 0 (Table 4), indicating that the cellular C density (expressed as carbon per unit volume) did not decrease with cell volume for the tested cultures, i.e. that small cells did not contain significantly more C per unit volume than larger cells (within the range assayed). Therefore, for each estimation method, we calculated the average and median C/V (considered as constant within the range of biovolume in this study) and compared this with the empirically determined C/V ratio of the mixture (Figure 2A). Dividing the empirically determined average

cellular C content of 1540 fgC cell^{-1} (obtained after correction for bacterial C by calculation) by the weighted average biovolume of 2.14 μm^3 (Table 2, Figure 2A), the average C/V ratio of the mixture was 717.5 fgC μm^{-3} (±12%). The average C/V ratio values determined in non-axenic conditions, after correction for bacterial C by calculation, and after flow cytometric cell sorting were 822.0 fgC μm^{-3} (±103%), 715.6 fgC μm^{-3} (±99%), and 555.0 fgC μm^{-3} (±52%) respectively. No significant differences (Student's t-test) were found between the 3 different average C/V ratio values and the average C/V ratio value measured for the mixture. However, since the data were not normally distributed and the extreme C/V ratio values greatly distorted the averages, we also determined the median C/V ratio values in non-axenic conditions, after correction for bacterial C by calculation, and after flow cytometric cell sorting of 484.0 fgC μm^{-3}, 444.5 fgC μm^{-3}, and 472.9 fgC μm^{-3}, respectively. The overall median C/V ratio was thus determined as 467 fgC μm^{-3} (±4%) (Figure 2A).

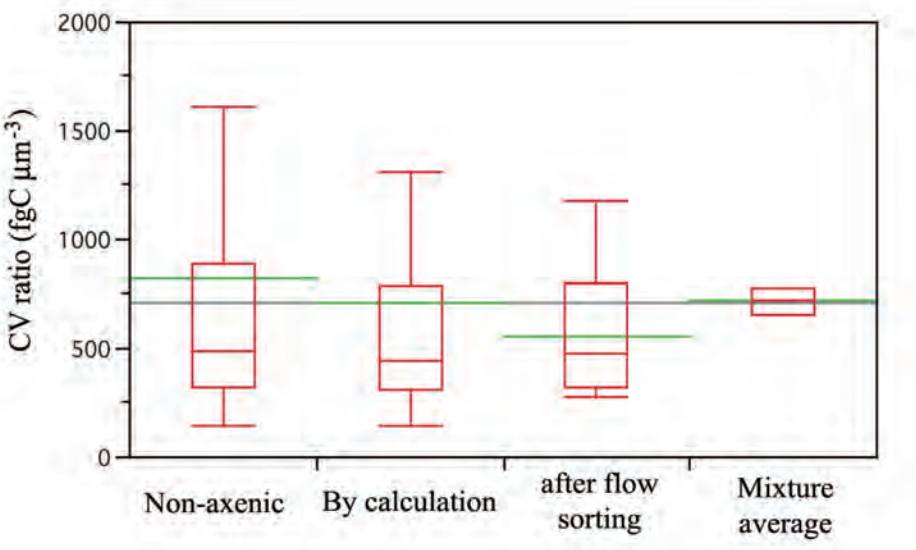


Figure 2A

Figure 2. Comparison of Picoeukaryote carbon content per unit of volume (fgC μm^{-3}). **(A)** Depending on the estimation method used in this study. The green horizontal lines correspond to the average value per method. The grey horizontal line corresponds to the overall average. **(B)** Depending on the ecosystem origin of the Picoeukaryotes isolates: coastal or open-ocean. The green horizontal lines correspond with the average value per method.

The average C/V ratios for groups of species designated as representative of coastal and open-ocean ecosystems (Table 1, Figure 2B) were 584 fgC μm^{-3} (±36.50%) and 1005 fgC μm^{-3} (±31.5%), respectively, but did not appear to be statistically different (Student's t-test). The 25^{th} and 75^{th} quartiles for the coastal C/V ratio were measured at 308 fgC μm^{-3} and 615 fgC μm^{-3}, respectively. For open-ocean species, the 25^{th} and 75^{th} quartiles were more distant, 309 fgC μm^{-3} and 1985 fgC μm^{-3}, respectively. The median values for groups of species from each ecosystem were 454 fgC μm^{-3} for coastal and 435 fgC μm^{-3} for open-ocean conditions.

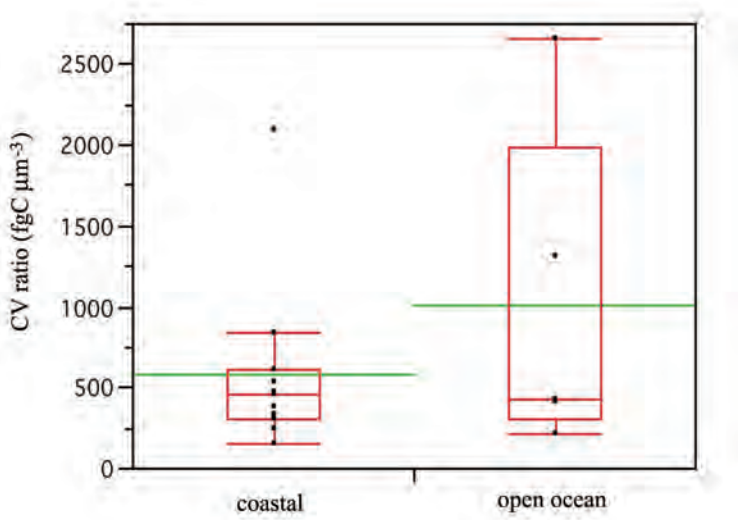


Figure 2B

DISCUSSION

A selection of unialgal pPeuk cultures representative of naturally abundant and ecologically important species was used in this study. In our culture conditions, the cell size (diameter) of cultures ranged from 1.38 μm for *Bathycoccus* to 5 μm for *Chrysochromulina sp*., with an average for the 16 strains of 2.73 μm. While the canonical cell size for picoplankton is 0.2-2 μm (Sieburth et al. 1978), most Picoeukaryotes are in fact within the size range 2-3 μm (e.g. Le Gall et al. 2008). The CV of measured cell diameters varied from 0.33% for *Minutocellus* sp. to 30% for *Florenciella parvula* RCC446 (Table 2). The CV was low for most cultures, indicating that the coulter counter was well adapted for determining small cell sizes. The high CV value recorded for *Florenciella parvula* may have been due to cellular dimorphism, as cytograms of red fluorescence versus volume discriminated two differently sized populations (average diameters 2.61 μm and 4.53 μm), confirmed also by light microscopy. For this reason, RCC446 was excluded from the mixture of pPeuk strains used to determine the average cell size and C and N content of a representative Picoeukaryotes fraction. The average cell diameter of 15 different cultures (1.60 μm ± 66.54%), calculated from the quantity of added cells and their respective sizes, was lower than the arithmetic average of measures of the 15 cultures, determined at 2.73 μm. This discrepancy was mainly due to the fact that cell sizes were not normally distributed, smaller cells contributing more to the mixture than larger cells. The weighted average value is coherent with the average size of natural pPeuk communities, measured to be 1.74 ± 0.13 μm in the eastern South Pacific (Grob et al. 2007).

The cellular C:N ratio varied from 3.06 (±1%) for *Pelagomonas calceolata* to 18.0 (±123%) for *Ochromonas* sp.. The average C:N value was 7.0 (±45%), close to the value of 6.6 often considered representative of nutrient replete cells (Goldman et al. 1979; Sakshaug et al. 1984). The C:N ratio was not correlated with cell size across the size range studied and was not impacted by growth rates. Apart from *Ochromonas* sp., no remarkable differences were observed between taxonomic types of algae. The very high CV of cell size (123%) measured for *Ochromonas sp.* means the average C:N value measured for this strain must be taken with caution. Nevertheless, the high C:N ratio for *Ochromonas* sp. could be the result of nutrient stress (Flynn et al. 1994) or may correspond to the fact that carbohydrates have been reported to be a large fraction (up to 70%) of total C content in Chrysophyceae (Moal et al. 1987). Starch and polyunsaturated fatty acids have been reported to be abundant in the eustigmatophyte *Nannochloropsis* (Lepère et al. 2009) and the pinguiophyte *Phaeomonas* (Kawachi et al. 2002), respectively, but C:N ratio values were not particularly high in these organisms (Table 2).

Staining with SybrGreen I allowed discrimination of algae and bacteria in plots of side scatter vs. green (DNA) fluorescence and green vs. red (chlorophyll *a*) fluorescence. Flow

cytometric cell sorting resulted in decreases in the proportion of bacterial cells with respect to algal cells by 74% for RCC497, 83% for RCC299, and more than 95% for the 9 other cultures. The ratio of bacteria/pPeuk concentration was the principal limitation for rendering the Picoeukaryotes cultures axenic. The highest bacteria/pPeuk value was observed for *Pelagomonas calceolata* RCC101, but this was still lower than average values in natural communities where there is a large variability, that we have measure to have a median value of 330 bacteria per pPeuk cell (unpublished data). This method could not be applied for cultures with very low cell density because a minimum number of cells were required per filter due to the detection limit of the CHN analyzer. Utilization of tangential flow filtration of samples could possibly have reduced this limit. After sorting, bacterial C always represented less than 1% of algal C. The 11 sorted pPeuk cultures were therefore considered axenic. While C content per pPeuk cell was expected to be lower after elimination of bacteria, this was not always the case. The measured C content after purification by sorting was sometimes higher than before sorting, particularly for smaller cells (Table 2). In contrast, C content of larger cells such as *Prasinoderma singularis* was under-estimated after cell sorting in comparison with other estimation methods. This may have been the result of an under-estimation of algal carbon if the cell sorting procedure damaged some cells.

There were no significant differences between the pPeuk C/V or N/V relationships following correction for bacteria by calculation or after cell sorting, indicating that both methods were suitable for establishing the relationship. The first attempts to describe the relationship between cellular C and biovolume were made by applying linear regression models to data from large diatoms, with cell volumes that ranged from 10^1 to 10^6 μm^3 (Mullin et al. 1966; Strathmann 1967). Use of non-linear relationships to describe cellular C and N content per unit volume has been reported as more appropriate (Verity et al. 1992; Menden-Deuer and Lessard 2000). In these studies, regression exponents of the power curve used to fit data of cellular C and N content with biovolume were significantly <1, indicating that cellular C and N densities (expressed as C or N per unit volume) decreased with cell volume, i.e. that small cells contained more C and N per unit volume than large cells. In this study, we used linear regressions to describe the relationship between cellular C and N content with biovolume. The slopes of the regressions described (equations 1 to 6) were not significantly different from 1. This can be explained by the low range of biovolumes in this study, varying only by one order of magnitude from 1.37 μm^3 to 68 μm^3 for RCC419 and RCC656, respectively, compared with other studies with much larger biovolume ranges, for example Verity et al. (1992) included cells from 10^1 to 10^3 μm^3. Rocha and Duncan (1985), Montagnes et al. (1994) and Pelegri et al. (1999) also found slopes not significantly different from 1 and considered cellular C to be constant with biovolume.

The average median C/V value established in this study at 467 fgC μm^{-3} (±4%) is clearly

higher than those reported in previous studies. In samples from the central Atlantic, Zubkov et al. (1998) used a CF of 1500 fgC cell^{-1} calculated from an average cell size of 6.8 μm^3 and a C/V value of 220 fgC μm^{-3}. With our overall median value of 467 fgC μm^{-3}, the estimation of pPeuk biomass would have been more than two-fold higher at 3176 fgC cell^{-1}, meaning that pPeuks would be considered to make a greater contribution to total biomass. Note that it has been suggested that the contribution of small phytoplankton to carbon export from surface layers might be much higher than previously expected (Richardson and Jackson 2007), as recently confirmed using a molecular approach in the eastern subtropical North Atlantic (Amacher et al. 2009).

The C/V values recorded for the different taxa in this study were sometimes different from those obtained for the same taxa by other authors. Durand et al. (2002) reported a value of 238 fgC μm^{-3} for *Micromonas pusilla*, similar to the value we measured for *Micromonas pusilla*Clade A (308 fgC μm^{-3}), but lower than that for *Micromonas pusilla* Clade C (470 fgC μm^{-3}). Grob et al. (2007) determined a C/V ratio for *Pelagomonas* of 692 fgC μm^{-3}, a value significantly lower than that we measured for *Pelagomonas* RCC101 (2660 fgC μm^{-3}). Such a discrepancy was also observed for *Pycnococcus*, with estimates of 2116 fgC cell^{-1} (Grob et al. 2007) compared to 7420 fgC cell^{-1} in this study. The C/V ratio of *Ostreococcus* was estimated to range between 233 and 247 fgC μm^{-3} (Worden et al. 2004), similar to our values measured between 146 fgC μm^{-3} and 324 fgC μm^{-3} depending on the correction method used. Grob et al. (2007), in contrast, found a higher value of 423 fgC μm^{-3}. Our new data add to the variability observed among pPeuk types, even between organisms that are phylogenetically closely related.

Since C/V values were constant over the range of volume measured (1-100 μm^3) but volume and hence C content varied drastically among the different pPeuk taxa selected, knowledge of the composition of the pPeuk community is clearly critical for determining the appropriate abundance-to-biomass CF. For instance, a community dominated by *Micromonas pusilla* Clade C would have a CF of ca. 800 fgC cell^{-1} (Table 2), whereas a community dominated by *Ostreococcus*would have a CF of 230 fgC cell^{-1}. In reality, pPeuk communities are composed of complex assemblages of diverse species with specific contributions that are largely unknown. We found no significant differences between the C/V in organisms considered representative of coastal or open-ocean environments, although the C/V of open-ocean species was highly variable (with a relatively large distance between the 25th and 75th quartiles, Figure 2B). High C/V values suggest adaptation to more oligotrophic conditions in contrast with communities in coastal waters where nutrients are more concentrated and the need for accumulating energy is lesser. Accumulation of C in cells has been assigned to an uncoupling between photosynthetic C assimilation and the C requirements for biomass production (Dubinsky and Berman-Frank 2001), forming reserves possibly mobilized when nutrients become available again.

In this study we used cultures representative of a relatively wide range of genera and classes of pPeuk and our results suggest that this strategy might be appropriate for determining CFs for pPeuk biomass estimation in field studies. It is still critical to better describe pPeuk community structure at a large scale, and more detailed information about the distribution of pPeuk groups is still needed, since the choice of pPeuk representative can drastically alter CF estimation.

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Short-time scale coupling of picoplankton community structure and heterotrophic activity in winter coastal NW Mediterranean Sea

Thomas Lefort and Josep M. Gasol

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ABSTRACT

We used flow cytometry to follow the diel variations of picoplankton community structure and heterotrophic activity in coastal NW Mediterranean surface waters during two successive 72 h periods in winter 2007. Heterotrophic bacteria always dominated numerically the picoplankton community structure (PCS), while photosynthetic Picoeukaryotes (pPeuk) dominated the photosynthetic fraction of the PCS during the first cycle and *Synechococcus* (Syn) dominated during the second. For each picophytoplankton group, pronounced diel patterns with significant periodicity of 24 hours were always observed for flow cytometrically determined side scatter (SSC) and pigment fluorescence. *Syn* and pPeuk grew during the light period and divided during the night. Opposite diel patterns were observed in *Prochlorococcus* since its growth occurred during the night. The diel periodic patterns of the different groups, and the overall PCS were strongly disrupted before the second cycle by a wind change event with associated rainfall and turbulence, suggesting that the shift observed in PCS resulted from the imbalances between growth and loss processes. Strong coupling between PCS and heterotrophic activity was observed. During the first cycle, activity was higher at night than at the light period, indicating that bacterial growth was phased with the diel variations of PCS. However, no diel patterns in bacterial abundance nor activity were observed during the second cycle, only similar increasing trends in bacterial activity and grazer abundance suggesting that grazing activity was a possible source of DOM release and consequently, was driving bacterial activity.

INTRODUCTION

Understanding the distribution of plankton, i.e. picoplankton, is one of the main goals of marine microbial ecology. In addition to the Picoeukaryotes (Johnson and Sieburth 1982), two phylogenetically closely related types of photosynthetic prokaryotes: *Synechococcus* (*Syn*) (Waterbury et al. 1979) and *Prochlorococcus* (*Pro*) (Chisholm et al. 1988; Chisholm et al. 1992) compose the picophytoplankton fraction (<3 μm). The concentrations of the three groups have often been shown to peak at different periods of the year, suggesting distinct environmental controls for each of these organism types (e.g. Partensky et al. 1999a). While mesotrophic regions are generally dominated by Picoeukaryotes, low productive oligotrophic waters are generally occupied by large numbers of *Prochlorococcus* and to a lesser extent by *Synechococcus* (Jacquet et al. 2002). These are ubiquitous in oligotrophic and mesotrophic regions (Olson et al. 1990; Campbell and Vaulot 1993; Partensky et al. 1996), but are generally more abundant in nutrient rich areas (Partensky et al. 1999a). A preference of *Prochlorococcus* for stratified over mixed waters has also been observed (Lindell and Post 1995; Vaulot and Partensky 1992). Although the individual geographic distributions of *Prochlorococcus* and *Synechococcus* is now well documented (Partensky et al. 1999a; Partensky et al. 1999b), less is known about the photosynthetic Picoeukaryotes (pPeuk), their low numerical contribution contrasting with their dominance in the picophytoplankton biomass of many marine ecosystems (Ishizaka et al. 1997; Li et al. 1992; Li et al. 1994; Worden et al. 2004). Yet, the comprehension of the factors driving the picoplankton group distribution and their relative contribution to total picoplankton biomass is essential for understanding the dynamics of the ecosystem. Using the comparative-analysis approach, Gasol et al. (1997) showed that the ratio of heterotrophic to autotrophic biomass (first introduced by Odum in 1971) tended to decrease with increasing levels of primary productivity and, additionally, Li et al. (2001) concluded that the heterotrophic and photoautotrophic components of the picoplankton tend to complement each other so that their total biomass is more conservative than either component alone.

The distribution of the different picoplankton groups has mostly been studied on relatively large time scales with sampling frequencies ranging from once per day to 1 per month, and only a few times at a higher frequency (i.e. several samples per day). Given that events of major ecological relevance often result from transient environmental perturbation (i.e. wind stress, turbulence, high irradiance…), and that the microbial life history more likely operates at short time frames, it is necessary to determine the significance of the short time scale to structuring the large scale patterns in microbial communities (Seymour et al. 2005).

Episodic physical forcing at short timescales is known to induce shifts in both phytoplankton and picoplankton community structures (Guadayol et al. 2009; Pannard et al. 2008; Thomas et al.

2010), and light has often been also identified as the most important driver of the diel variability. Most phytoplankton species divide at specific times of the day (Gouch 1905), and even large phytoplankton such as diatoms and dinoflagellates follow diel cycles (Swift and Durbin 1972; Smayda 1975). Jacquet et al. (1998) showed that the *Synechococcus* cell cycle was phased with the daily light cycle, possibly enforced by a "clock" controlled by genetic factors (Johnson et al. 1996). Synchronization and phasing of cell growth for both *Synechococcus* and pPeuk was measured from dawn to dusk during the winter in the northwestern Mediterranean Sea and in the Alboran Sea (Jacquet et al. 1998; Jacquet et al. 2002). However, differences were reported in the equatorial Pacific where the division of *Synechococcus*, *Prochlorococcus* and Picoeukaryotes did not proceed at the same time (Vaulot and Marie 1999). Whether or not such phase differences between groups are linked to the differential sensitivity of each group to light (Sommaruga et al. 2005) remains unclear, but it was suggested that the *Prochlorococcus* cell cycle is tightly coupled to the irradiance levels (Jacquet et al. 2001).

Moreover, the relative stability of picoplankton group cell concentrations measured on a daily and a weekly scale suggests that mortality generated by grazing and viral lysis balances cell growth and division (Landry et al. 1995). Differential grazing on *Synechococcus*, *Prochlorococcus* and pPeuk has already been described (Worden et al. 2004) and different factors that translate into preferential grazing on some bacteria have been identified, including cell size (Gonzalez et al. 1990), motility (Matz and Jürgenz 2005), surface properties (Matz and Jürgenz 2001), phylogenetic affiliation (Jezbera et al. 2005), food quality as estimated from C:N:P ratio (Shannon et al. 2007), cell viability (Landry et al. 1991) or membrane integrity (Massana et al. 2009). Thus, a detailed knowledge of grazing is needed to understand microbial diel variability and the resulting consequences on ecosystem functioning.

Tight coupling between phytoplankton and bacteria should result in bacteria also following circadian cycles. As a consequence of this link, a peak of bacterial activity at noon/afternoon should directly follow a peak of DOM originated from primary production (Fuhrman et al. 1985; Gasol et al. 1998; Herndl and Malacic 1987). Conversely, absence of daily coupling between phytoplankton and bacteria would imply that bacteria are not very much dependant of the DOM produced by phytoplankton, and instead support its growth and activity from DOM coming either from non-diel grazing pressure (Nagata et al. 2000) or from allochtonous sources. Evidences for diel patterns in bacterial abundance and activity have been reported from the coastal NW Mediterranean (Ghiglione et al. 2007, Gasol et al. 1998), but how picophytoplankton variability is coupled with bacterial single cell activities has not yet been analyzed.

For that objective, we followed the diel variations of picoplankton abundance by flow

cytometry sampling with a high frequency (4 hours intervals) during two cycles of 72 hours in winter 2007 in a NW Mediterranean coastal station, period of the year that presented the higher chlorophyll *a* levels. Combined with flow cytometry, we used molecular probes testing for bacterial activity, bacterial membrane integrity and heterotrophic nanoflagellate abundance, to determine to what extent picophytoplankton was coupled with heterotrophic bacteria, and how this coupling could be altered at the short time scale by physical forcing, such as that provoked by shifts in wind direction and strength.

MATERIAL AND METHODS

Sampling sites- Two diel cycles were studied during two successive three-day periods in February- March 2007 (from 20th to 23rd February 2007 and from 26th February to the 1st of March 2007) at the Blanes Bay Microbial Observatory, a shallow (20 m depth) oligotrophic coastal station in the NW Mediterranean Sea, located 800 m offshore of Blanes, Catalonia, Spain (41º39.90'N, 2º48.03E). The sampling of surface water was performed at 0.5 m depth with polycarbonate carboys at a frequency of 6 samplings per day (every 4 hours). The samples were kept in the dark until analyses at the laboratory (less than 20 min from sampling). The first sampling of the two cycles (CDN01 and CDN 20, respectively) began at 10:00 A.M. Only one sample (CDN 14) could not be performed due to sea conditions. The temperature and salinity of the waters were measured with a SAIV A/S 204 CTD probe. Irradiance measurements during the sampling were obtained from the nearby station of Malgrat de Mar (Catalan Meteorological Service, www.meteo.cat), located at 5 km from the sampling station and at 4 m above sea level. The station recorded arithmetically averaged hourly air temperature and relative humidity at 1.5 m above ground, vector-averaged hourly wind speed and direction and global irradiance at 2 m, and accumulated rainfall at 1 m. Wave height data were collected from a scalar buoy (DATAWELL, Waverider) placed at 41º 38.49´N, 2º 48.56'E over a depth of 74 m (XIOM Network, www.boiescat.org). Chlorophyll *a* concentration was determined from 150 mL of seawater filtered through GF/F filters (Whatman) extracted in acetone (90% v/v), and fluorescence was measured with a Turner Designs fluorometer.

Picoplankton abundances- Determination of picoalgal and bacterial abundance was performed by flow cytometry (Gasol and del Giorgio 2000; Marie and Partensky 2006). For picophytoplankton, the samples were analyzed without addition of fixative and run at high speed (ca. 100 µl min^{-1}), three populations (*Prochlorococcus*, *Synechococcus*, Picoeukaryotes) were discriminated according to scatter and fluorescence signals. For non-phototrophic bacteria, we choose to estimate the abundance following the NADS Viability protocol (see below), to avoid using fixatives. However, abundances were estimated also by fixing 1.2 ml samples with a 1% paraformaldehyde + 0.05% glutaraldehyde solution, and deep-freezing in liquid N_2. Afterwards the samples were unfrozen, stained with SybrGreen at a 10x dilution and run at low speed (ca 15 µl min^{-1}). Cells were identified in plots of side scatter versus green fluorescence.

Heterotrophic nanoflagellate abundances were measured following the Rose *et al.* (Rose et al. 2004) protocol. From a stock solution of 1 mM Lysotracker Green (Molecular Probes), 1 µl was added to 99 µl of <0.2 µm MilliQ, and 3.8 µl of this diluted Lysotracker stock were added to 0.5 ml of the sample, generating a 75 nM Lysotracker final concentration. We analyzed the samples

as in Rose et al. (2004), using a combination of side scatter and green and red fluorescence plots. Samples were run alive at high (ca. 100 µl min^{-1}) speed. Concentrations were obtained from weight measurement of the volume analyzed.

Carbon Conversion factors for biomass calculation- The *Synechococcus*/ Picoeukaryotes biomass ratio was obtained by transforming abundances with standard biovolume to C conversion values: 250 fgC $cell^{-1}$ and 1540 fgC $cell^{-1}$ for respectively *Syn* (Campbell et al. 1994) and Peuk (Lefort et al. Chapter I).

Bacterial single-cell activity- Measurements of the different physiological status of bacteria were done in two ways: I) Highly active prokaryotes, as those able to reduce 5-cyano-2,3-diotolyl tetrazolium chloride (CTC; Polysciences). CTC turns into a red fluorescent formazan that is detectable by epifluorescence and flow cytometry (Sherr et al. 1999; Sieracki et al. 1999). Sample aliquots (0.4 ml) were amended with 5 mM CTC (from a fresh stock solution at 50 mM) immediately following collection and were incubated for 90 min in the dark at room temperature. CTC-positive (CTC^{+}) cells were enumerated by flow cytometry using the FL2-versus-FL3 dot plot (Gasol and Arístegui 2007). For these analyses, we used a high speed (ca. 100 µl m^{-1}) and a threshold set in red fluorescence. ii) Cells with intact membranes were enumerated using the NADS viability protocol, based on the combination of the cell-permanent nucleic acid strain SybrGreen I (Molecular Probes, Eugene, OR) and the cell-impermeant propidium iodine (PI, Sigma Chemical Co.) fluorescent probe. We used a 10x SG1 and 10 μg ml^{-1} PI concentrations. After simultaneous addition of each stain, the samples were incubated for 20 min in the dark at room temperature and then analyzed by flow cytometry. SG1 and PI fluorescence were detected in the green (FL1) and red (FL3) cytometric channels, respectively. A dot plot of red versus green fluorescence allowed distinction of the "live" cell cluster (i.e., cells with intact membranes and DNA present) from the "dead" cell one (i.e., with compromised membranes) (Grégori et al. 2001; Falcioni et al. 2008). Fluorescence and Side scatter parameters were standardized to reference Polysciences 1 µm beads

Data transformations and statistical analyses- To perform the Fisher's Kappa statistic (Davis 1941; Fuller 1976), we completed the time series with the missing CDN14 values. For that purpose, we forecasted the CDN14 values calculating the arithmetical average between the surrounding values CDN13 and CDN15. Therefore, we tested the null hypothesis that the values in the series were drawn from a normal distribution with variance 1 against the alternative hypothesis that the series had some periodic component. Kappa is the ratio of the maximum value of the periodogram, $I(f_j)$, and its average value. The null hypothesis is rejected if this probability is less than the significance level. All conducted in JMP 7 (SAS institute Inc).

RESULTS

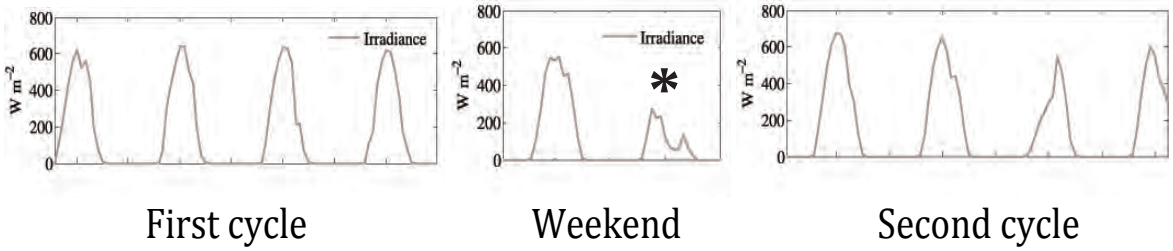
Background Environmental parameters- The two successive diel cycles were sampled in the winter of 2007 at the Blanes Bay coastal station. Water temperature was 13ºC (close to the minimum of the year) and salinity close to 38.30 psu, both parameters varied little over the period of observation (Table 1). Chlorophyll *a* concentration was only measured at the beginning of each cycle and increased from the first cycle to the second (from 0.47 to 0.89 μg L^{-1}, Table 1). The main wind direction was N/NW (340º) during the two cycles (Figure 1B), frequently interrupted by shifts in speed and direction from North to South/SW. During the second half of the day 25th of February (between the two cycles), a pronounced change from North to South occurred concomitantly with light rainfall (not shown) and a decrease in irradiance (*, Figure 1A),

		Average (±SD)		Coefficients of variation	
	Parameters	First Cycle	Second cycle	First Cycle	Second cycle
Env. parameters	Chlorophyll *a* (μg l^{-1})	0.47 ± 0.02 *	0.89 ± 0.03*	n.d	n.d
	Temperature (ºC)	13.43 ± 0.04	13.36 ± 0.01	n.d	n.d
	Salinity (psu)	38.27 ± 0.03	38.30 ± 0.01	n.d	n.d
Synechococcus	Abundance (10^3cells mL^{-1})	5.76 ± 0.81*	15.70 ± 0.30*	14%	19%
	FL2 (rel. units)	0.96 ± 0.05*	0.87 ± 0.05*	5%	6%
	FL3 (rel. units)	1.39 ± 0.04*	1.32 ± 0.04*	3%	3%
	SSC (rel. units)	1.16 ± 0.07*	0.86 ± 0.10*	7%	12%
Prochlorococcus	Abundance (10^3cells mL^{-1})	5.40 ± 0.10*	12 ± 0.17*	19%	14%
	FL3 (rel. units)	0.62 ± 0.07*	0.56 ± 0.05*	12%	10%
	SSC (rel. units)	0.24 ± 0.03*	0.21 ± 0.02*	14%	8%
Picoeukaryotes	Abundance (10^4cells mL^{-1})	1.09 ± 0.40*	1.38 ± 0.30*	40%	20%
	FL3 (rel. units)	1.63 ± 0.07*	1.57 ± 0.06*	4%	4%
	SSC (rel. units)	0.80 ± 0.04*	0.75 ± 0.05*	6%	6%
Heterotrophic. Bacteria	Abundance (10^5cells mL^{-1})	7.75 ± 1.13	8.27 ± 0.51	15%	6%
	Live + Dead cells (10^5cells mL^{-1})	7.20 ± 0.70	7.53 ± 0.90	9%	12%
	CTC+ (10^4cells mL^{-1})	4.70 ± 0.90	5.70 ± 0.17	19%	20%
	CTC+ (%)	6 ± 0.70*	6.50 ± 2.00*	11%	32%
	HNA (%)	59 ± 3.00	58 ± 2.00	5%	5%
	NADS-Live (%)	84 ± 3.15	86 ± 5.00	10%	11%
HNF (Lysotracker)	Abundance (10^3 cells mL^{-1})	1.14 ± 0.80	0.76 ± 0.40	68%	53%
Syn/PEUK	biomass Ratio (10^{-1})	0.96 ± 0.29*	1.88 ± 0.31*	30%	17%

Table 1. Average values and coefficients of variation of the different environmental, picoplankton community structure, and activity parameters. SD=Standard deviation. Coefficients of variation were calculated as (standard deviation)/(Mean).

* indicates significantly different values (t-tests , $p<0.05$).

A



B

In %	N	NE	E	SE	S	SW	W	NW
First cycle	26.29	0.43	3.02	4.74	12.93	12.93	23.71	15.95
Weekend	30.77	2.56	7.69	4.27	14.53	9.40	18.80	11.97
Second cycle	25.41	0.66	7.26	5.61	12.87	11.22	21.12	15.94

C

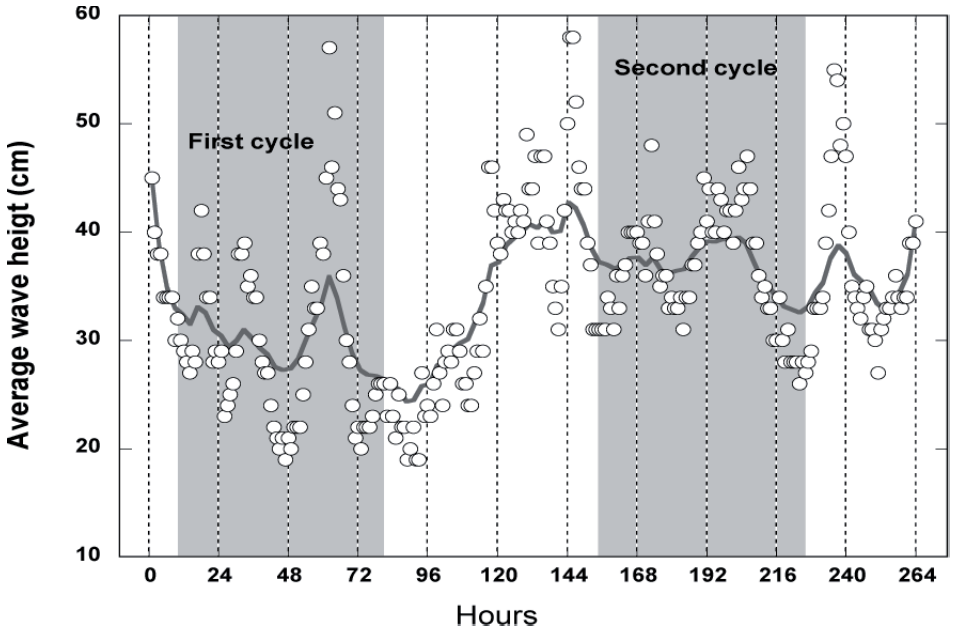


Figure 1. (A) Irradiance measurements (upper panels) during the first cycle (from the 20th to the 23rd of February), during the second cycle (from the 26th of February to the 1st of March 2007; and between the two cycles (from the 24th to the 25th of February 2007) and main wind direction (lower panel) expressed in percentage of time, * indicates the episode of increased turbulence, lower irrediance and shift in wind direction before the second cycle **(B)**. The weather data come from the station of Malgrat de Mar (Catalan Meteorological Service, http:// www.meteocat.com) **(C)** The smoothed average wave height measured by a scalar buoy throughout the sampling period (XIOM Network, http:// www.boiescat.org), The lines were obtained using a smooth fit in software Kaleidagraph vs 3.6.2 (Synergy Software).

rapidly followed by an increase of the turbulence at the onset of the 26th of February (Figure 1C). Compared with the first cycle, turbulence stayed at higher levels during the second cycle.

Picoplankton community Structure- Heterotrophic bacteria constituted the major component of the Picoplankton community structure during the two cycles (Figure 2). The average bacterial concentrations during the first and second cycle were 7.75 (±1.13) 10^5 cells ml^{-1} and 8.27 (±0.51) 10^5 cells ml^{-1} (Table 1). During the first cycle, picophytoplankton community structure appeared clearly dominated by photosynthetic Picoeukaryotes (pPeuk) for which the average concentration was 1.09 (±0.40) 10^4 cells, followed by *Synechococcus* and *Prochlorococcus* with 5.76 (±0.81) 10^3 cells ml^{-1} and 5.40 (±0.10) 10^3 cells ml^{-1} respectively (Table 1 and Figure 2). During the second cycle (week 2), a shift in community composition followed the change in wind direction and strength. *Synechococcus* dominated community structure with an average concentration of 1.57 (±0.30) 10^4 cells ml^{-1} (representing a 172% of increase when compared

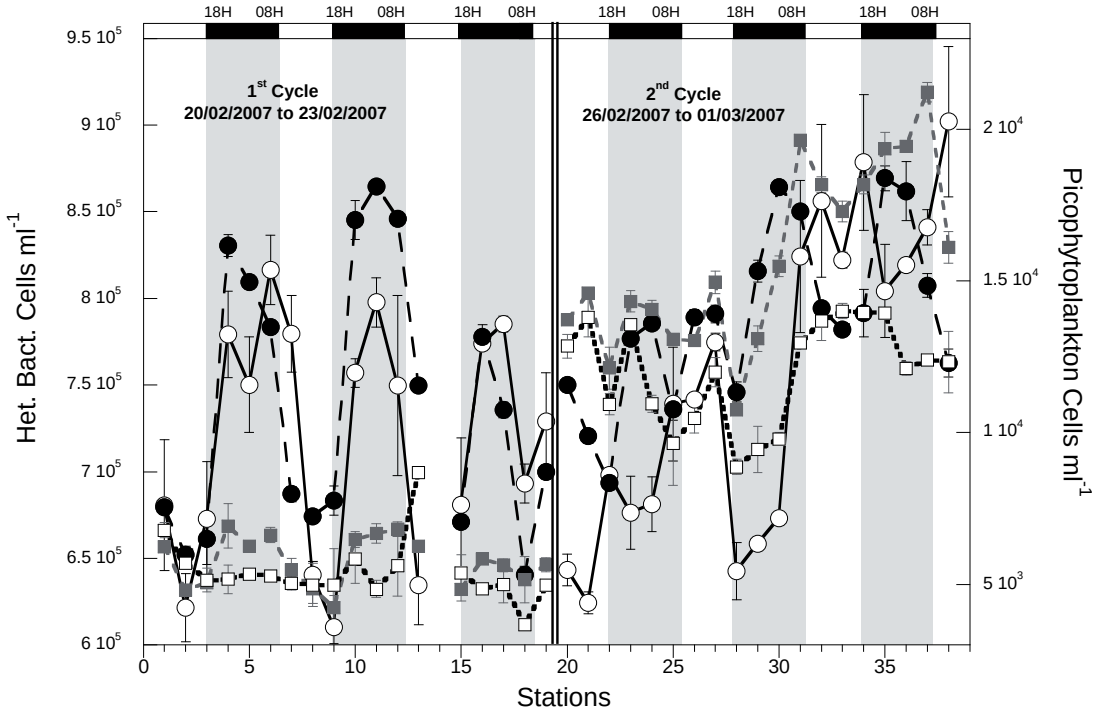


Figure 2: Diel variations of Picoplankton group abundances as measured by flow cytometry. The Y left axis corresponds to the heterotrophic bacterial concentration (❍) and the Y right axis to the picophytoplankton abundances: (●) for Picoeukaryotes; (■) for *Synechococcus*; (❒) for *Prochlorococcus*. The grey areas correspond to dark period (from 18h00 to 7h00) also indicated by solid bars on the top axis; the error bars correspond to the range of variation of the duplicate samples.

with the average found the first week), closely followed by pPeuk and *Prochlorococcus* with the average concentrations of 1.38 (±0.30) 10^4 cells ml^{-1} and 1.20 (±0.17) x 10^4 cells ml^{-1} respectively (Table 1 and Figure 2)

***Synechococcus* diel patterns-** As a general tendency, *Synechococcus* concentration increased during the dark period, and decreased during the light period. During the first cycle (From 20th to 23rd February 2007)**,** *Synechococcus* abundance followed a clear diel cycle with a significant periodicity of 24 hours (Fisher's Kappa, $p<0.05$) (Table 2, Figure 2). Their concentration increased strongly during the first part of the dark period (from 6:00 to 10:00 pm), followed by a plateau until dawn. After dawn, a pronounced decrease of *Synechococcus* concentration was observed until a minimum reached at dusk.

		Kappa Periodicity	
		First Cycle (hours) N=19	Second cycle (hours) N=19
Synechococcus	Abundance	24h*	Undefined
	FL2	24h*	Undefined
	FL3	24h	Undefined
	SSC	24h	19h
Prochlorococcus	Abundance	Undefined	Undefined
	FL3	24h	24h*
	SSC	Undefined	Undefined
Picoeukaryotes	Abundance	24h*	24h
	FL3	24h*	24h*
	SSC	24h*	Undefined
Het. Bact	Abundance	Undefined	15h
	Live+Dead	24h*	Undefined
	CTC+ abundance	Undefined	nd
	CTC (%)	Undefined	nd
	HDNA (%)	24h	15h
	Live (%)	Undefined*	Undefined
HNF (Lysotracker)	Abundance	24h	15h
Syn/PEUK	Ratio biomass	Undefined	12.6

Table 2. Fisher's Kappa statistic tests. * indicate significant periodic variations ($p<0.05$)
"Undefined" indicates that no periodicity could be measured from the periodograms.
N for number for observations.

In comparison with the first cycle, a less pronounced diel pattern in *Synechococcus* abundance was observed during the second diel cycle, with no significant and defined periodicity (Figure 2, Table 2). A large diel abundance variation of *Synechococcus* concentration was measured (14% - 19%), mostly resulting from the strong increase during the second night of observation (Figure 2). *Synechococcus* abundance recovered a diel pattern towards the end of the second cycle, more exactly during the third light period of the second cycle (28th of February), when its concentration decreased with the pattern observed during the first week.

Cell-specific pigment content (as measured by the standardized FL2 and FL3 parameters) followed a clear diel pattern during the two cycles, with a significant periodicity of 24 hours (Fisher's Kappa, $p<0.05$) during the first cycle (Figure 3A, Table 2). However, this pattern was opposite to that observed for abundance (Figure 2). It generally increased from dawn to reach a maximum at dusk, corresponding with the accumulation of pigments during the growth process occurring during the lit period of the day. The decrease began just after dusk and reached a minimum at noon with a stationary period until dawn. No significant differences between cycles were measured for the fluorescence parameters (Table 1).

A

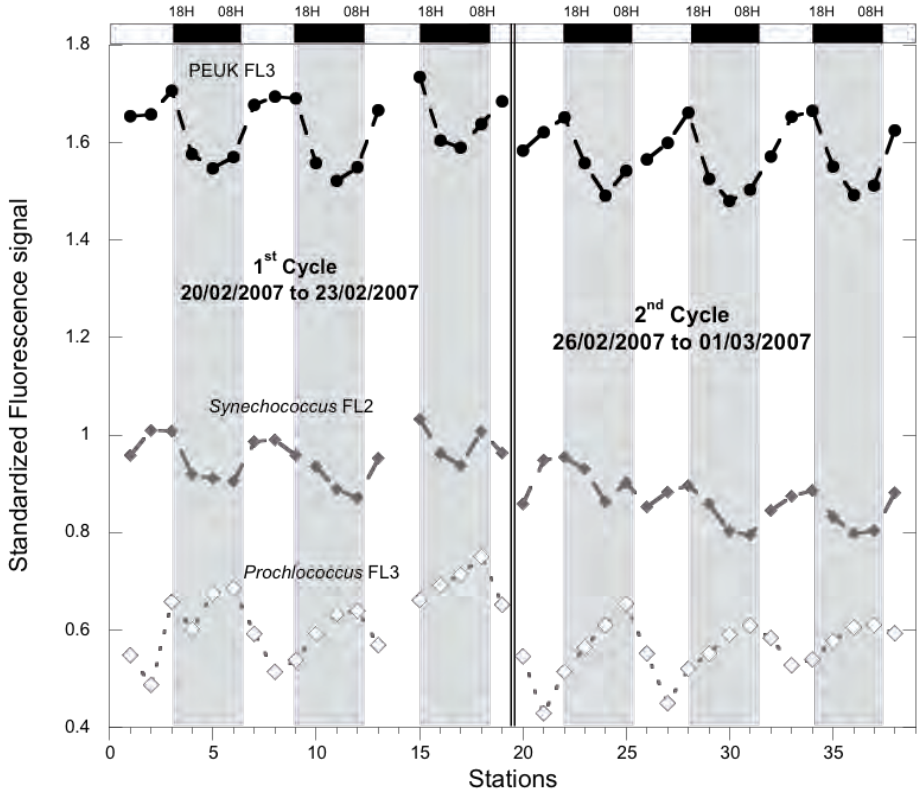


Figure 3. (A) FL3 Red Fluorescence (relative units) of Picoeukaryotes and *Prochlorococcus*, FL2 fluorescence (relative units) of *Synechococcus*, all standardized according to the fluorescence of Polysciences 1 μm beads ;

B

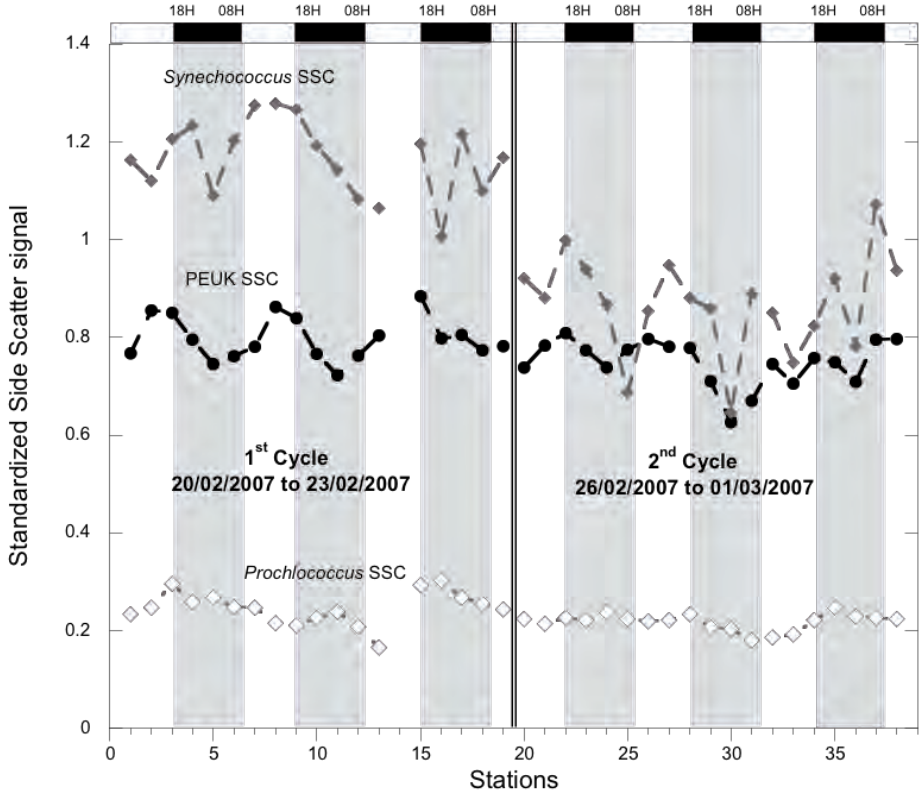


Figure 3. (B) Side scatter parameter for Picoeukaryotes, *Synechococcus* and *Prochlorococcus*. All standardized according to the beads SSC. The grey bars represent dark periods.

A less pronounced diel pattern was observed when looking at the side scatter parameter (SSC, a surrogate of cell size) (Figure 3B). Following a pattern opposite to that of abundance, this parameter had a minimum around midnight, at the expected moment of cell division. Note that the average SSC of *Synechococcus* during the second cycle was significantly lower (t-test, $p<0.001$) than the one measured during the first cycle with values of 0.86 (±0.10) and 1.16 (±0.16) respectively (Table 1), indicating possibly that 2 different populations of *Synechococcus* were sampled during the two weeks.

***Prochlorococcus* diel patterns**- No pronounced diel pattern of *Prochlorococcus* abundance were observed during the two cycles (Figure 2) without any significant periodicity (Table 2). During the second week, the average *Prochlorococcus* concentration was higher than the one measured the first week (Table 1). The diel abundance variation measured at 14% during this second period was, on the contrary, lower than that of the first week calculated at 19%. As previously observed for *Synechococcus* abundance, the average *Prochlorococcus* abundance

increased by 122% between the first and the second week (Table 1). In contrast to abundance, the FL3 Parameter followed a significant 24 hours periodicity (Table 2). A minimum of fluorescence was reached at midday and increased from midday until dawn (Figure 3A). A decrease of fluorescence was observed during the day. While significant differences between cycles were measured for FL3 values, very similar daily coefficients of variation of 10 and 12% were seen (Table 1).

PPEUK diel patterns- The concentration of Picoeukaryotes followed a clear diel pattern during the first week of the experiment with a significant periodicity of 24 hours (Fisher's Kappa, $p<0.05$) (Figure 2, Table 2). Abundance started to increase from dusk to the middle of the dark period, and showed a two-step decrease. The first stage of the decrease began from the middle of the dark period to dawn, followed by a more pronounced decrease from dawn until the end of the light period (Figure 2). The diel variation of abundance was particularly high and calculated at 40% (Table 1). During the second cycle, a less pronounced diel pattern was noticed, and no significant periodicity was observed in Picoeukaryotes abundance (Fisher's Kappa, $p<0.05$), (Figure 2). While the average concentration of the second week was 26.5% higher than the first week, we measured smaller variability (coefficient of variation of 20%) (Table 1).

A pronounced diel pattern was observed during the two cycles in pPeuk pigment content (FL3 parameter, Figure 3A) with a significant 24 hours periodicity (Fisher's Kappa $p<0.05$). This pattern was opposite to the one described for pPeuk abundance and showed a minimum at noon and a maximum at dusk. Significant but weak differences were observed between the per cycle global means ($p<0.05$). A significant diel pattern was observed in pPeuk SSC only during the first cycle with a periodicity of 24 hours (Fisher's Kappa, $p<0.05$) (Figure 3B).

Synechococcus to pPeuk Biomass ratio (*Syn*:pPEUK)- We calculated the *Syn*:pPEUK ratio using standard conversion factors to translate abundances to biomass as a way to follow the diel changes of picophytoplankton community structure. This ratio followed a diel pattern, particularly pronounced during the first cycle, slightly less during the second (Figure 4), but no significant periodicity could be defined for the first cycle (Table 2). A minimum *Syn*:pPEUK ratio was always observed during the night between 22h00 and 02h00. This ratio decreased from midday to midnight and increased from midnight to midday (or until dusk the third day of the first week). During the second cycle, this ratio tended to decrease during the first half of the second cycle but appeared to follow a day-night pattern once again during the second part of the second cycle. The per cycle average biomass ratio *Syn*:pPeuk increased greatly the second week with a value at 18.8 (±3.1) as compared to 9.6 (±2.9) measured in the first diel cycle (Figure 4, Table 1). Note that the

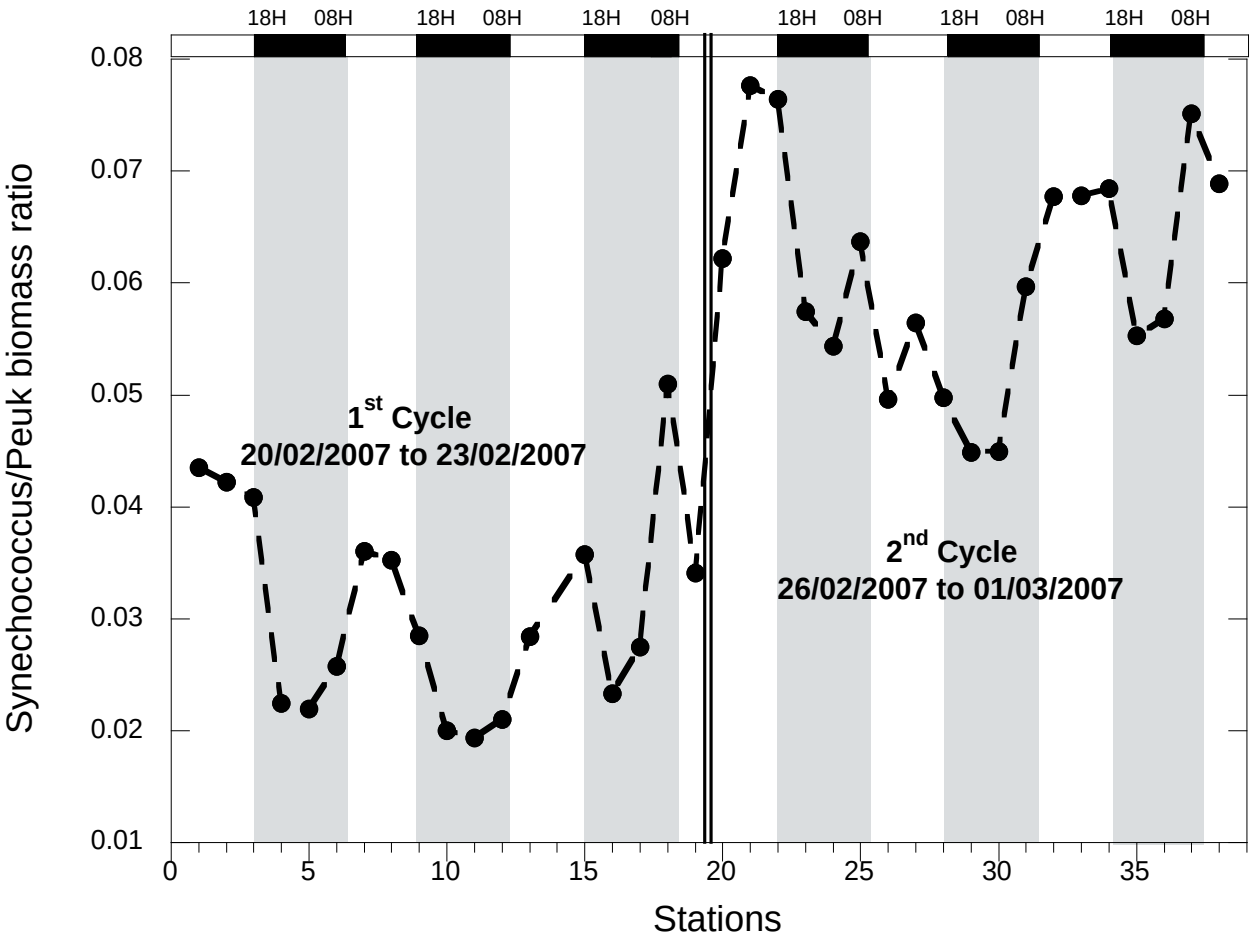


Figure 4. Diel variations in the ratio biomass of *Synechococcus* / biomass of Picoeukaryotes during the two cycles, calculated from abundances (as measured by flow cytometry) translated into biomass using standard conversion factors. (250 fgC cell^{-1} for *Synechococcus*, Campbell et al. (1994) and 1540 fgC cell^{-1} for Picoeukaryotes, (Lefort et al, chapter I).

variability decreased from 30% to 17% (Table 1).

Group division rates- We estimated the in situ division rates for each picophytoplankton group calculating the day-to-day variations of the ratio of the minimum to the maximum light scattering (SSC) parameter as proposed by Binder et al. (Binder et al. 1996) and Vaulot and Marie (Vaulot and Marie 1999) (Figure 5). The amplitude of the SSC variation varied from one day to the next one, and varied differently for the different groups. The highest division rates were estimated for *Prochlorococcus* during the first cycle, followed by *Synechococcus* and Picoeukaryotes.

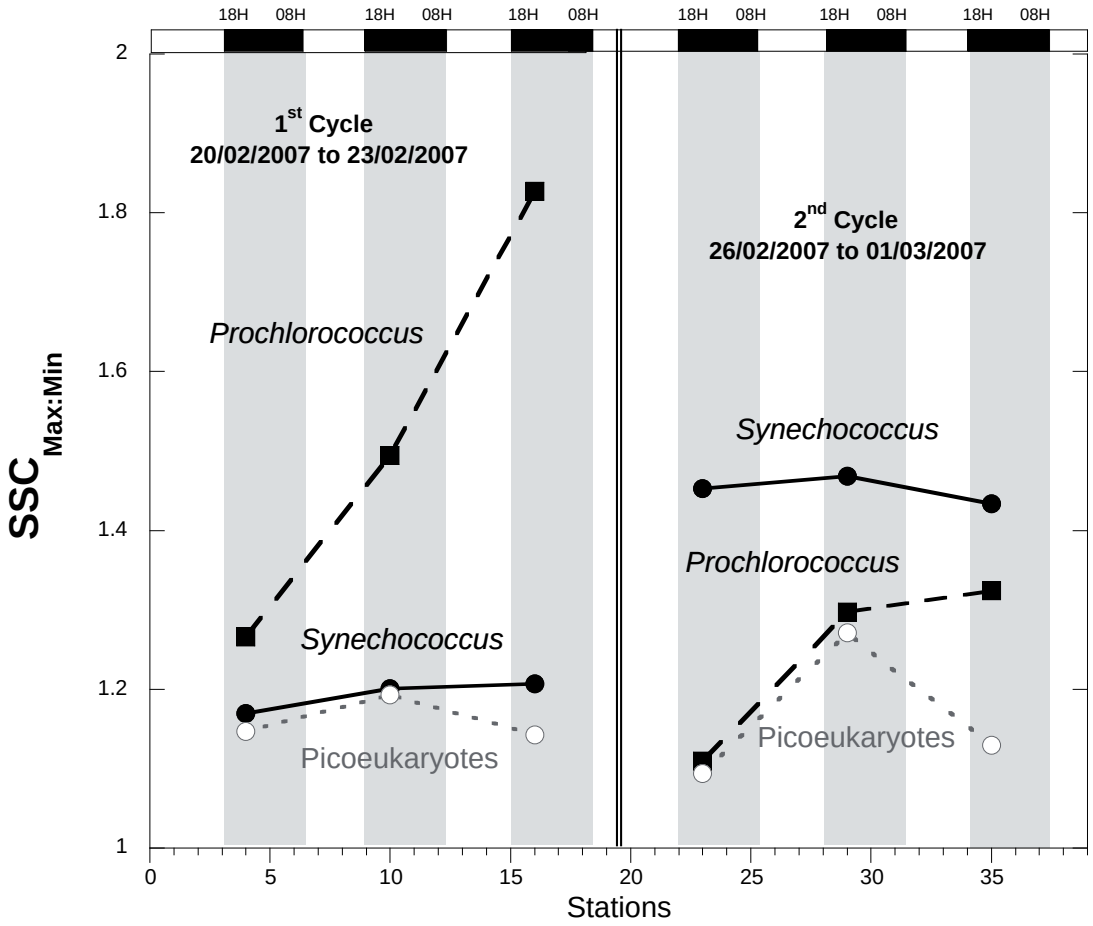


Figure 5. Ratio of the maximum to the minimum side scatter (SSC) as a proxy for the in situ division rate of *Synechococcus*, *Prochlorococcus*, and Picoeukaryotes.

During the second cycle, the highest $SSC_{Max:Min}$ were measured for *Synechococcus*, followed by *Prochlorococcus* and Picoeukaryotes. Considering that a $SSC_{Max:Min}$ value of 2 for *Prochlorococcus* in the Equatorial Pacific corresponded to 1 division per day as formulated by Vaulot et Marie (Vaulot and Marie 1999), *Prochlorococcus* showed large rates all along the first cycle, reaching almost 1 division per day at the end of the first cycle, but this was paradoxically not translated into an increase in cell abundances (Figure 2).

Heterotrophic bacteria diel patterns- Bacterial abundance was estimated by two different methods, a first one by adding the live and dead bacterial cell abundances (see material and methods) obtained with the NADS method on unfixed cells, and the second one by fixation, storage in liquid nitrogen and SG staining. No significant differences were observed between

the two per cycle averages values with both methods of estimation (Table 1). The clearest diel patterns were observed with the former method, particularly during the first cycle with a significant periodicity measured close to 24 hours (Fisher's Kappa, $p<0.05$) (Figure 2, Table 2). Bacterial concentration increased from dusk until midnight, and then decreased until noon-dusk. While the diel range of variation of bacterial abundance estimated by the NADS method was of 9%, more variability was measured in bacterial abundance when measured by SG staining (Table 1). Less pronounced patterns and no significant periodicity were measured the second week for both estimations of bacterial abundance, and only a general increase was observed (Figure 2, Table 2).

Heterotrophic activity- The contribution of HNA cells to total bacterial abundance (%HNA) and the abundance of actively respiring cells (CTC+ cell abundance) followed during the two cycles the same pattern observed with bacterial abundance (Figure 6), but the periodicity was not significant (Table 2). The peaks of activity occurred around midnight during the first week (Figure 6), with an important increase in the second night period of the second cycle corresponding also with the increase in bacterial and *Synechococcus* abundance. The percentage of CTC+ cells (%CTC) appeared highly correlated with %HNA and the different picoplanktonic

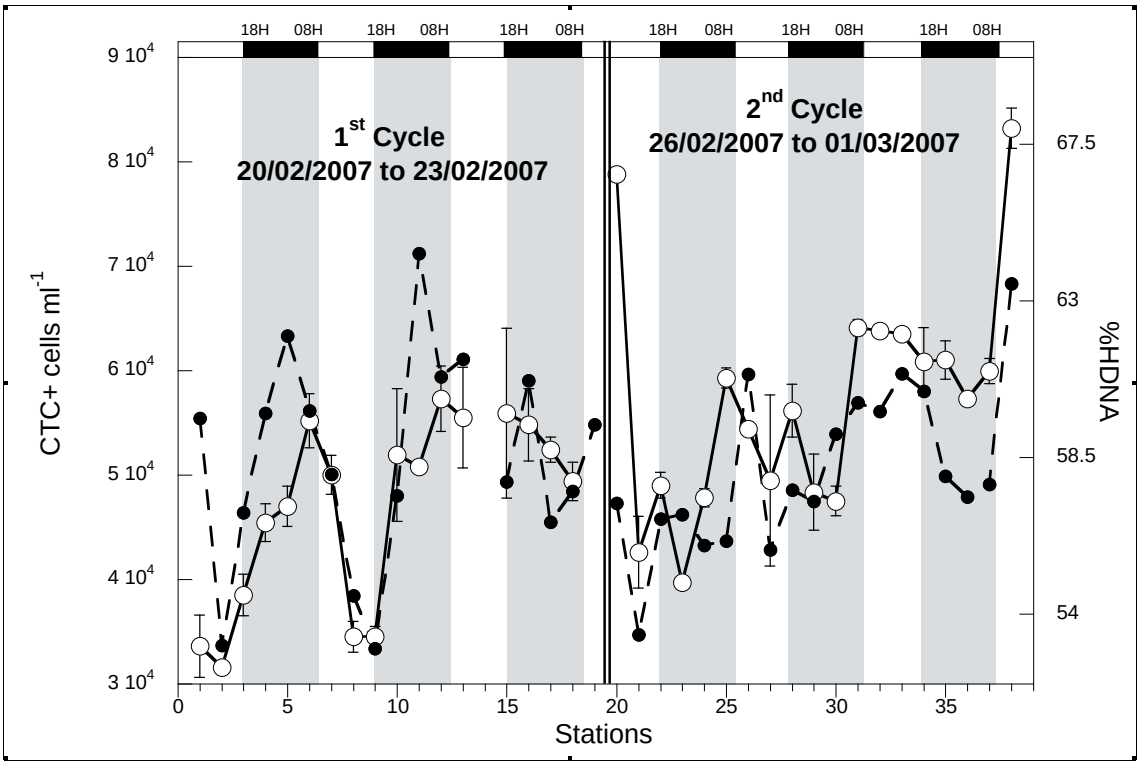


Figure 6. Concentration of CTC positive bacterial cells (●) with the scale on the left axis and HNA percentages (dash black line) with the axis on the right. The grey bars represent dark periods.

A (N=18)

Cycle 1	Syn abund.	Pro abund.	pPEUK abund.	Bact abund.	Hnf abund	%HNA%	%CTC+
Syn abund.	-						
Pro abund.	0.11	-					
PEUK abund.	**0.85**	0.05	-				
Bact. abund	**0.80**	-0.26	**0.72**	-			
Hnf abund	**-0.54**	-0.15	-0.22	-0.34	-		
%HNA	**0.72**	0.18	**0.65**	**0.61**	-0.40	-	
%CTC+	0.28	0.11	**0.54**	0.33	0.25	0.46	-
% (NADS)Live	-0.10	0.06	0.12	-0.12	0.25	-0.08	**0.50**

B (N=19)

Cycle 2	Syn abund.	Pro abund.	pPEUK abund.	Bact abund.	Hnf abund	HNA%	%CTC
Syn abund.	-						
Pro abund.	**0.60**	-					
PEUK abund.	**0.67**	0.12	-				
Bact. abund	**0.75**	**0.45**	0.36	-			
Hnf abund	0.27	0.20	0.15	-0.14	-		
%HNA	0.39	0.18	0.30	**0.69**	0.00	-	
%CTC+	0.09	0.06	-0.14	0.37	0.40	**0.53**	-
%(NADS)Live	-0.12	-0.07	0.07	-0.51	0.10	**-0.50**	-0.28

Table 3. Pearson correlation coefficients between Picoplankton group abundances and heterotrophic activity parameters as measured by flow cytometry during the first cycle (**A**) from the 20th to 23rd of February and during the second cycle (**B**) from the 26th of February to 1st of March 2007. Bold figures indicate significant correlations (Pearson's correlations, $p<0.05$). Abbrevations: Abund.= abundance Syn= *Synechococcus*, Pro= *Prochlorococcus*, pPeuk=photosynthetic Picoeukaryotes, HNF=Heterotrophic nanoflagellates, %HNA=percentage of High nucleic Acid content bacteria, N=Number of observations.

group abundances, indicating an important phasing between these parameters (Table 3). Moreover, %HNA was also significantly positively correlated with pPeuk and bacterial abundances, but negatively correlated with Heterotrophic nanoflagellate abundance (HNF).

HNF diel patterns- During the first cycle, HNF concentration increased during the light periods (Figure 7), although the periodic variations were not significant (Table 2). Less periodicity was observed during the second cycle, but after a pronounced decrease of HNF abundance observed the first day of the second cycle, a general trend of increase was finally observed. Note that while negative correlations were calculated between HNF abundances and the different picophytoplankton group abundances (significant only for *Synechococcus,* Table 3), positive relationships (but not significant) were observed with %CTC and %Live cells during the first and second cycle, suggesting a possible preference of HNF grazing activity for actively growing bacterial cells with intact membranes.

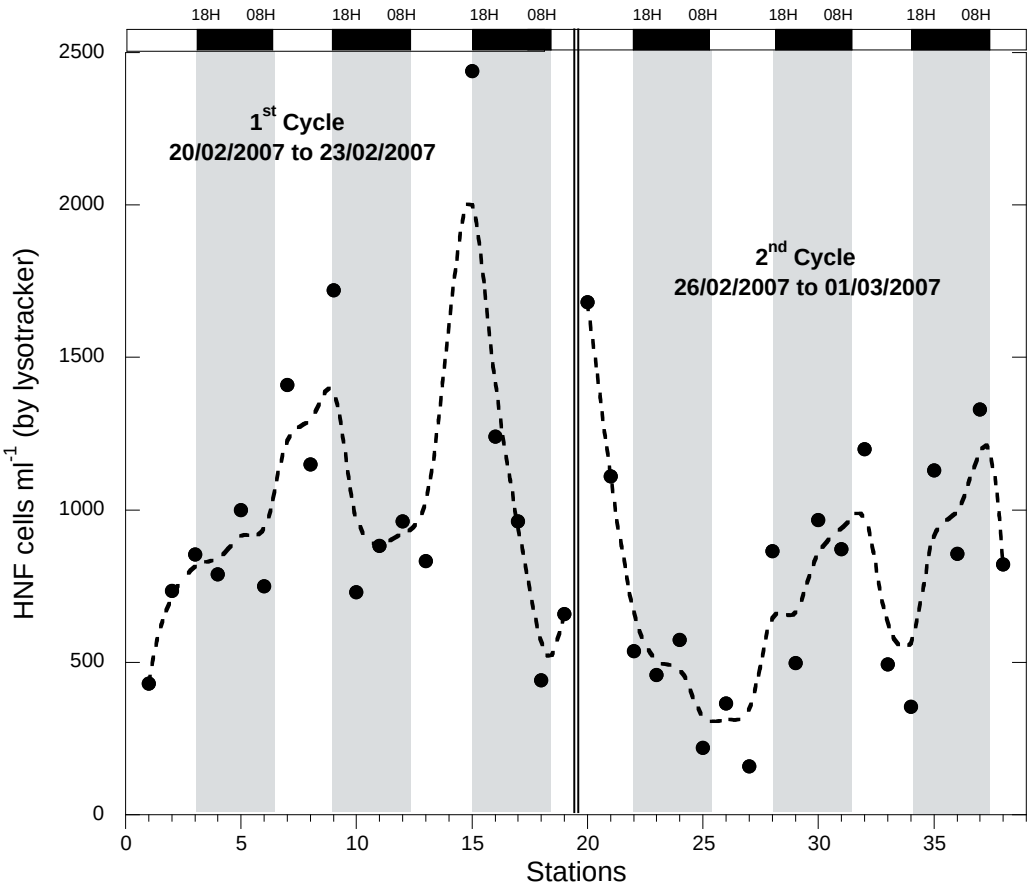


Figure 7. Concentration of heterotrophic nanoflagellates (HNF) after Lysotracker staining (solid black line) during the two cycles. The lines were obtained using a smooth fit with software Kaleidagraph vs 3.6.2 (Synergy Software). The grey bars represent dark periods.

DISCUSSION

Improved knowledge of the diel patterns in microbial parameters and the resulting diel variability may inform us about the factors controlling the growth and loss processes of marine microbes. Photosynthesis occurs only during the light parts of the diel cycle, and the cellular division of microbes commonly occurs at specific moments of the diel cycle (e.g. Vaulot and Marie 1999), although not necessarily at the same time for all organisms, nor in all oceanographic settings (e.g. Jacquet et al. 2001). The photosynthetic release of DOM is dependent upon the light cycle (Mague et al. 1980; Furhman et al. 1985; Pausz et al. 1999), and the grazing activities of most zooplankters are also circadian (Atkinson et al. 1992; Jakobsen and Strom 2004; Wikner et al. 1990) and so, the rates of DOM supply to heterotrophs are likely to follow diel patterns. Factors that affect the single-cell physiological status and activity level of marine bacteria, such as ultraviolet radiation, bacterivory and viral lyses are also known to follow diel variations (Jeffrey et al. 1996; Christaki et al. 2002; Wikner et al. 1990; Winter et al. 2004).

Not all studies of diel variability encounter the above-mentioned periodicities, and in some cases microbial abundance and activity seem to vary at random. Other than lack of sensitivity of the used methods, it is interesting to describe what environmental factors facilitate that microbial populations vary with diel periodicity in some cases and not in others. Coastal communities fon instance may not show evidences of coupled microbial diel variability if heterotrophic communities depend on land- or benthos-derived materials instead of using phytoplankton-derived primary production.

We were particularly interested in the diel variability of the structure of the picoplankton community (understood as the differential contributions of each organism type), and in the linkage between this structure and the heterotrophic activities of the bacteria measured at the single-cell level, which could also be expected to vary following the phytoplankton and the light regime. We chose to perform the experiment at the likely time of the phytoplankton bloom to maximize the likelihood of observing coupled variability of picophytoplankton and bacteria.

In brief, our results have shown 1) consistent diel variability of all picoplankton populations, including heterotrophic bacteria and HNF, 2) differences in the time of duplication and growth of different picophytoplankton groups, and 3) coupling between picophytoplankton variability and single-cell bacterial activities. We furthermore observed how a relatively small variation in weather patterns changed considerably the structure of the microbial community and disrupted most diel cycles, which started to recover a couple of days after the disruption.

Diel patterns in the picophytoplankton growth and division- During the two cycles, diel patterns were observed for the different Picophytoplankton group fluorescence and scatter parameters, with a periodicity close to 24 h for the majority of the parameters studied. *Synechococcus* and Picoeukaryote growth, as measured by the increase of SSC and fluorescence (FL2 and FL3), occurred during the light period, indicating that light drove the synthesis and accumulation of carbon and pigment, followed during the night by division, producing smaller cells with lower scatter (Durand and Olson 1998). These measured diel variations are not particular but are apparently common for *Synechococcus, Prochlorococcus,* and Picoeukaryotes communities across systems (Vaulot et al. 1996; Jacquet et al.,1998; 2001,;2002; Vaulot et Marie 1999; Seymour et al. 2005; Durand et al. 2002).

However, the increase of *Prochlorococcus* FL3 fluorescence and cell size occurred during the night period instead of at light. A minimum of chlorophyll *a* fluorescence (FL3) was measured at midday for *Prochlorococcus* and was concomitant with the maximum irradiance measured daily. On the contrary, no particular bleaching of fluorescence for *Synechococcus* and Picoeukaryotes was measured during the light period of the diel cycle. Vaulot and Marie (Vaulot and Marie 1999) measured similar patterns in the equatorial Pacific and observed only for *Prochlorococcus* fluorescence some quenching during the light period at the surface (in samples particularly exposed to high irradiance levels) and an increase of fluorescence at depth (Vaulot and Marie 1999), suggesting that *Synechococcus* and Picoeukaryotes were more protected against light damage than *Prochlorococcus,* something that can be explained by a much thicker thykaloid layer in the former genus *Synechococcus* and more complex photoprotective mechanisms in eukaryotes (Sommaruga et al. 2005; Llabrés and Agustí 2006). But no other decrease of FL3 for *Prochlorococcus* during the night was observed, suggesting that such day light minimum was more likely related to division rather than photochemical quenching, our study suggests a specific timing for *Prochlorococcus* division.

Moreover, this specific behavior found for *Prochlorococcus* fluorescence and size increasing during the night period, could also suggest that other processes were supporting cell growth, more likely promoted by heterotrophy (by incorporation of organic matter) rather than by photosynthetic activity. It has been shown for example that both *Synechococcus* and *Prochlorococcus* are capable to assimilate amino acids in surface waters of the South Atlantic Subtropical front (Zubkov and Tarran 2005) and also that *Prochlorococcus* followed pronounced diel patterns in ^{3}H-leucine and ^{35}S-methionine uptake with a minimum occurring at midday as shown in the Atlantic (Mary et al. 2008). In spite that organic matter uptake by freshwater *Synechococcus* has been seen to follow diel patterns (Chen et al. 1991; Vila-Costa et al. 2006), no significantly different uptake rates between night and day were measured for *Synechococcus* during our sampling in Blanes Bay

(Ruiz-González et al. 2011).

Disruption of the diel patterns in Picoplankton community structure- Chlorophyll *a* concentration increased from one week to the next concomitantly with the shift in picophytoplankton community structure, dominated numerically by pPeuk during the first diel cycle and by *Synechococcus* during the second. Besides, these changes in community structure were preceded on the 25th of February by shifts in wind direction, rainfall and turbulence conditions. If it has been shown that *Synechococcus* cell cycle was relatively little impacted by strong hydrological variability when compared with other picophytoplankton groups (Jacquet et al. 2002), the supremacy of *Synechococcus* during the second cycle could also be promoted by the resuspension by wind and turbulence that changed nutrients availability, differentially modifying the activities of the different marine organisms as it has been shown elsewhere (Cotner 2000; Garstecki et al. 2002), dramatically increasing *Synechococcus* growth rates (Agawin et al. 2000; Agawin et al. 2003; Lindell and Post 1995) over those of *Prochlorococcus* and Picoeukaryotes, as corroborated by the higher division rates measured for *Synechococcus* during the second diel cycle in comparison with other picophytoplankton groups (Figure 5).

In comparison with the first cycle, the diel patterns of abundances during the second cycle appeared disrupted, particularly during the first and second day of observation, with a tendency for the recovery of the diel patterns towards the third day. Comparing with abundances, the pigment fluorescence and the SSC parameter of *Prochlorococcus*, *Synechococcus* and pPeuk presented a pronounced and very stable diel pattern during the two cycles, suggesting that in spite of the shift in community structure provoked by the turbulence and wind event, the single-cell biology was still following a regular day-night pattern.

The fact that only the diel patterns in abundances were altered and not the other parameters indicate more likely an imbalance between growth and loss processes. As already commented by Seymour et al. (2005), different underlying factors can explain the loss processes in picophytoplankton communities, including grazing by heterotrophic nanoflagellates (HNF) (Dolan and Simek 1999), and also viral lysis (Suttle and Chan 1994). In view of the fact that the diel variations in picophytoplankton community structure indicate that loss processes do not occur at a uniform rate (Vaulot et Marie 1999), that grazing activity by HNF could vary with the picophytoplankton cell cycle (Christoffersen 1994; Dolan and Simek 1999; Christaki et al. 2002) and that diel variability of viral infection has been also demonstrated (Weinbauer et al. 1995), thereof, disruption of the diel periodicity in HNF or viruses abundance and activity could result in an increase in prey abundance. Effectively, Ruiz-González et al. (Ruiz-González et al. 2011) showed patterns of maximal grazing activity on pPeuk populations at nights and particularly

pronounced during the first cycle, but weakened during the second cycle. Similarly, the strong variability in HNF abundance showing decreasing trends during the first day of the second cycle directly after the wind event was likely to explain such disequilibria between rates since it resulted in a general increase of all picoplankton group abundances.

Whether or not the observed changes between weeks in community structure are linked with the sampling of different water masses over the period covering the diel cycles is difficult to ascertain and exclude. No major variations of temperature or salinity were measured during this study suggesting that we followed a relatively stable water mass. However, a relatively and significantly lower light scattering measured the second week for *Synechococcus* might also suggest that we followed two different populations, a first one with high SSC values and low division rates (as estimated from the ratio of the maximum to the minimum SSC over one day) during the first week, and a second with smaller cells, but presenting higher division rates, possibly enhanced by the changes in water mass characteristics.

Coupling between heterotroph and phototroph parameters- During the first cycle, bacterial abundance followed pronounced diel patterns, strongly phased with the relative activity measurements (number of CTC+ cells as well as %HNA) peaking around midnight and strongly correlated with *Synechococcus* and Picoeukaryotes concentration. During the second cycle, only bacterial abundance was correlated with all the picophytoplankton groups (Pearson's tests, $p<0.05$), and no coupling with bacterial activity estimators was found. In view of the fact that the abundance of active cells has been related with bacterial production and cell growth (Lovejoy et al. 1996; del Giorgio et al. 1997; Choi et al. 1996; Sherr et al. 1999), then, it is reasonable to consider that bacteria were more active during the night period, an idea supported by the observation of bulk and group-specific bacterial production particularly enhanced during the dark period (Ruiz-González et al. 2011).

The tight phasing between picophytoplankton parameters and bacterial abundance or activity found during the first cycle could indicate that the release of dissolved organic matter originating from phytoplankton growth and division processes was directly used to support heterotrophic activity (Nagata et al. 2000). Similarly, the pronounced diel patterns in bacterial production measured during the first cycle (Ruiz-González et al. 2011) and the extremely high variability associated to its diel fluctuations (calculated to be 37% during the two cycles as measured by ^{3}H-Leucine incorporation) supports also the idea that bacteria rapidly responded to diel changes in organic matter release from phytoplankton (Hagström et al. 2001). Ruiz-González et al. (2011) observed that the weekend turbulence event affected little bacterial community structure and activity, except for *Gammaproteobacteria* uptake activity, which increased during

the second cycle and followed the same increasing patterns than those measured for the different picophytoplankton groups. Pinhassi et al. (Pinhassi et al. 2004) hypothesized that the shift of bacterial community structure occurring after a turbulence event was more likely driven by the shift suffered by phytoplankton community structure than by the physical stress alone.

It is known that DOM issued from egestion by grazing activity can represent up to 65% of DOM and most of bacterial C demand (Nagata et al. 2000). However, no night peaks of CTC+ or %HNA, nor a night increase of bacterial production (Ruiz-González et al. 2011) were observed during the second cycle, only the same trend of increase of both parameters with HNF abundance (only corroborated by a remarkably high but not significant correlation between CTC+ and %HNA with HNF abundance) strengthened the idea that bacteria were more likely using the dissolved organic matter released from grazing activity to support their growth rather than the excreted primary production.

Implications of the short time scale variability on patterns- Consequence of the shift in community structure between weeks likely to be provoked by the turbulence event between the diel cycles, the ratio between the two major contributors to picophytoplankton biomass (*Syn*:pPeuk) increased by two fold from one week to another, from 0.03 to 0.06, and something that points out the importance of transient meteorological events in structuring coastal planktonic communities (Guadayol et al. 2009) and that significant ecological events often results from episodic physical forcing operating at short timescales (Seymour et al. 2005).

Similarly, the variability of this ratio was also important at the daily scale since the same two-fold increase was measured during both the first and second cycle and was driven by the differences in the diel pattern amplitudes. Using microscopy to count the number of cells grazed per HNF, it appeared that the number of Picoeukaryotes grazed (mostly *Micromonas* at this period of the year, details not shown) increased all along the first cycle (Ruiz-González et al. 2011). A differential grazing pressure operating at the diel scale on Picoeukaryotes and *Synechococcus* would then likely contribute to the differential group contribution to total picoplankton biomass.

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Patterns in picoplankton community structure: multi-scale spatial and temporal variability in the NW Mediterranean Sea during late summer

Thomas Lefort, Clara Ruiz-González, Martí Galí, Ruben Sommaruga, Rafel Simó and Josep M. Gasol

03

ABSTRACT

The temporal and spatial variability of Picoplankton Community Structure (PCS) and heterotrophic activity were studied by flow cytometry and radioactive tracers during a cruise performed in the NW Mediterranean. Variability was measured at a short time scale in diel cycles performed in a coastal and an offshore station and was compared to the large time scale variability estimated from two years of survey at the Blanes Bay microbial Observatory (the coastal station). *Synechococcus* dominated numerically in coastal and surface waters and was the main contributor to picophytoplankton biomass in all stations, followed by Picoeukaryotes at the coastal and slope stations. The maximum *Prochlorococcus* contribution was constrained within oceanic well-stratified situations. While Picoeukaryote cell numbers exhibited the highest spatio-temporal variability, the lowest was found for bacterial abundance. When we compared the different sources of variability, we found that the largest one observed was at the spatial scale, vertically promoted by water column stratification, and horizontally by the differences in trophy between stations. Coastal stations presented high bacterial abundance and activity but low spatio-temporal variability. On the contrary, offshore waters presented lower bacterial abundances and activities but higher spatio-temporal variability. Finally, opposite patterns between the *Synechococcus* to Picoeukaryotes biomass ratio and chlorophyll *a* levels were observed not only spatially, but also at both the short-term and large temporal scale, representing a possible good candidate variable to act as ecological indicator of the trophic state.

INTRODUCTION

The picoplanktonic (< 2-3 μm) community in aquatic environments is formed by the heterotrophic bacteria and Archaea, and the picophytoplankton, in turn composed by *Synechococcus* (Waterbury et al. 1979), *Prochlorococcus* (Chisholm et al. 1988) and Picoeukaryotes (Johnson and Sieburth 1982). The large-scale temporal and spatial distribution patterns of these groups have a strong seasonal component (Campbell et al. 1997; Jacquet et al. 1998; Li 1998; Grégori et al. 2001; Li and Dickie 2001) and their relative contribution to picoplankton community structure varies not only with ecosystem trophic level (Zhang et al. 2008), but also with temperature and with stratification of the water column (Bouman et al. 2011). While *Synechococcus* have been shown to dominate in nutrient-rich and coastal environments (Partensky et al. 1999), *Prochlorococcus* often dominate numerically in warm, more oligotrophic and well-stratified waters and generally extend much deeper than *Synechococcus* (Partensky et al. 1999). In comparison with photosynthetic prokaryotes, eukaryotic picophytoplankton (pPeuk) are relatively less abundant, but can dominate in terms of biomass in a variety of ecosystem (Li et al. 1992; 1993; Worden et al. 2004). Different controlling factors are underneath these distribution patterns, and macro-ecological studies have shown that not only temperature, but also nitrate and chlorophyll *a* concentration contribute up to 66% of the variance in picophytoplankton abundance (Li 2007). Heterotrophic bacteria are also known to increase following chlorophyll *a* and temperature at **large scales** (e.g. (Li et al. 2004), but not necessarily in a given study area (Li 2009). The fact that consistent distribution patterns of heterotrophic bacterial abundance and bacterial activity have been identified across a range of trophic levels (as estimated from chlorophyll *a* concentration) (Cole et al. 1988; Billen et al. 1990 Ducklow and Carlson 1992; Bird and Kalff 1984), has given support to the idea that bacteria use mainly the dissolved organic matter produced by phytoplankton and grazers to support their heterotrophic activities (Nagata 2000; Moran et al. 2002) .

In comparison, the factors contributing to the variability in PCS and heterotrophic activity at **shorter spatio-temporal scales** have been less studied. Picoplanktonic group abundance has been shown to fluctuate drastically over short distances in the Celtic Sea (<1 km), indicating that the magnitude of variation in the PCS patterns at the short spatial scale might be greatly underestimated in comparison to larger spatio-temporal patterns (Martin et al. 2005). Similarly the relevance of diel variability is commonly disregarded as compared to monthly or annual variability. However, several studies have shown that *Synechococcus*, *Prochlorococcus* and Picoeukaryotes abundances follow daily variations, with diel oscillations of their pigment content (e.g. Jacquet et al. 1998; Jacquet et al. 2002; Lefort et al. submitted; Vaulot et Marie, 1999) and generally associated to a synchronized pattern of cell division, but this short-term variability has still not been compared to the variability at other scales. Similarly, bacterial activity can vary over short periods, likely

due, for example, to variations in phytoplankton extracellular release of DOC (Gasol et al. 1998; Ruiz-González et al. 2012; Lefort et al. Chapter II). Light drives these variations resulting from the balance between growth rates (linked to light, nutrient availability and nutrient quality) and mortality rates (linked to grazer, viral activity or physical stresses such as UV radiation). Short time-scale variations in bacterial activity have been observed to be at least as large as those created by the seasonal variations (Ruiz-González et al. 2012).

The links between the spatio-temporal shifts occurring in both PCS and heterotrophic activities and the shifts in structure and function of whole ecosystems are unclear. If some ecological parameters (such as abundance or activity of one or all picoplanktonic groups) can be used to estimate the changes occurring in ecosystem conditions (Karr 1991; Beaugrand 2005) then these microbial community structure or activity variables might capture the complexity of the ecosystem (Paerl et al. 2003), and be useful as indicators, one of the conditions being that they must be simple enough to be routinely and easily measured (Dale and Beyeler 2001), something that picoplankton abundances and activities are.

We describe here picoplankton group distribution and heterotrophic production across different spatio-temporal scales with the aim of i) identifying patterns in picoplankton group distribution and activity at each spatio-temporal scale (including the short time scales), ii) quantify and compare variability at each scale, and iii) to determine the links between the variability and different environmental and ecological factors, such as stratification and trophic level (as estimated from chlorophyll *a* concentration).

To address these issues, we followed the different group abundances (also including viruses) and bacterial activity using flow cytometry and incorporation of radioactive tracers, during a cruise performed in the North Western Mediterranean Sea in September 2007 (mainly) and in some additional samplings. We measured for each variable the coefficients of variation as estimators of parameter variability and we compared the values at different spatio-temporal scales: the temporal scale was divided into 2: the **diel scale** (with two diel cycles performed at a coastal station and one at an open-ocean station) and a **larger temporal scale** (seasonal year-round variations in the same parameters). The **spatial** scale was analyzed during the transect across 5 stations from coastal to deep ocean sites, comparing the **vertical** profiles with the **horizontal** distribution of the different picoplankton groups.

MATERIAL AND METHODS

Sampling - Diel cycles- Two diel cycles were sampled in September 2007 from onboard the oceanographic vessel "García del Cid", during the cruise "MODIVUS" in the NW Mediterranean Sea (Figure 1). The first one was performed during 56 hours from the 18th to the 20th September 2007 at a coastal station (the Blanes Bay Microbial Observatory, Station C), a shallow (20 m depth) oligotrophic coastal site, located 800 m offshore of Blanes (41º39.90'N, 2º48.03E). The second diel cycle was done at an offshore Station D (40º39´4.7"N, 2º51´1.6" E), in the deepest point of the Catalan Sea. Its duration was shortened to 44 hours from the 23rd to the 25th February 2007, because of sea conditions. The samples were collected at a frequency of 6 per day (every 4 hours) with Niskin bottles mounted on a rosette with a CTD. While 2 different depths were sampled at station C (at surface: 5 m and 15 m), 4 different depths were sampled at station D (5 m, 25 m, 48 m, 65 m). We also include the data from two other diel cycle studies performed in winter at Station C (20-22 February 2007 and 26 February to 1st of March 2007) with the same sampling frequency (published in Lefort & Gasol, submitted).

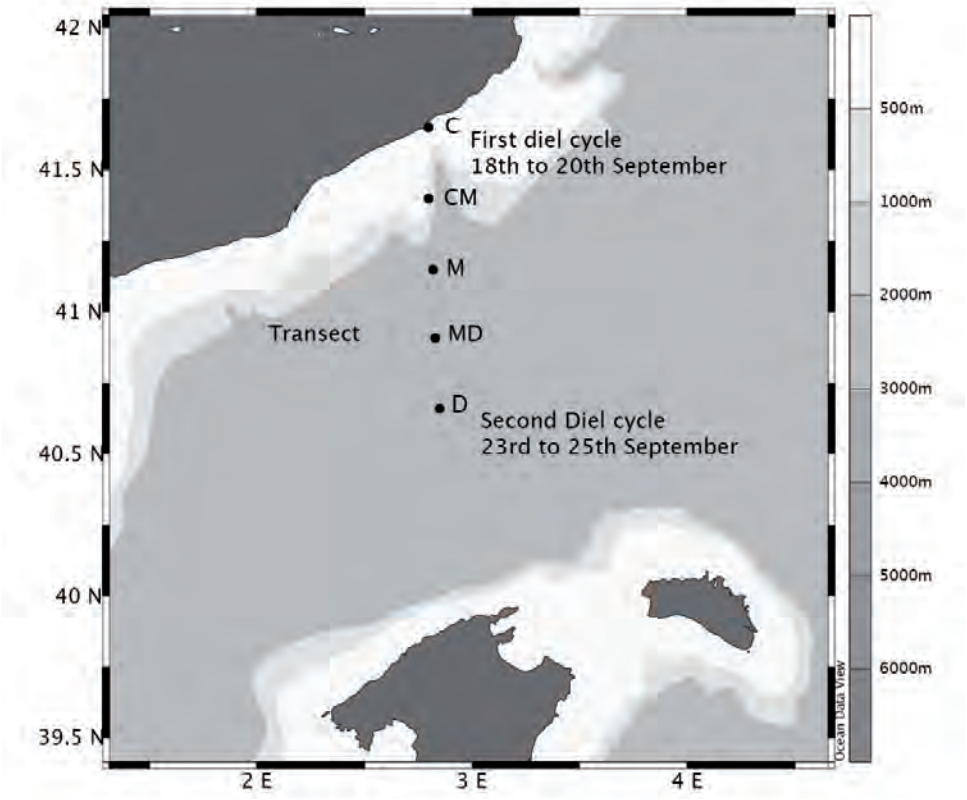


Figure 1. Bathymetric Map of the different sampling sites during oceanographic cruise MODIVUS, Blanes Bay coastal station C was the first station of the transect, D was the last one. Note the Blanes submarine canyon close to station CM. The diel cycles were performed at stations C and D.

Spatial study- Between the two summer diel cycles, a coast to offshore transect was sampled in September 2007, and five vertical profiles taken at stations C, CM, M, MD, D (Figure 1). All the samples were kept permanently in the dark until analysis, which was done onboard.

General samples- Temperature and salinity were obtained with a SAIV A/S 204 CTD probe, except in the spatial and diel cycle studies of September 2007 in which a CTD SBE 9plus was used. Chlorophyll *a* concentration was determined from 150 mL of seawater filtered through GF/F filters (Whatman) extracted in acetone (90% v/v), and fluorescence measured with a Turner Design fluorometer.

Sampling - Seasonal survey at the Blanes Bay microbial observatory (BBMO)- To compare the diel scale with the long time scale variability, we followed picoplankton community structure and heterotrophic activity at the Blanes station C (BBMO) from January 2007 to November 2009. The samples were taken monthly, collected with polycarbonate carboys and processed by flow cytometry at the ICM facilities, 2 h after sampling. The samples were always kept at dark until analysis.

Picoplankton and virus abundances- Algal and bacterial abundance were determined using flow cytometry (Marie and Partensky 2006; Gasol and del Giorgio 2000). Three populations from the photosynthetic fraction of the picoplankton (<3 μm) (*Prochlorococcus, Synechococcus,* Picoeukaryotes) were discriminated according to scatter and fluorescence signals, for which the samples were run at high speed (at ca. 100 μl min^{-1}) without additional fixative. For non-phototrophic bacteria, abundance was estimated following two different methods, a first one with the use of fixative and the second one with the NADS protocol in unfixed samples. Bacterial abundances during the long term survey at BBMO were measured taking 1.2 mL samples which were preserved with 1% paraformaldehyde + 0.5% glutaraldehyde (final conc.), and kept frozen at –80ºC until analysis by SybrGreen I (dilution x10,000) staining and flow cytometric analysis (Becton-Dickinson FACSCalibur flow cytometer). Bacteria were detected by their signature when plotting side scatter (SSC) versus green fluorescence (FL1) and FL1 vs. red fluorescence (FL3) (Gasol and del Giorgio 2000) and converted to abundances measuring the volume of sample before and after sample passage. Bacterial abundances during the oceanographic cruise were also performed with the NADS Viability protocol, based on the combination of the cell-permanent nucleic acid strain SybrGreen I (Molecular Probes, Eugene, OR) and the cell-impermeant propidium iodine PI (Sigma Chemical Co.) fluorescent probe. We used a 1:10 SG1 and 10 μg ml^{-1} PI concentrations. After simultaneous addition of each stain, the samples were incubated for 20 min in the dark at room temperature and then analyzed by flow cytometry. SG1 and PI fluorescence were detected in the green (FL1) and orange-red (FL3) cytometric channels, respectively. A dot plot of red versus

green fluorescence allowed distinction of the "live" cell's cluster (i.e., cells with intact membranes and DNA present) from the "dead" cell one (i.e., with compromised membranes) (Grégori et al. 2001, Falcioni et al. 2008). Total cell abundances were the addition of the "live" and "dead" cells.

Viruses- Viral abundance was also determined by flow cytometry. Subsamples (2 ml) were fixed with glutaraldehyde (0.5% final concentration), quick frozen in liquid nitrogen and stored at -80°C as described by Marie et al. (1999). The samples were stained with SYBRGreen I, and run at a medium flow speed following standard protocols (Brussaard 2004).

Bacterial single-cell activity- We measured the abundance of highly respiring prokaryotes, i.e. those able to reduce 5-cyano-2,3-diotolyl tetrazolium chloride (CTC; Polysciences). CTC turns into a red fluorescent formazan that is detectable by epifluorescence and flow cytometry (Sherr et al. 1999a; Sieracki et al. 1999). Sample aliquots (0.4 ml) were amended with 5 mM CTC (from a fresh stock solution at 50 mM) immediately following collection and were incubated for 90 min in the dark at room temperature. CTC-positive (CTC^+) cells were enumerated by flow cytometry using the FL2-versus-FL3 dot plot (see Gasol and Arístegui 2007). For these analyses, we used a high speed (ca. 100 µl m^{-1}) and a threshold set in red fluorescence.

Bacterial heterotrophic production (BHP)- BHP was estimated every 4 hours from both, radioactive ^{3}H-leucine and ^{3}H-thymidine incorporation. For leucine we used the ^{3}H-leucine incorporation method described by Kirchman et al. (1985) adapted to microfuge tubes. Briefly, 4 aliquots (1.2 mL) and 2 TCA-killed controls were incubated with radiolabeled leucine (40 nmol L^{-1}, final conc., 160 Ci $mmol^{-1}$) for about 1.5 hours in the dark at in situ temperature. The incorporation was stopped by adding 120 µl of cold TCA 50% to the samples, which were stored at –20ºC until processing by the centrifugation method of Smith and Azam (1992). Bacterial production was also measured as ^{3}H-thymidine incorporation following Fuhrman and Azam (1980) also in microfuge tubes. Samples were incubated with 10 nmol L^{-1} ^{3}H-thymidine (final concentration) and processed like the ^{3}H-leucine samples.

Standard cell to carbon Conversion factors for biomass estimation- To translate cell abundance into biomass, different CF were chosen for *Prochlorococcus*, *Synechococcus* and Picoeukaryotes, with respectively 53 fgC $cell^{-1}$ and 250 fgC $cell^{-1}$ (Campbell et al. 1994) and 1540 fgC $cell^{-1}$ for pPeuk (Lefort et al. Chapter I).

Integration of the data- To compare *Synechococcus*, *Prochlorococcus* and Picoeukaryotes abundances, we calculated the integrated average at stations C and D. To estimate the diel variability, we followed the variations of the ratio of the depth-integrated average (every 4 hours) to the diel average. For the transect, the data were integrated over the photic zone for the picophytoplankton

(0-125 m) as well as for bacterial and virus abundance, CTC activity, and bacterial production. No depth integration was done with the long-term survey performed at BBMO since only one depth had been sampled (5 m).

Statistical analysis- To estimate the variability of each abundance or activity parameter, we calculated the respective coefficient of variations (CV) expressed for the diel scale as the standard deviation of the depth-integrated values divided by the diel averages, For the variability of the vertical profiles, CVs were expressed as the standard deviation of the depth-integrated values divided by the depth-integrated average of the station, for every station of the transect. For the horizontal variability, CVs were expressed as the standard deviation of the different depth integrated averages measured at each station, divided by the transect average. The CVs measured during the diel cycles were compared to the CVs measured during the long time-scale survey. To perform correlation analyses, we run Pearson correlations that summarized the strength of the linear relationships between each pair of response variables. All conducted in JMP 7 (SAS institute Inc).

RESULTS

To describe the patterns in picoplankton group distribution, we calculated for each parameter the depth integrated average at different spatio temporal scales in 5 stations from the coastal station C to open-ocean station D (C, CM, M, MD, D, Table 1A). We estimated the variability generated by the distributions, calculating the coefficients of variation (CV) for every picoplankton group as a proxy of the amplitude of variation around the integrated average (Table 1B).

Mesoscale variability of Picoplankton community structure (horizontal variability- Heterotrophic bacteria dominated numerically the picoplankton at all the stations of the transect and at all the scales of observation (Table 1A). Despite no strong differences in the distribution were measured, as evidenced by the low variability (average of only 27%, Table 1B), bacterial concentration was relatively higher at the coastal station C than at station D (Table 1A), with a maximum at the slope station CM of 8.6 x 10^5 cells.ml^{-1} (±10%) that followed the isopycnal 27 kg m^{-3} to surface waters of station MD. This density dome, which possibly indicates an upwelling event or a cyclonic vortice, frequent in this region (La Violette et al. 1990), was confirmed in satellite images (details not presented) and separated a patch of lower bacterial concentration at station D with 4.3 x 10^5 cells.ml^{-1} (±71%) (Figure 2D). Compared to the low variability found in the bacterial abundance parameter, much higher variability was measured in bacterial activity, ranging from 43% to 53% (close to two fold) either when estimated for the percent of actively respiring cells (%CTC) or for ^{3}H-Thymidine or ^{3}H-Leucine incorporation rate values.

Synechococcus followed similar spatial distribution patterns than heterotrophic bacteria as revealed by the strong correlation found between their abundances (Pearson tests, N=30, $p<0.005$, Suppl. Table 1C), but higher coefficients of variation were observed horizontally from coastal station C to offshore station D (Table 1B). *Synechococcus* dominated numerically the picophytoplankton community structure at coastal station C that was characterized by lower salinity and temperatures than the offshore station D (Figure 2A, Figure 2E). At this station, *Synechococcus* cell concentrations ranged from 6.8 x 10^4 to 8.0 x 10^4 cells.ml^{-1} at 15 and 28 meters depth respectively for a depth integrated average of 5.9 x 10^4 cells.ml^{-1} (Table 1A). Another maxima of *Synechococcus* abundance of 5.9 x 10^4 cells.ml^{-1} was observed at the surface in station MD, which corresponded also with the density dome (Figure 2A and 2E).

Prochlorococcus dominated numerically the photosynthetic fraction of the picoplankton in more offshore conditions with a depth integrated average of 7.1 x 10^4 cells.ml^{-1}, particularly at the DCM of Station D, with a cell concentration of 1.2 x 10^5 cells.ml^{-1} (Table 1A, Figure 2B).

	STATION C			STATION CM	STATION M	STATION MD	STATION D		ST C, CM, M, MD, D
DEPTH AVERAGED PARAMETER	DIEL CYCLE	LONG TERM SURVEY	VERTICAL PROFILE	VERTICAL PROFILE	VERTICAL PROFILE	VERTICAL PROFILE	VERTICAL PROFILE	DIEL CYCLE	TRANSECT
SYNECHOCOCCUS 10^4 cells. ml^{-1}	**5.94**	2.45	**6.23**	1.69	**3.06**	2.02	1.49	2.16	2.90
PROCHLOROCOCCUS 10^4 cells. ml^{-1}	2.48	**8.33**	2.21	**2.67**	2.79	**2.86**	**7.10**	**6.55**	**3.54**
PICOEUKARYOTES 10^3 cells. ml^{-1}	1.95	3.55	1.46	1.55	0.42	0.33	0.82	0.79	0.92
HET. BACTERIA 10^5 cells. ml^{-1}	**7.78**	**7.29**	**8.53**	**7.30**	**8.02**	**7.07**	**6.19**	**6.42**	**7.42**
CTC+ CELL ABUNDANCE 10^4 cells. ml^{-1}	5.67	---	6.29	7.76	4.57	2.59	3.13	3.80	4.87
BACTERIAL ACTIVITY pmol Tdr L^{-1} h^{-1}	10.91	NA	12.54	8.36	3.47	8.99	3.78	7.43	7.43
BACTERIAL ACTIVITY pmol Leu L^{-1} h^{-1}	41.10	49	69.15	35.83	26.96	21.41	27.29	18.42	36.13
VIRUS ABUNDANCE 10^6 ml^{-1}	8.33	N.A	6.60	6.43	5.82	4.29	6.22	5.81	5.87
SYN:PPEUK BIOMASS RATIO	6.17	2.34	6.94	1.77	11.80	9.89	2.93	4.58	8.89
CHLOROPHYLL A µg L^{-1}	0.17	0.48	0.14	0.35	0.22	0.17	0.21	0.26	0.22

A

Table 1. Depth averaged cell concentration (cells. ml^{-1}) (**A**) and variability (Coefficient of variation) (next page) (**B**) of the different picoplankton groups and activity indices considered. The averages were calculated from the integrated data over the photic zone. The coefficient of variation (CV) of each parameter was calculated dividing e.g. the standard deviation of the diel depth integrated value by the diel average. For the long-term survey at station C, Picoplankton group abundances at the Blanes bay station (5 m) are averaged from January 2007 to November 2009 over the whole period. NA: not available.

PARAMETER VARAIBILITY (±%CV)	STATION C			STATION CM	STATION M	STATION MD	STATION D		ST C, CM, M, MD, D	AVERAGE VARIABILITY
	DIEL CYCLE	LONG TERM SURVEY	VERTICAL PROFILE	VERTICAL PROFILE	VERTICAL PROFILE	VERTICAL PROFILE	VERTICAL PROFILE	DIEL CYCLE	TRANSECT	
SYNECHOCOCCUS	14%	97%	31%	61%	79%	137%	60%	16%	68%	63%
PROCHLOROCOCCUS	40%	114%	20%	140%	72%	61%	97%	18%	**57%**	69%
PICOEUKARYOTES	72%	92%	31%	158%	66%	82%	150%	18%	62%	81%
HET. BACTERIA	13%	27%	19%	30%	31%	46%	49%	13%	12%	27%
CTC+	23%	---	23%	33%	70%	82%	66%	33%	44%	47%
BACTERIAL ACTIVITY TDR	70%	---	66%	36%	62%	38%	42%	56%	51%	53%
BACTERIAL ACTIVITY Leu	46%	87%	38%	64%	38%	57%	32%	46%	53%	43%
VIRUS ABUNDANCE	76%	N.A	10%	57%	102%	57%	69%	28%	16%	52%
SYN:PPEUK BIOMASS RATIO	35%	125%	11%	106%	100%	125%	58%	17%	64%	71%
CHLOROPHYLL A	66%	77%	37%	101%	54%	47%	122%	85%	37%	70%
SCALE VARIABILITY AVERAGE	46%	88%	29%	79%	67%	73%	75%	33%	46%	

B

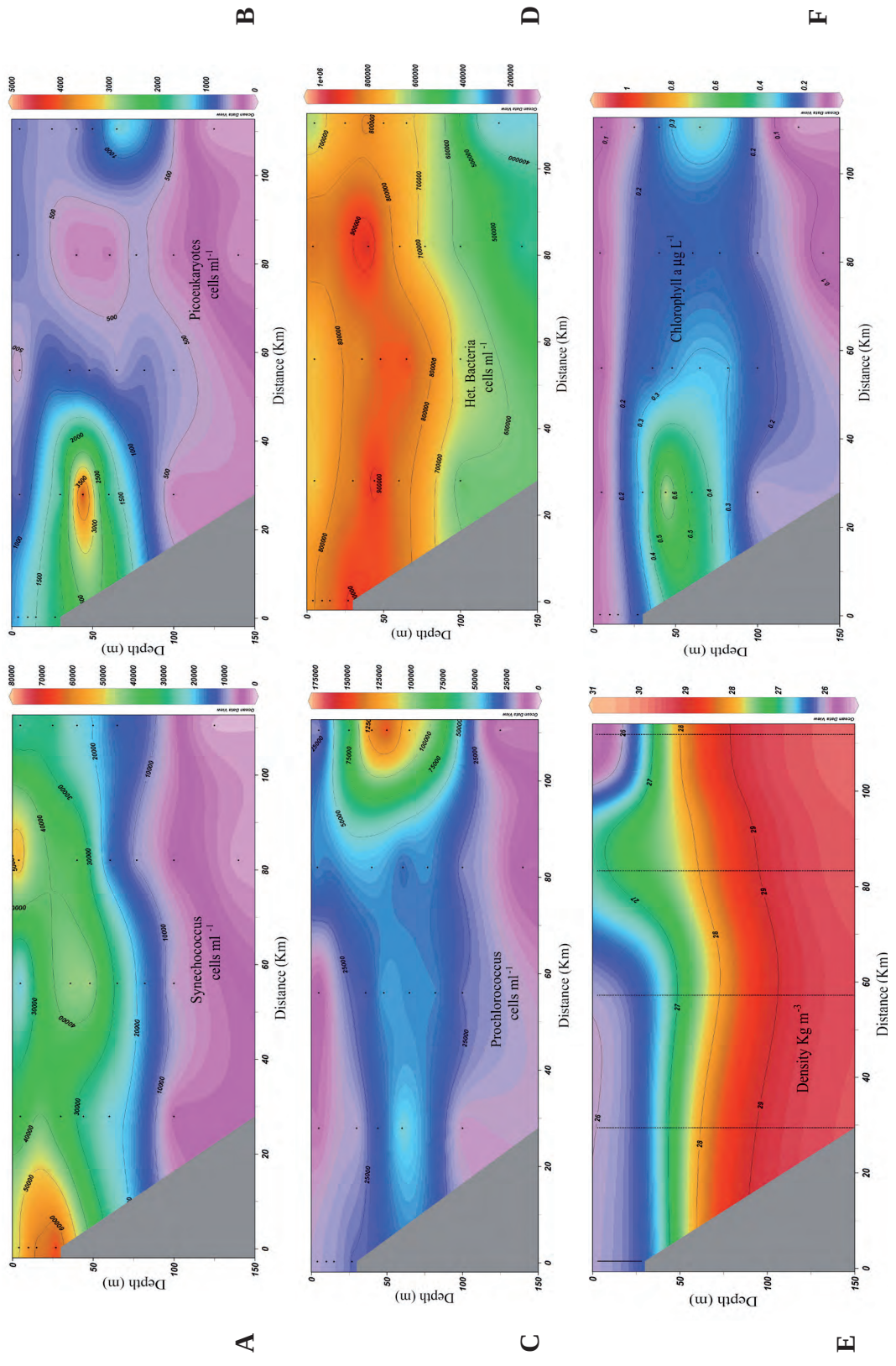


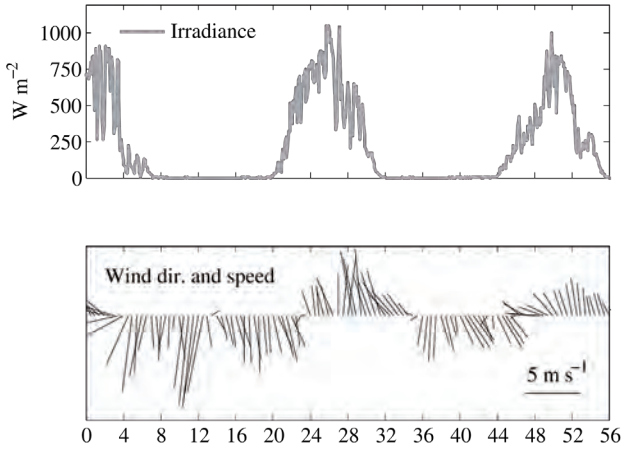
Figure 2. Picophytoplankton group abundance during the Transect from station C to station D, *Synechococcus* cell concentration (cells ml^{-1}) (**A**), *Prochlorococcus* cell concentration (cells. ml^{-1}) (**B**), Picoeukaryotes cell concentration (cells. ml^{-1}) (**C**), Heterotrophic bacterial abundance (cells ml^{-1}) (**D**), In situ water density (kg m^{-3}) (**E**) and chlorophyll *a* concentration (µg. l^{-1}) (**F**). Only the 150 first meters are shown.

On the contrary, the lowest *Prochlorococcus* abundances were measured at the surface layers of stations CM and M (Figure 2B).

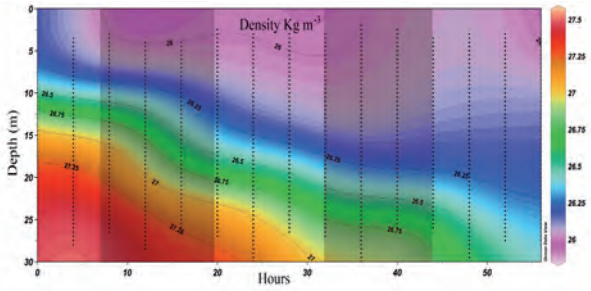
Contrasting with the low variability found with the distribution of heterotrophic bacterial abundance, strong differences in Picoeukaryotes abundance were measured across the different stations of the transect, generating high variability that reached an average CV of 81% across the different scales of analysis (Table 1B). Two pronounced maxima in Picoeukaryotes abundance were found at the two different peaks of chlorophyll *a* concentration: a first one at 44 m of the slope station CM (slope of the continental shelf) with 7.8 10^3 cells.ml^{-1}, and a second at 65 m of the most offshore oceanic influenced station D (Table 1A, Figure 2C, Figure 2F). On the contrary, the lowest pPeuk cell abundances were measured at the surface waters of more offshore stations M, MD and D (Table 1A).

Vertical variability of picoplankton community structure- We measured the vertical variations of the different picoplankton group abundances in each station of the transect (Table 1B). The highest source of variability was measured vertically with an average of 64% among the different stations and appeared enhanced by stratification of the water column, since the lowest vertical variability was measured at the well-mixed station C (Table 1B). On the contrary, well-stratified stations such as CM at the slope presented the highest variability, with 158% of variation for Picoeukaryote abundance, 140% and 61% for respectively *Prochlorococcus* and *Synechococcus* abundances (Table 1B). At station MD, *Synechococcus* vertical variation reached 137%, consequence of the peak of concentration measured at the surface layer, resulting possibly from the mesoscale event reported above.

Diel variability in picoplankton community structure- We performed several diel cycles to estimate and compare the short-temporal variability in two different ecosystem types (in coastal conditions at station C and at offshore in station D). The short time scale variability was higher at coastal station C than at offshore station D with averages of 46% and 33% respectively (Table 1B), amplified by a wind burst and a shift in wind direction from W to N that occurred concomitantly with a deepening of the water mass at the beginning of the first diel cycle performed at station C, as shown by the progressive decrease of the in situ density and the 26.5 kg m^{-3} isopycnal (Figure 3A, 3B), At the coastal station C, the highest diel variability was measured for viruses and Picoeukaryotes abundance at 76% and 72% respectively; the lowest was measured for bacteria at 13% and *Synechococcus* at 14% (Table 1B). To precise whether the different picoplankton group abundances and activity parameters were following diel patterns, we measured for each parameter the ratio to its diel average, dividing each depth-integrated parameter (measured every 4 hours) by the diel average (covering 56 hours and 44 hours at station C and D respectively) and tested for



A, B



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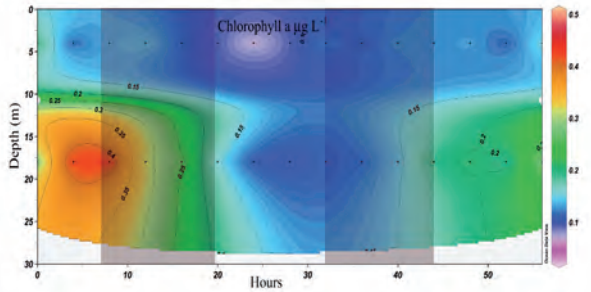
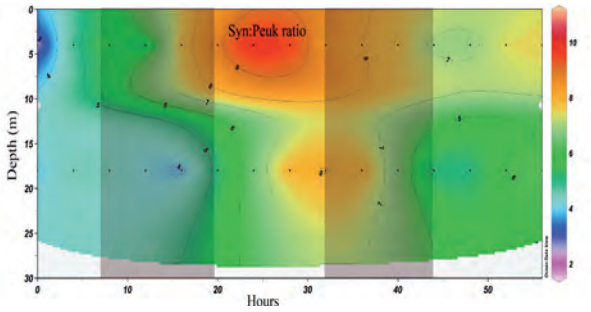


D



E

Figure 3. Irradiance levels (W m^{-2}) measured during the diel cycle at station C (**A**). Wind speed and direction during the diel cycle at station C (**B**). In situ density (kg m^{-3}) measured during the diel cycle at station C and chlorophyll *a* concentration (µg. l^{-1}) (**D**) and ratio of *Synechococcus* to Picoeukaryotes biomass (**E**). Time starts from 0 and corresponded to 12:00 at station C the 18th of September 2007. Rectangular shadows correspond to dark periods of the day. Chlorophyll *a* concentration in stations C and D was calculated from CTD fluorescence data.

significant differences between per day and per night ratio averages (t-tests, $p<0.05$).

In spite of the shift in water density at station C (Figure 3B), which rendered the patterns less obvious, *Synechococcus* and Picoeukaryotes abundances were significantly higher during the night that during the day (n=15, t tests, $p<0.05$). In general, depth integrated abundances decreased during the day and increased at night (reaching a maximum at midnight or a few hours later) (see suppl. Figure 1). Synchrony of the diel variations was observed for all the different parameter, including bacterial activity as estimated by ^{3}H-Thymidine or ^{3}H-Leucine incorporation rates, as shown by the significant positive correlations between heterotrophic activities and the different group abundances (Suppl. Table 1A). In comparison, neither significant differences were measured between the light and the dark periods for group abundances at station D nor any particular synchrony of the diel variations was observed in the different parameters measured (Suppl. Table 1B and 2).

Short versus large temporal variability of PCS patterns- To identify patterns in picoplankton community structure at both short and large temporal scales, we studied the concentrations of the main contributors to picophytoplankton biomass following the diel variations of the ratio of *Syn:*pPeuk biomass. We did it at station C (Figure 3E), since no significant diel patterns in the different parameters were found at station D. This was compared with 3 years of survey of the same area. The variability measured for all the parameters was always higher at the large temporal scale than at the diel scale, with particularly high values for the ratio of *Syn:*pPeuk biomass that reached 125% (Table 1B) at the seasonal scale. During the diel cycle at station C, the ratio of *Syn:*pPeuk biomass at station C ranged from 1.5 to 11, varying from 35% around a depth integrated average of 6.17 (Table 1A, 1B) that resulted mostly from the differences of diel variation amplitudes measured between Picoeukaryotes and *Synechococcus* abundances, Picoeukaryotes varying much more than *Synechococcus* abundance at station C (±72% compared to ±14%) (Table 1B). This ratio reached its maximum during the light period at the sea surface (4 m) during the second day of the diel cycle (t= 25 h), concomitant to a minimum of chlorophyll *a* concentration of 0.02 $\mu g\ l^{-1}$ (Figures 3D and 3E). On the contrary, the minimum ratio values corresponded to the maximum chlorophyll *a* concentration of 0.51 $\mu g\ l^{-1}$ at 18 m measured during the first hours of the diel cycle.

Scaling the variations of this ratio with those of chlorophyll a concentration over two years of observation at station C, we found strong seasonality revealed by opposite patterns between maximum and minimum ratio values and chlorophyll *a* concentrations (Figure 4). Indeed, the minimum values of the ratio were concomitant with maximum chlorophyll *a* concentration, indicating that the relative contribution of Picoeukaryotes to the picoplankton community

decreased relative to *Synechococcus* during the bloom periods (more particularly during spring).

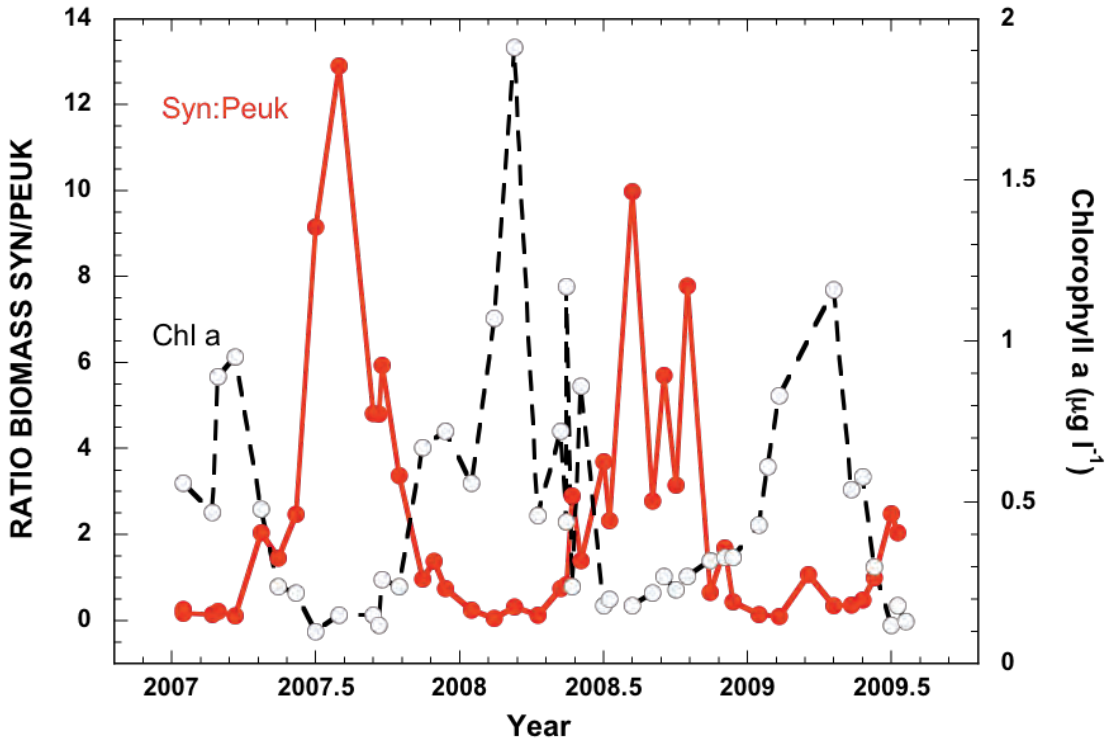


Figure 4. Variations of the ratio biomass of *Synechococcus* to biomass of Picoeukaryotes and chlorophyll *a* concentration during 2 years of observation at station C (From January 2007 to November 2009).

DISCUSSION

Picoplankton group variability- Patterns in picoplankton community structure (understood as the differential contribution of each organism type to the whole community) result from the structuring effect of interconnected physical and biological processes, which generally occur simultaneously but at different scales (Ducklow 1984; Dickey 1991). These interconnected factors participate greatly in the distribution of the different picoplankton groups, and are relatively well described for *Synechococcus* and *Prochlorococcus* (Partensky et al. 1999) but less well known for pPeuk and heterotrophic bacteria.

Our analysis indicates that each Picoplankton group varied differently across the different scales of analysis, and Picoeukaryote abundance showed the highest average variability and heterotrophic bacteria the lowest (Table 1B). Neither bacteria nor pPeuk communities (followed in this study by cytometry) constitute homogeneous groups but are both composed by several phylogenetic groups that likely vary differentially. While we followed the pPeuk at the bulk level by flow cytometry, without information about phylogenetic composition and relative group contribution to community structure, it is likely that the high variability measured in pPeuk bulk abundance resulted from the shifts occurring in community structure across the different stations of the transect. Several studies have shown that pPeuk community structure vary according to oceanic region, nutrient characteristics of the water masses (coastal or open-ocean, eutrophic or oligotrophic) and time of the year (see review by Worden and Not 2008). For instance, Prasinophyceae (Archaeplastida) typically dominate pPeuk communities in coastal waters (Not et al. 2004), while Prymnesiophyceae (Haptophyta), and to a lesser extent Chrysophyceae and Pelagophyceae (Heterokontophyta), contribute more to pPeuk communities in more open-ocean ecosystems(Mackey et al. 2002; Fuller et al. 2006; Liu et al. 2009).

In contrast, the relatively lower bacterial abundance variability, an average of 27% (Table 1B), suggests that bacterial community structure was spatially and temporarily more stable. Comparative studies of picoplankton community structure in different ecosystems have shown that spatial variability in the bacterial heterotrophic biomass is less strong than in autotrophic picoplankton (Zhang et al. 2008), Massana et al. (2004) also noticed such discrepancy between community variability and observed a high temporal variability of pPeuk assemblages compared to the small seasonal changes that tend to occur in bacterioplankton not only in the same site that had been studied by fingerprinting by Schauer et al. (2003) but also at the SOLA station (Banyuls-sur-mer, France), a site relatively close from the Blanes Bay station (Ghiglione et al. 2005).

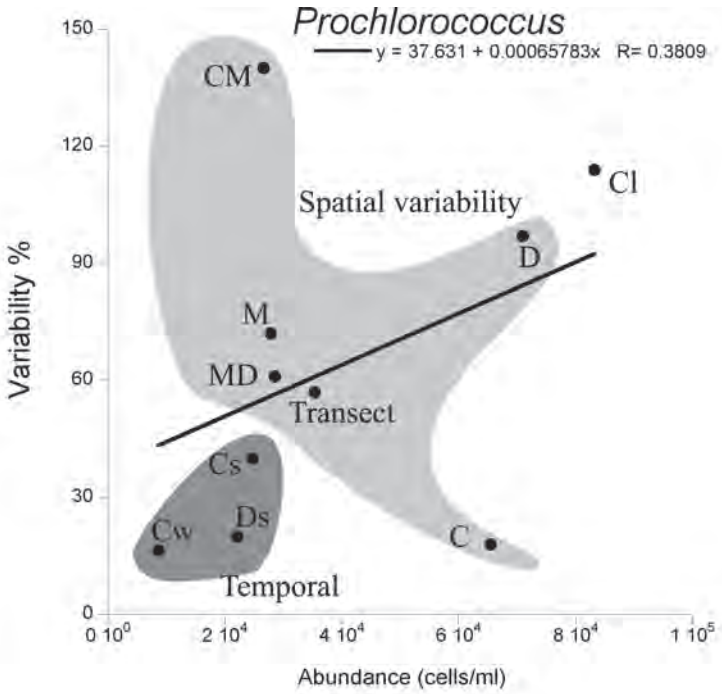
However, a constant overall bacterial concentration or stability of phylogenetic group

contribution to community structure does not imply necessarily the stability at the OTU level. Indeed, while almost similar bacterial community structures were measured by FISH among the different stations of this same transect (Crespo et al. unpublished), dominated by SAR11 and *Bacteroidetes* in the coastal conditions and by SAR11 and *Gammaproteobacteria* in open-ocean conditions), strong differences in bacterial OTUs composition (as estimated by pyrosequencing) was found from a station to another by Pommier et al. (2010). Several studies have suggested that the limits of bulk bacterial and bacterial group concentration are set by ecological parameters such as grazing and trophic level (Gasol and Duarte 2000; Li et al. 2004, chapter IV). If we consider the possibility that the high average variability found for pPeuk abundance resulted from more pronounced grazing pressure or competition with other algae for nutrients, then the stability of the overall bacterial concentration more likely indicated resilience of the bacterial community as a whole.

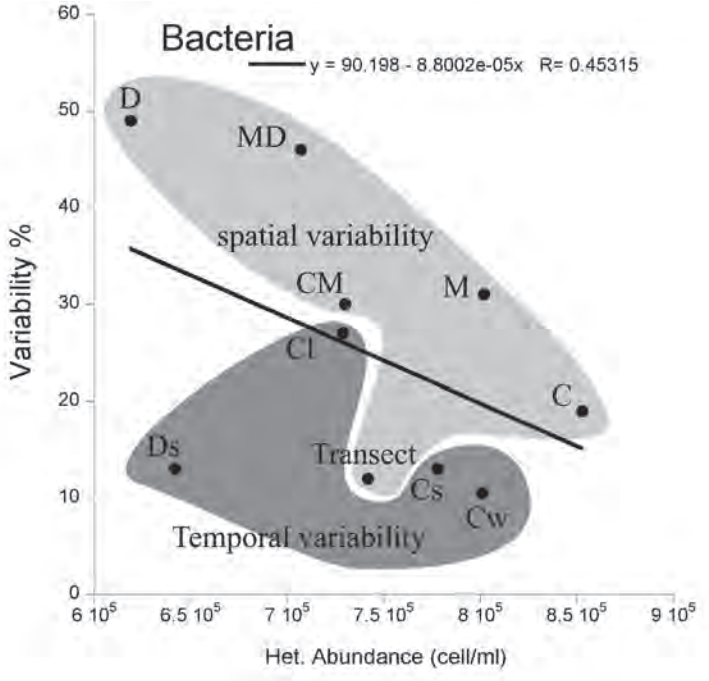
Ecosystem variability- To determine whether the variability observed was related to the type of ecosystem (Coastal station C and more Offshore influenced stations), we plotted for each picoplankton group their coefficient of variability (Table 1B) against their corresponding depth-integrated average concentrations measured at the different spatio-temporal scales (Table 1A, Figure 5). In this representation, as the Y and X scales share a common term (i.e. the CV are the standard deviation divided by the depth-integrated average), the relationships described should be taken as indicative and no statistical prediction is possible.

However, it is interesting to observe that *Prochlorococcus* and heterotrophic bacteria followed two opposite patterns. While the variability of *Prochlorococcus* increased significantly with increasing cell abundance, the variability of bacterial abundance decreased with increasing bacterial cell densities (Figure 5A and 5B). Such opposite patterns indicate that the maximum *Prochlorococcus* concentration occurred at specific and narrower periods of the day or of the year and also at narrower spatial locations (at strongly stratified spots). The opposite pattern was observed for bacteria since a lower bacterial variability was in general measured at coastal station C and was associated with high cell densities. On the contrary, high variability was measured in offshore stations, and was associated with lower bacterial cell densities.

This relative stability of the bacterial concentration at high cell densities in coastal ecosystems could indicate that shifts in bacterial community structure in coastal conditions, generated by the decrease of the abundance of a particular bacterial phylogenetic group, would be concomitantly balanced by the increase of abundance of another group. Moreover, the decrease of bacterial abundance variability from coastal station C to offshore station D was concordant with the parallel decrease of bacterial richness and evenness as estimated by pyrosequencing by Pommier

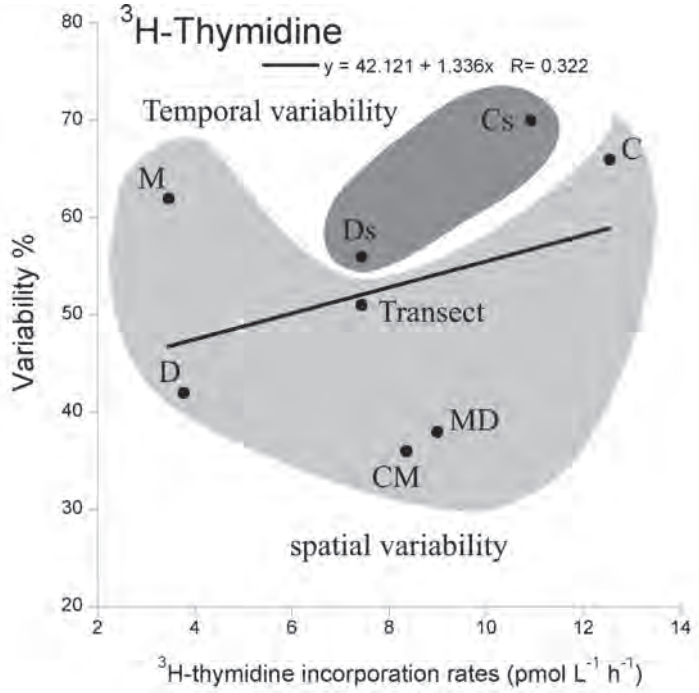


5A

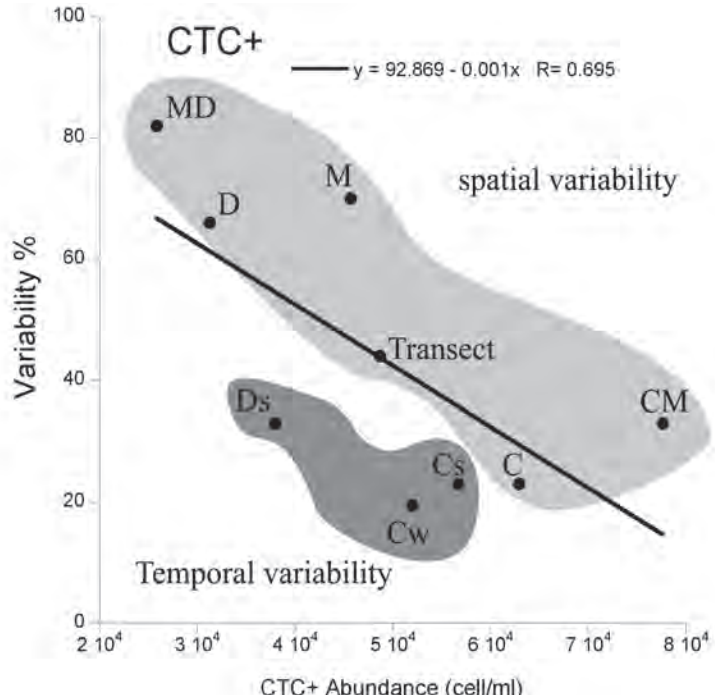


5B

Figure 5. Relationships between the spatio-temporal variability of the Picophytoplankton groups and bacterial activity with abundances or bacterial activity level. *Prochlorococcus* (**A**), Heterotrophic bacteria (**B**), ^{3}H-thymidine incorporation rates (**C**), CTC cell abundance (**D**).



5C



5D

C, CM, M, MD, D for the different vertical averages and variability. "**Transect**" for the horizontal average and variability, "**Cl"** for the long-term average and variability at station C, "**Cw"** and "**Cs"** for the diel cycle averages and variability during winter 2007 and summer 2007 at station C, "**Ds"** for the diel cycle performed the summer 2007 at station D. "**Cw"** values were taken from (Lefort et al. Chapter II).

et al. (2010) from coastal to offshore during the same transect. This would suggest that the spatio-temporal variability of bacterial community structure was enhanced at low bacterial richness and evenness, and on the contrary, that the high number of different bacterial OTUs composing the heterotrophic bacterial community at station C offered the optimum conditions for ecological homeostasis, in agreement with the idea that stable ecosystems tend to have higher diversity as suggested by Pommier et al. (2010). It is important to note that bacterial cell concentration was integrated only for the photic zone, the low bacterial concentration in deeper layer was not taken into account but would have considerably increased the spatial vertical variability, resulting in the increase of the strength of the positive relationship found between variability and cell densities, (until a Pearson's R= 0.92, details not shown).

Vertical sources of variability- The vertical scale was the major source of variability for all the groups, likely enhanced by the stratification of the water column. While *Synechococcus* dominated more in coastal and surface waters, *Prochlorococcus* dominated numerically in offshore well-stratified waters, particularly at the DCM. Temperature and water column stability have been shown to participate greatly in the spatial and temporal patterns of the overall size structure of phytoplankton communities (Li 2002; Bouman et al. 2003). Opposite patterns have been shown between the *Prochlorococcus* preference for well stratified nutrient depleted waters and the *Synechococcus* numerical dominance during periods of vertical mixing in a variety of ecosystems such as the subtropical waters (Campbell et al. 1997; Bouman et al. 2011a); or in the Western North Atlantic (Zinser et al. 2007). The relatively high surface area to volume ratio typical of *Prochlorococcus* in comparison to the other picophytoplankton groups has been suggested to indicate an ecological advantage in oligotrophic conditions (Raven et al. 2005; Partensky and Garczarek 2010). However, stratification not only influences the supply of nutrient from deep waters, but also regulates the light environment of cells within the mixed layer. Thus, it is possible that the spatial variability measured in *Synechococcus* and *Prochlorococcus* distributions resulted from either different capacities to resist high irradiance exposure and rapid changes in light Conditions (Six et al. 2007) or the possible photo-inhibition of some of these groups (Vaulot and Marie 1999; Sommaruga et al. 2005; Llabrés and Agustí 2006; 2010).

Several studies have highlighted the particular aptitude of Picoeukaryotes for growing in physically dynamic environments when compared with cyanobacteria (Lindell and Post 1995; Campbell et al. 1998; Steinberg et al. 2001). During the transect, Picoeukaryotes appeared to contribute more at the DCM of the slope station CM where the concentration of NO_3 was particularly high (data not shown), and was coherent with the findings of Bouman et al. (2011) that showed that the abundance of picoeukaryotes increased in deeply mixed and weakly stratified waters, with moderate to high concentrations of inorganic nitrogen.

Importance of the short spatio-temporal scale- Physical processes, including wind-induced mixing (Figure 3B) impacted greatly water column stratification and induced short spatio-temporal variability of picoplankton group distribution. At the short temporal scale, wind greatly altered the regularity of the variations at coastal station C, increasing particularly the measured variability of Picoeukaryotes and *Prochlorococcus* abundances, with CVs of ±72% and ±40% respectively (Table 1B) compared to only 18% at offshore station D. Taylor and Howes (1994) suggested that events of major ecological significance are likely to result from episodic environmental perturbations, more particularly if we consider that the time frames of relevance to the life history of marine organisms are relatively short (Seymour et al. 2005). We observed also (**Chapter II**) at station C during winter 2007 one of such disturbances at the short time scale during a wind event, with picoplankton community structure that varied after possible resuspension of bottom sediments and decrease of light availability. Wind has also been reported as a principal factor of environmental heterogeneity in a deep Polynesian atoll lagoon (Thomas et al. 2010), and is likely to affect other parameters of the water mass such as temperature, positively linked with *Synechococcus* abundance (Waterbury et al. 1986; Chang et al. 1996; Tsai et al. 2005). However, it is also possible that different water masses were sampled over time during the diel cycles, generating strong variability at the short spatial scale and representing a potential source of errors for the interpretation of eulerian time-series as suggested by Martin et al. (2005) and shown in their analysis of a transect in the Celtic Sea.

Heterotrophic bacterial abundance and activity variability- The variability of the different estimates of heterotrophic activity observed at the different spatio-temporal scales (Table 1B) was 1.60 to 1.96 fold higher than the variability measured in bacterial abundance, these differences being more particularly pronounced at coastal station C than at offshore station D. Several studies have shown similar discrepancies in the variability of activity as compared to that of abundance or biomass (Cole et al. 1988; Ducklow 2000; Sherr et al. 2001), indicating that in situ bacterioplankton assemblages can undergo relatively rapid shift-up or shifts-down in metabolism depending on local environmental conditions (del Giorgio and Cole 2000). At large spatio-temporal scales, comparative analyses revealed that bacterial production increased faster than bacterial abundance (Gasol and Duarte 2000) and that the strength of this link increased in more productive environments, suggesting that bacteria use algal-derived carbon more efficiently in eutrophic waters, since the maintenance energy costs might appear to be highest in oligotrophic systems (del Giorgio and Cole 1998).

The discrepancies measured in our study at the different scales between the variability of bacterial abundance versus the variability found in bacterial activity indicate that different controlling factors occurred across the different stations. Gasol et al. (2002) showed that while

bacterial abundance and growth in the most oligotrophic environments are likely regulated by top down control (i.e. protists grazing), such control in more productive waters is more likely through changes in bacterial phylogeny, size and activity community composition. In this study, the lowest spatio-temporal variability in bacterial abundance and highest spatio-temporal variability in bacterial activity were both measured in coastal station C, while the opposite pattern was measured in offshore station D. This indicated that contrarily to bacterial activity, bacterial concentration was less influenced by top down control in the coastal station than in more oligotrophic waters.

Moreover, opposites trends of variability were found between bacterial production and CTC+ cell number at the diel scale at both station C and D (Figure 5C and 5D), and vertically at station CM (Table 1B). Such discrepancies in the magnitude of variations of the different bacterial activities suggest that different bacterial metabolic processes are targeted by each method. Several studies have shown that heterotrophic bacterial activity follows diel patterns (i.e. Gasol et al. 1998; Ruiz-González et al. 2011). Contrasting with Fouilland and Mostajir (2010; 2011) that argued that bacteria do not depend on primary production in oligotrophic waters but from other sources of carbon at the relatively short time scale (days and week), the relatively large variability measured at the diel scale in ^{3}H-thymidine and 3H-leucine incorporation rates at both coastal station C and offshore station D indicates that some of the bacterial populations in a community can rapidly respond to enhanced substrate availability on a time scale of hours by increasing rates of cell-specific activity and growth (Sherr et al. 1999; del Giorgio and Cole 2000), in agreement with the comment by Morán and Alonso-Sáez (2011) stating that bacterial metabolism is strongly dependent on the local primary production and the organic matter released in situ by primary producers as previously formulated by Baines and Pace (1991). Since several studies have shown that the CTC method targets the cells with the highest respiration rates (Sherr et al. 1999b; Sieracki et al. 1999; Smith and del Giorgio 2003), the discrepancies measured at the diel scale between the low variability in CTC+ cell number and the high variability of bacterial production would indicate that production processes vary more rapidly than bacterial respiration processes, again a pattern that seems logical: maintenance processes being more stable than growth.

Ecological indicators of shifts in ecosystem picoplankton structure- Opposite temporal and spatial patterns were found between the ratio of *Synechococcus* to pPeuk biomass and chlorophyll *a* concentration levels, at both short and large temporal scales. While water-column stratification has been shown to be one of the main factors promoting shifts in phytoplankton community structure in coastal and temperate waters at the seasonal scale (Cushing 1989), in picoplankton community structure in subtropical waters (Bouman et al. 2011), Zwirglmaier et al. (2007) showed that *Synechococcus* generally presented no obvious depth preference, but did show highly specific distribution at the horizontal scale. Early considered as possible biological marker

of shifts in water mass properties, *Synechococcus* abundance indicated advection of a warm matter mass in Polar Regions (Gradinger and Lenz 1989) and high ratio values of cyanobacteria to eukaryotes abundances were used to characterize saline intrusions of subtropical origin (Jochem and Zeitzschel 1993). Despite associations between picoplankton community structure and water mass properties have been since established in large spatial scale surveys (Li 1995; Zubkov et al. 2000; Li and Harrison 2001; Tarran et al. 2001), little is still known about Picoeukaryotes distribution and their link with water mass characteristics. Looking at the contribution of each group to picophytoplankton community (considered here as composed by *Synechococcus*, *Prochlorococcus* and Picoeukaryotes) in terms of C biomass at each station (Figure 6), the major contributor to picophytoplankton biomass was *Synechococcus*, which ranged from 54% at the slope (station CM) to 89% at the station M. The second contributor to PCS were generally the pPeuk at both coastal and slope stations (C and CM) with 12% and 32% respectively, the second most important contributor in more oligotrophic stations (M, MD, D) was *Prochlorococcus*, that reached 26% of total PCS biomass at station D (Figure 6).

However, this overall domination by *Synechococcus* expressed by calculating an average ratio *Syn*:pPeuk of 8.89, varied at 64% horizontally (Table 1A and 1B). The ratio *Syn*:pPeuk

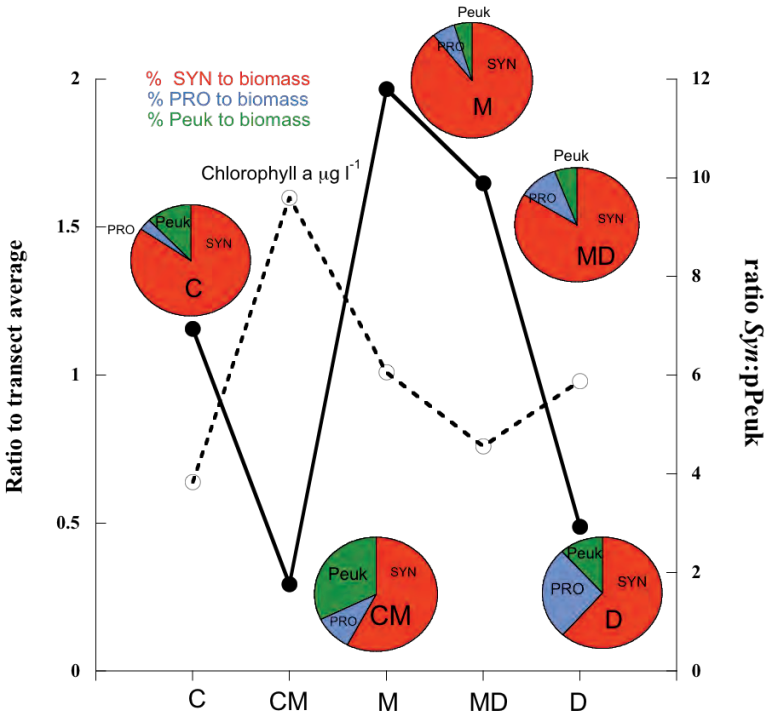


Figure 6. Ratio of chlorophyll *a* concentration to the transect average (depth integrated for the first 125 m) and ratio *Synechococcus*:Picoeukaryotes biomass with the percentage biomass contribution of each picophytoplankton group (*Synechococcus*, *Prochlorococcus* and Picoeukaryotes) in the pie charts.

decreased from 6.94 (±11%) at station C to a minimum of 1.77 (106%) at station CM (Table 1) where maximum chlorophyll *a* concentrations were measured and confirmed by the increase of the ratio of chlorophyll *a* to its transect average (Table 1A and Figure 6). These horizontal variations, driven by the differences between the ecosystem characteristics of coastal station C, slope station CM, or more offshore influenced stations highlighted opposite trends: while pPeuk contribution to biomass increased from coastal station C to the slope CM, *Synechococcus* contribution decreased (Table 1A). Similar to these results, Calvo-Díaz et al. (2004) showed during transects along the N and NW Iberian peninsula shelf that *Synechococcus* prevalence in picoplankton community structure was associated with low chlorophyll *a* levels whereas the total and relative abundance of pPeuk increased with phytoplankton biomass. Note that high values of the ratio *Syn*:pPeuk were also measured at the sea surface at station MD, a region that corresponded with the eddy-associated upwelling event (Figure 2E). Similar findings by Tarran et al. (2001) demonstrated that eddy waters contained higher contributions of pPeuk and heterotrophic bacteria.

Conclusions- The large variability measured in the different heterotrophic activities at the different spatio-temporal, particularly pronounced at the diel scale, as well as the synchrony of the variations with the picophytoplankton groups, indicate the tight coupling that can occur between bacteria and primary producers. On the contrary, the stability measured in bulk bacterial abundance was poorly indicative of the shifts that can occur at the group or the OTU level in changing environments, suggesting that further study of variability of the bacterial community structure at narrower phylogenetic level would be welcome. Similarly to chlorophyll *a* concentration, used in several comparative analysis as indicator of phytoplankton biomass or trophic level, the distribution of the different picoplankton groups, here indicated by the ratio of *Syn*:pPeuk throughout the transect, was not random but linked to the ecological characteristics of the water masses.

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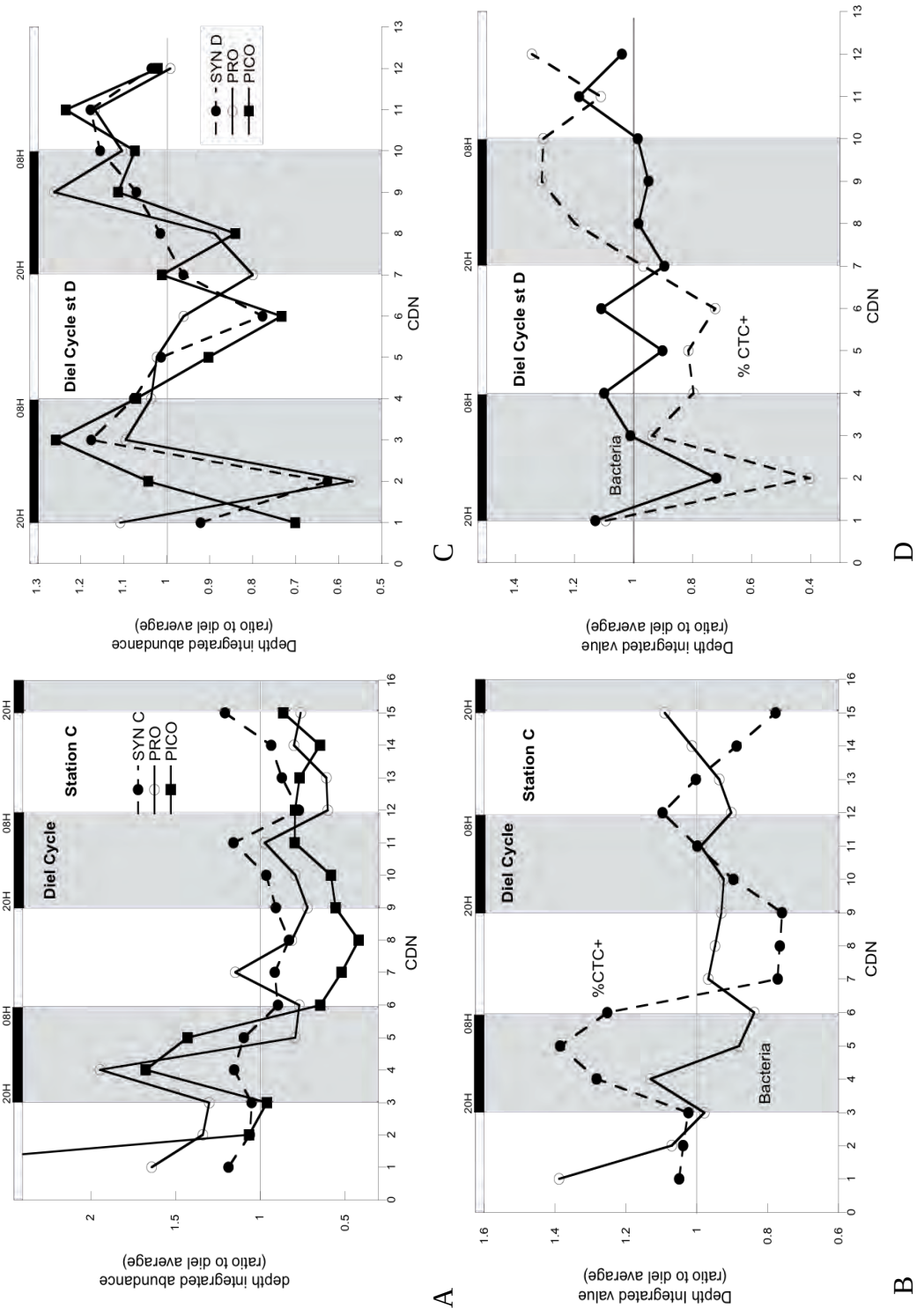


Figure 1. Ratio to the diel average (depth Integrated) at Station C and D of the picophytoplankton groups (**A** and **C**) and of bacterial abundance and percentage of CTC+ bacterial cells (**B** and **D**).

N=13 to 15

DIEL CYCLE ST C

	SYN	*PRO*	PPEUK	RATIO BIOMASS SYN : PPEUK	HET.BACTERIA	CTC+	BP TDR	BP LEU	VIRUSES	WIND SPEED M- S^{-1}	IRRADIANCE W m^{-2}
SYNECHOCOCCUS		**0.59***	**0.60***	-0.49	**0.64***	0.57*	**0.70***	**0.77****	-0.45	0.05	-0.32
PROCHLOROCOCCUS			**0.66***	**-0.55***	**0.71****	0.68*	**0.56***	0.40	-0.41	**0.51***	-0.02
PICOEUKARYOTES				**-0.84****	**0.83****	0.84**	**0.60***	0.44	-0.15	0.27	-0.13
RATIO BIOMASS SYN : PPEUK					**-0.56***	**-0.91****	-0.45	-0.22	-0.16	-0.28	0.07
HET. BACTERIA						0.56*	**-0.54***	**0.74***	-0.20	0.26	-0.18
CTC+							0.38	0.17	-0.05	0.26	-0.08
BP TDR								**0.89****	-0.37	0.21	**-0.65***
BP LEU									-0.42	-0.03	**-0.61***
VIRUSES										-0.26	0.23
WIND SPEED											-0.02
IRRADIANCE											

Table 1A

Table 1. Matrix of Bonferroni-corrected Pearson's correlation coefficients between the different parameters measured during the diel cycle performed at station C (**A**), at station D (**B**), and during the transect from station C to D (**C**). *Syn* For *Synechococcus* cell abundance (cells ml^{-1}), *Pro* for *Prochlorococus* abundance (cells ml^{-1}), and pPeuk for Picoeukaryote cell abundance (cells ml^{-1}). BP for Bacterial activity as measured by Thymidine incorporation (pmol TdR l^{-1} h^{-1}) and Leucine incorporation (pmol Leu l^{-1} h^{-1}). N for number of observations. One asterisk indicates significant correlations ($p< 0.05$) while two asterisks indicate probabilities that are below the Bonferroni-corrected probability equivalent to $p=0.05$ (which for the set of correlations is $p=0.003$ for **A**, $p=0.004$ for **B** and $p=0.001$ for **C**). v

Table 1B

DIEL CYCLE ST D

N=12	*Syn*	*Pro*	PPeuk	Ratio biomass Syn : PPeuk	Het.Bacteria	CTC+	BP Tdr	BP Leu	viruses	Wind speed m- s^{-1}	Irradiance W m^{-2}
Synechococcus	**0.76***	0.55	0.34	0.48	**0.66***	0.13	-0.18	**-0.60***	0.22	0.20
Prochlorococcus		0.21	0.51	**0.67***	**0.63***	-0.08	0.09	-0.15	0.07	0.22
Picoeukaryotes			**-0.59***	-0.09	0.22	0.52	-0.05	**-0.69***	0.27	-0.03
Ratio biomass Syn : PPeuk				**0.58***	0.41	-0.45	-0.01	0.19	-0.03	0.18
Het. Bacteria					0.54	-0.33	-0.14	0.28	0.31	0.14
CTC+						0.18	0.05	-0.39	0.45	-0.08
BP Tdr							**0.75***	**-0.62***	0.51	-0.50
BP Leu								-0.10	0.27	-0.49
N=12 Viruses									-0.36	0.08
Wind speed										-0.21

Table 1C

TRANSECT

N=28 to 47	*Syn*	*Pro*	PPeuk	Ratio biomass Syn : PPeuk	Het.Bacteria	CTC+	BP Tdr	BP Leu	virus
Synechococcus	0.09	0.17	**0.52***	**0.69****	0.18	**0.52***	**0.71****	**0.54***
Prochlorococcus		0.06	0.05	**0.37***	0	0	0.06	**0.65****
Picoeukaryotes			-0.2	**0.44***	0.16	0.29	**0.68****	0.46
Ratio biomass Syn : PPeuk				**0.52***	0	0	0.04	0.31
Het. Bacteria					0	**0.58****	**0.75****	**0.92****
%CTC+						0	-0.1	0
BP Tdr							**0.75****	**0.48****
BP Leu								**0.70****
Virus								

	Station C Day / Night n=15	station D Day / Night n=12
Synechococcus (cells ml^{-1})	+ (n.s / n.s)	n.s (+ / -)
Prochlorococcus (cells ml^{-1})	n.s (n.s / n.s)	n.s (+ / -)
Picoeukaryotes (cells ml^{-1})	+ (n.s / n.s	n.s (+ / n.s)
Synechococcus : PPeuk biomass	n.s (n.s / n.s)	n.s (n.s /-)
Het. Bacteria (cells ml^{-1})	n.s (n.s / n.s)	- (n.s / n.s)
HNA	n.s (n.s / n.s)	n.s (n.s / n.s)
live	n.s (n.s / n.s)	n.s (n.s / n.s)
dead	n.s (+ / n.s)	- (n.s / n.s)
ctc+ abundance	+ (n.s / n.s)	n.s (n.s / n.s)
ctc%	+ (n.s / n.s)	n.s (n.s / n.s)
BP Leu. incorp.	n.s (n.s / +)	(n.s / n.s)
BP Tdr. Incorp.	n.s (n.s / +)	+ (n.s / n.s)
Viruses	n.s (n.s / n.s)	n.s (n.s / n.s)

Table 2. Diel cycles at stations C and D during cruise MODIVUS. (+) and (-) indicate significantly increasing or decreasing values from day to night (t-tests, $p<0.05$). Signs inside parenthesis : (+/-) indicates significantly increasing values during the day, and significantly decreasing during the night. (-/+) indicates significantly decreasing values during the day, significantly increasing during the night. N.s for non significant differences.

Patterns in marine bacterial group distribution, as measured by FISH, in relation to chlorophyll, temperature and salinity

Thomas Lefort and Josep M. Gasol

04

ABSTRACT

We used marine literature data on bacterial (sub) group abundances as determined by fluorescent in situ hybridization (FISH or CARDFISH) to test whether the ecological variability of the different subgroups was similar to that of the bacterial community as a whole. Patterns are described between the major groups (*Alpha-*, *Beta-*, *Gammaproteobacteria* and *Bacteroidetes*, as well as *Rhodobacteraceae* and SAR11) and environmental variables such as chlorophyll a concentration, salinity and temperature, distinguishing between coastal (i.e. over the continental platform) or open ocean environments. Coastal ecosystems exhibited significantly higher relative abundances (average % of DAPI counts) of *Bacteroidetes* (25%), Beta- (14%), *Gammaproteobacteria* (12%) and *Rhodobacteraceae* (6%) while significantly higher contributions of *Alphaproteobacteria* and SAR11 (32%) were enumerated on averaged offshore. Multiple regression analyses showed significant effects of both chlorophyll a and temperature on total and SAR11 absolute abundances (expressed as cells ml^{-1}) and significant effects of chlorophyll a and salinity levels on absolute abundances of *Betaproteobacteria* and *Rhodobacteraceae.* Single Parameter Analysis was applied to assess the "preferred" range of environmental parameters for the different bacterial group abundances and revealed "preference" of *Gammaproteobacteria* for increasing temperatures and the opposite for *Betaproteobacteria*. Significantly different relationships were found between bacterial group absolute abundances and chlorophyll a concentration, with significantly different log-log slopes that ranged from 0.13 (±0.04) for SAR11 to 0.53 (±0.08) for *Betaproteobacteria* at the global scale, from -0.11 (±0.08) for SAR11 to 0.52 (±0.08) for *Betaproteobacteria* in coastal and from -0.06 (±0.12) for *Gammaproteobacteria* to 0.48 (±0.06) for Bacteroidetes in open-ocean conditions. The different slopes measured for the different groups across trophic levels, suggest different metabolic aptitudes for utilizing algal-derived DOC, differing with the type of ecosystem.

INTRODUCTION

Bacteria (here taken to mean the ensemble of non-phototrophic prokaryotes) do not constitute a uniform pool and their patterns of variability can be studied at different levels, either considering "bacteria" as a whole, or dividing the community into the phylogenetic groups that comprise the pool. Evidences of a trophic coupling between phytoplankton and bacteria in most marine ecosystems were first found empirically, by observing a strong relationship and a significant correlation between bacterial and phytoplankton biomass (as estimated from chlorophyll *a* concentration, Bird and Kalff 1984; Cole et al. 1988; Gasol and Duarte 2000). This link between autotrophs and heterotrophs would imply that bacteria use mainly the dissolved organic matter produced by phytoplankton to support growth by bacterial production (Nagata et al. 2000; Morán et al. 2002). Studies based on the analysis of large data sets have also demonstrated the generality of such a link. Gasol and Duarte (2000) showed how bacteria and chlorophyll *a* are related with a positive relationship and a log-log slope <1 between heterotrophic and autotrophic biomass, indicating that bacterial biomass vary proportionally less than chlorophyll *a*. This relationship was shown to differ among habitats type such as open-ocean which had more heterotrophic biomass per unit autotrophic biomass than coastal ecosystems (Gasol et al. 1997; Gasol and Duarte 2000). It also varied between freshwater and marine habitats (Simon et al. 1992). Li et al. (2004), using a comparative analysis approach between 13.973 paired data of bacterial abundance and chlorophyll *a* concentration, showed that the relationship was upper-limited at high trophic levels, indicating a macro-ecological limit to bacterial abundance in highly productive waters.

The emergence of molecular techniques based on the detection of 16S rDNA sequences have shown that the bacterial community is composed by members of a variety of phylogenetic groups. Fluorescence In Situ hybridization (FISH), allows quantifying the contribution to the bacterial community of the different bacterial groups, independently of the biases associated with PCR amplification (e.g. Wintzingerode et al. 1997). Based on the staining of the bacterial small subunit (16S rRNA) rRNA ribosome sequences after hybridization with specific probes, this technique was initially limited by the difficulty of detecting small and slow growing bacterial cells. But new hybridization protocols considerably reduced the limit of detection (Schönhuber et al. 1997; Pernthaler et al. 2002). The different protocols have allowed, e.g. to differentiate the contribution of different bacterial groups in distinct habitats such as freshwaters or marine ecosystems (Glockner et al. 1999) and also to describe the spatio-temporal dynamics of bacterial community structure in a variety of natural habitats (Alfreider et al. 1996; Llobet-Brossa et al. 1998; Murray et al. 1998; Simon et al. 1999; Kirchman et al. 2005). These studies have shown that the *Alphaproteobacteria* group dominates in marine coastal waters (e.g. Kirchman et al.

2005), contrasting with *Betaproteobacteria* found more abundantly in freshwaters (Glockner et al. 1999; Bouvier and del Giorgio 2002). The SAR11 cluster, a distinct branch within the *Alphaproteobacteria* phylum, has been shown to dominate surface ocean bacterial communities in nutrient-depleted areas such as oligotrophic waters of the Sargasso Sea (Morris et al. 2002) but also in coastal Mediterranean waters in spring and summer (Alonso-Sáez et al. 2007). The *Rhodobacteraceae* group of marine *Alphaproteobacteria* appears in most marine environments (Buchan et al. 2005), and is generally more abundant in bacterial communities associated with marine algae (Buchan et al. 2005). The *Bacteroidetes* constitutes one of the major picoplankton groups and appear to dominate in a variety of ecosystems such as cold waters (Simon et al. 1999; Abell and Bowman 2005) and in coastal waters (Eilers et al. 2001; O'Sullivan et al. 2004; Alonso-Sáez et al. 2007), accounting for as much as half of all bacterial cells counted by FISH in California coastal seawater samples (Cottrell and Kirchman 2000) and in some offshore conditions (Simon et al. 1999; Abell and Bowman 2005; Schattenhofer et al. 2009) particularly associated to phytoplankton blooms (Simon et al. 1999).

The geographical distribution of the different bacterial groups does not vary at random but is likely controlled by different environmental factors. Alonso-Sáez et al. (2007) showed that the relative contribution of *Bacteroidetes*, *Rhodobacteraceae* and *Gammaproteobacteria* increased in more productive waters of the subtropical North East Atlantic Ocean. Baltar et al. (2007) reported concomitant decreases of bacterial group relative abundance and pronounced changes in bacterial community structure along a transect performed in the North Atlantic from coastal to offshore waters. Seasonal studies of bacterial assemblage structure in some habitats such as the NW Mediterranean coastal waters (Alonso-Sáez et al. 2007), the English Channel (Mary et al. 2006), or the California coast (Fuhrman et al. 2006) indicate that a variety of environmental parameters likely participate in the spatio-temporal variability observed in bacterial community structure. For instance, patterns of bacterial group distribution along a gradient of salinity have been observed, with a shift in the dominance of *Betaproteobacteria* in ecosystems influenced by freshwater inputs to a predominance of *Alphaproteobacteria* in more open water conditions (Bouvier and del Giorgio 2002; Kirchman et al. 2005).

However, all these studies describe bacterial group distribution and their controlling factors in particular habitats, and only few have analyzed community structure at a larger, more global scale. Selje et al. (2004), using both quantitative PCR and FISH, reported distinct patterns in the large-scale distribution of SAR11 and the *Roseobacter* clades. At the global ocean scale, Wietz et al. (2010) used CARD-FISH to analyze latitudinal and biome-related patterns in bacterial group distribution, showing differing bacterial communities between colder and warmer oceans. Little is still known about the macro-ecological patterns of distribution of the different bacterial groups,

but we already have now a reasonable number of studies and a relatively large data set about bacterial subgroup concentrations that allow for a general macro-ecological study. Thus, we set to explore statistically this database to demonstrate whether well-constrained relationships at the phylogenetic group level exist between bacterial abundance and ecological factors and are similar, or different, from those existing at the bulk community level. For this endeavor, we collected most published data and we describe how the different bacterial subgroup abundances vary with chlorophyll *a* concentration, temperature and salinity. We test the null hypotheses that the bacterial group abundances exhibit the same patterns along gradients of chlorophyll a, temperature and salinity in coastal and open ocean ecosystems, as those of the bacterial community as a whole. One additional hypothesis that we explore is that the different bacterial subgroups show differing patterns, and that the one previously identified between the environment and bulk bacterial abundance is an emergent property of the planktonic ecosystem.

MATERIAL AND METHODS

Data collection

We electronically searched within the ISI Web of Knowledge (Thomson Reuters) for publications including the keywords: marine bacterial community composition, bacterial community structure, Fluorescence in situ hybridization (FISH), catalyzed reporter deposition-FISH (CARD-FISH), marine bacterial assemblages. We completed our selection of studies going through the cited literature sections of these papers to locate publications that had not been detected in the electronic searches. We found 33 different studies corresponding to 31 published papers to which we added one series of unpublished CARDFISH results of bacterial group abundance in Blanes Bay, NW Mediterranean from 2008 to 2010 (T. Lefort and C. Ruiz-González, unpublished) and one communication (Carlson et al.) electronically available at: *icomm.mbl.edu/oocs_summaries/OOCS_**Carlson**/OOCS_**Carlson**_final.ppt*.

All these studies (Table 1) presented bacterial subgroup abundances or, at least, total bacterial abundance and % contribution of each group, in addition to the necessary ancillary data (at least chlorophyll *a*, temperature and salinity when available).

We centered our study on the bacterial groups that have been previously described as major contributors to total bacterial community structure, in particular, *Alphaproteobacteria* (with a particular focus on SAR11 and *Rhodobacteraceae*), *Gammaproteobacteria*, *Bacteroidetes*, *Betaproteobacteria* and *Eubacteria* to compare with total bacterial abundance.

FISH and CARDFISH Procedures- From the 34 studies selected, 16 used basically a "FISH" protocol, as in Cottrell and Kirchman (2000, 2003), and 18 used a "CARD-FISH" protocol as described by Pernthaler et al. (2004). To target the bacterial groups, the bacterial probes considered in most publications were: ALF968 (5'-GGT AAG GTT CTG CGC GTT-3') for *Alphaproteobacteria* (Glöckner et al. 1999), GAM42a (5'-GCC TTC CCA CAT CGT TT-3') for *Gammaproteobacteria* (Manz *et al*., 1992), and CF319a (5'-TGG TCC GTG TCT CAG TAC-3') for the class *Flavobacteria* of phylum *Bacteroidetes* (Manz et al. 1996), BET42a for *Betaproteobacteria* (Manz et al. 1992) and the probe EUB338 I-III (5'-GCT GCC TCC CGT AGG AGT-3') as a general probe mixture targeting all *Bacteria* (Amann et al. 1990) (Daims et al. 1999). In most studies, a negative control with the EUB antisense probe NON338 (5'-ACT CCT ACG GGA GGC AGC-3') (Wallner et al. 1993) was used to determine non-specific binding.

REFERENCES	TECHNIQUE TYPE	SAMPLING SITES	PROBES CONSIDERED
Alderkamp et al. 2006	CARD-FISH	Coastal North Sea	CF319, Ros537
Wietz et al. 2010		Global survey	EUB338, ALF968, BET42a, GAM42a, CF319
Alonso Sáez et al. 2007		NW Mediterranean coastal waters (Blanes Bay)	EUB338, ALF968, BET42a, GAM42a, SAR11-441R, ROS537
Obernosterer et al. 2007		South Pacific Ocean	EUB338, ALF968, GAM42a, CF319A
Piccini et al. 2006		Southwestern Atlantic	EUBI-III, ALF968, GAM42a. BET42a, CF319, SAR11-441
Stoica and Herndl 2007		NW Black Sea	EUB338, ALF968, BET42a, GAM42a, ROS537 AND ROS1029
Garcés et al. 2007		NW Mediterranean coastal waters	EUB338, ALF968, BET42a, GAM42a, ROS538
Mary et al. 2006		English Channel	EUB338 I-II-III, ALF968, SAR11-152R, SAR11-542R, RSB67, GAM42a, CF319a
Garneau et al. 2006		Canada Arctic shelf	EUB338, ALF968, BET42a, GAM42a, CF319a
Alonso Sáez et al. 2006		NW Mediterranean coastal waters (Blanes Bay)	EUB338, ALF968, BET42a, GAM42a, SAR11-441R, ROS537
Ruiz-González et al. (unpublished)		NW Mediterranean coastal waters (Blanes Bay)	EUB338-II-III, ALF968, BETA42a, CF319, SAR11-441R, ROS537, GAM42a
Alonso Sáez et al. 2008		Western Arctic	EUB338-II-III, ALF968, BETA42a, CF319, SAR11-441R, ROS537, GAM42a
Baltar et al. 2007		NW Africa upwelling to Canary Coastal Transition zone	EUBI-II-III, SAR11-441R, CF319a, ROS537, GAM42a
Teira et al. 2008		Ría de Vigo, Atlantic	EUB338, ALF968, BET42a, GAM42a, CF319a, SAR11-441R, ROS537
Schattenhofer et al. 2009		Atlantic	EUB338 I-III, SAR11-441R, GAM42a, CF319a
Topping et al. 2006		Scotia Sea, Antarctica	EUB338, ALF968, SAR11-486, SAR11-542R, ROS537, GAM42a, CF319a
Lin et al. 2006	FISH/CARD-FISH	Eastern sub-basin of the Cariaco system and Black Sea	EUB338, ALF968, BET42a, GAM42a, CF319
Lin et al. 2008		Cariaco system	EUB338, ALF968, BET42a, GAM42a, CF319
Zhang et al. 2006	FISH	South China Sea	EUB338, ALF968, BET42a, GAM42a, CF319
Simon et al. 1999		Southern Ocean	EUB338, ALF968, BET42a, GAM42a, CF319
Wells et al. 2003		Canadian archipelago	EUB338, CF319
Longnecker et al. 2006		Oregon, Newport (West US), North Pacific	EUB338, ALF968, BET42a, CF319, GAM42a
Vila et al. 2004		Northern gulf of Mexico	EUB338, ALF968, ROS536, GAM42a, CFB319
Kirchman et al. 2003		Delaware estuary	EUB338, ALF968, BET42a, GAM42a, CF319a
Agawin et al. 2006		NE Subarctic Pacific	EUBI-II-III, ALF968, ALF1B, BETA42a, GAM42a, CF319a
Yokokawa and Nagata 2005		North Pacific coast	EUB338, ALF968, BET42a, GAM42a, CF319
Zubkov et al. 2002		Northern north Sea	EUB338, ALF968, GAM42a, CF319a, RSB67
Cottrell and Kirchman. 2000		California Coast	EUB338, ALF968, BET42a, GAM42a, CF319a, SAR11-A1
Lin et al. 2007		Eastern sub-basin of the Cariaco system	EUB338, ALF968, BET42a
Castle and Kirchman 2004		Delaware estuary and Chesapeake Bay	EUB338, ALF968, BET42a, GAM42a, CF319
Yokokawa et al. 2004		Delaware estuary	EUB338, ALF968, BET42a, GAM42a, CF319
Cottrell et al. 2006		Mid Atlantic bight and North Pacific gyre	EUB338, ALF968, SAR11, CF319, ROS537
Kirchman et al. 2005		Delaware Estuary	Eub338, Alfa968, ROS537, Beta42, Gam42a, CF319
Carlson et al. (unpublished)*		BATS. Sargasso Sea	SAR11, CF319, ROS537

The data from BATS station by Carlson et al. were extracted from icomm.mbl.edu/oocs_summaries/OOCS_**Carlson**/OOCS_**Carlson**_final.ppt

Table 1. Data used in this study, oligonucleotide probes considered as well as the type of Fluorescence in situ Hybridization protocol.

In addition, the relative abundance of the SAR11 and *Rhodobacteraceae* clusters was also analyzed mostly using the probes SAR11-441R (Morris et al. 2002) and Ros537 (5´-CAACGCTAACCCCTCC-3´) (Eilers et al. 2001). However, other probes were also used to target SAR11, such as SAR11/486 (5´-GGACCTTCTTATTCGGGT-3´) (Fuchs et al. 2005) and SAR11/542R (5´-TCCGAACTACGCTAGGTC-3´) (Morris et al. 2002). Probe RSB67, specific for *Alphaproteobacteria* subgroup *Rhodobacteraceae* was also used in some studies (5'-CGCTCCACCCGAAGGTAG-3' (Zubkov et al. 2001) (see details in Table 1).

Extraction of the data- Most data were from the surface ocean layer (except for the CARIACO basin data set, Table 1). Relative abundances were expressed in terms of % contribution to total DAPI counts or to total flow cytometric counts, while absolute abundances were expressed in number of cells per milliliter. The subgroup concentrations were paired with chlorophyll *a* concentration added to other environmental variables such as temperature (ºC), and salinity when information was available. Some data were obtained from graphs and digitized with the use of GraphClick vs. 3 (Arizona Software). Considering the break shelf as the limit between coastal and open ocean ecosystems, 20 of the studies were considered to be coastal environments (of the 20, 4 studies were clearly river-influenced or estuary habitats), 6 were open-ocean, and 6 contained both coastal and open-ocean data.

Chlorophyll *a* measurements- Chlorophyll *a* concentration (μg ml^{-1}) defined operationally as the pigment amount detected from particles retained on glass-fibre membranes, by spectrophotometry or by High-performance liquid chromatography (HPLC), was used as a proxy of ecosystem trophy. All chlorophyll *a* concentrations corresponded to field measurements performed on the day of sampling. For the study of Castle and Kirchman (2004), chlorophyll *a* concentrations were not directly available but were estimated from the particulate beam attenuation coefficient (cp) results following the relationship:

Chla (μg. L^{-1}) = 2.6 cp – 0.014 as proposed by Behrenfeld and Boss (2006).

Standardization of FISH and CARD-FISH results- As explained above, we combined the data obtained with the FISH and the MARFISH protocols. It is well known that, in most ecosystems, the CARD-FISH protocol produces higher counts, as is more sensitive than the FISH protocol (e.g. Pernthaler et al. 2002). In order to be able to compare the relative abundances (% of DAPI) and the absolute cell concentrations (calculated from the total DAPI) measured by FISH and CARDFISH, we conducted a standardization of the relative abundances, assuming that all the FISH and the CARDFISH estimates came from the same global "population", and that both

methodologies had sampled enough to obtain a fair representation of the contribution of each group to the global community. For each bacterial group studied, one-way Anova and t tests were conducted on the whole data set to test whether measurements of bacterial group contribution to total bacterial community structure by FISH or CARD-FISH were significantly different. When significant differences were observed, we corrected the FISH values accordingly. E.g. for EUB+ cells, we could assume that the average 30% obtained by FISH and the average 59% obtained by CARD-FISH are both estimates of the same data. In that example case, we would bring the 30 to 59% and we would thus multiply all the EUB values by the factor 59/30. Concentrations were then computed from the percentages and the total DAPI. When no significant differences were observed between techniques, no percentage transformations were conducted. Note that this procedure assumes that the unlabelled cells by FISH that could be labeled by CARDFISH are distributed equally among all bacterial groups, and that the discrepancy between the two methodologies stands from different degrees of activity spread equally within all subgroups. This is likely not the case (large *Gammaproteobacteria* cells might be better detected by FISH than small SAR11 cells), but we have no other ways of accounting for these differences.

We ignored specific variations in protocols (even though they are relevant, Bouvier and del Giorgio 2003). The detection of target cells by FISH is known to vary drastically among the published literature, ranging from 1% to 100% of variations for *Eubacteria* across 51 different published reports (Bouvier and del Giorgio 2003). Not only methodological factors such as the type of fluorochrome or the stringency conditions can significantly influence the performance of FISH, but ecological factors such as ecosystem type (coastal, open-ocean, freshwaters….) explain also a large amount of variability in target detection (Bouvier and del Giorgio 2003).
We also ignored variability in probe coverage, but the known limitations of current probes should be taken into account (e.g. Amann and Fuchs 2008). Many of the group-specific probes used to target the major taxonomic groups do not have 100% group coverage and no outgroup hits, and potential for false identification exists (e.g. CF319a was designed to cover the *Flavobacteria* (90%) and *Shingobacteria* (90%) but is much less efficient at targeting the *Bacteroidetes* (30%) (Amann and Fuchs 2008).

Multivariate analysis- Both relative (% of DAPI/total prokaryotes) and absolute abundances (cells ml^{-1}) were used to describe the relative importance of chlorophyll *a* concentration, temperature and salinity. To avoid the differences generated by the scale of the independent variable (chlorophyll *a*, temperature, salinity) and to allow for the comparison of the relative impact of each independent variable in multivariate models, we used standardized Beta-coefficients in multiple regression models. All analyses were performed with the JMP (version 5.0.1) statistical

software package (SAS Institute, Inc). The graphs of Figure 1 were done with the software Aabel 2.4 (Gigawiz Ltd. Co) with a 7*7 moving average.

Linear regression analysis and analysis of covariance- To compare slopes of relationships between log-transformed standardized absolute abundances of each bacterial group and independent variable (e.g. chlorophyll *a*), equations of the regressions are presented as log (Y) = a + b log (X) with Y= cells ml^{-1}; a=intercept; b=slope; X =independent variable (e.g. chlorophyll a in $\mu g\ l^{-1}$). In order to test whether the slopes and intercepts of the relationships are significantly different, Student's t-tests were conducted after applying model I regression analyses following the methods explained by Zar (1999). To compare linear regressions and test for heterogeneity of slopes, ANCOVA tests were also performed. All conducted in JMP 5.0.1 software (SAS Institute Inc).

Analysis of bacterial group distribution- The collected data of bacterial group relative abundances and environmental parameters such as chlorophyll *a* concentration, temperature and salinity were used to estimate the "preferred" range of environmental parameters for each bacterial group using a Quotient-Rule Analysis (QRA, Somarakis et al. 2006). Each environmental variable was divided into regular intervals for which the frequencies of occurrence were calculated and expressed in percentage. The number of intervals in every environmental variable was set to ensure that maximum occurrence per interval did not exceed 20% of all measurements. For every bacterial group, the log transformed cell concentration within each interval of environmental parameter was calculated and then was expressed as a percentage of the group abundance over the full range of environmental variable. Then, for every interval the quotient values were estimated with the equation:

$$Q = \frac{\textit{bacterial}\text{ group abundance (\%)}}{\text{frequency of occurence of environmental variable (\%)}}$$

Quotient values were smoothed using a 3-point running mean and then plotted against environmental factors, reflecting "preference" (quotient values >1) or "avoidance" (quotient values <1) for a specific variable range (interval). A non-parametric Kolmogorov-Smirnov test of goodness of fit (Zar 1999) was used to compare the cumulative frequency distribution of bacterial groups per category of environmental variable against the distribution histograms of that environmental variable. The null hypothesis (H_0) considers that the observed bacterial group distribution should be at random along that environmental variable.

RESULTS

Environmental parameters- The data set analyzed was quite representative of the world's oceans: chlorophyll *a* concentrations in coastal environments had an average value of 3.97 μg l^{-1} and ranged from 0.05 μg. l^{-1} to 103.15 μg.l^{-1} (N=266). A shorter range and lower chlorophyll *a* average, 0.48 μg l^{-1}, was measured in open-ocean conditions, 0.001 μg.l^{-1} - 10.01 μg. l^{-1} (N= 197, Tables 1 and 2). The large chlorophyll *a* values were measured in the Southern China Sea and in the NW African upwelling.Temperature ranged from -1.3ºC and -0.67ºC in coastal and open-ocean environments respectively, to 28.5ºC and 30.1ºC (Tables 1 and 2). Salinity of coastal waters ranged from 0.08 recorded in Delaware estuary (Kirchman et al. 2003) to a maximum at 38.7 measured at the Blanes Bay station in NW Mediterranean (Alonso-Sáez et al. 2007). In open-ocean waters, a minimum was measured in Canada Arctic shelf (Garneau et al. 2006) at 20.3 and a maximum at 37.43 observed in the Western Arctic (Alonso-Sáez et al. 2008) (Tables 1 and 2).

Range and average bacterial group contribution to bacterial community structure (BCS) across ecosystems- At the global scale, SAR11, *Alphaproteobacteria* and *Bacteroidetes* were the largest contributors to BCS with relative abundance averages (expressed as % of DAPI counts) of 29% (±44%), 26% (±53%) and 21% (±71%) respectively (Table 2). Almost similar contributions were found for *Betaproteobacteria* and *Gammaproteobacteria* with respectively 13% (±84%) and 11.5% (±95%). The average bacterial group relative contributions and their coefficient of variation (± CV) showed opposite patterns, low relative contributions were associated with high variability (as estimated from CVs) (Table 2).

Alphaproteobacteria relative abundance in coastal ecosystem was on average 25%, for a range of 1-85% (Tables 1 and 2). In offshore conditions, there was a higher relative contribution of *Alphaproteobacteria* to BCS, 32% (t-tests, n_{Alpha}=351, $p<0.001$) but with a lower range of variability 5-59%. SAR11 contributed 25% on average in coastal conditions, ranging from 0-59%. In offshore, an almost similar range of variations 0-67% was measured but with significantly higher average contribution, 32% (t-test, n_{SAR11} = 252, $p<0.001$). In comparison with *Alphaproteobacteria* and SAR11, the average relative contribution of *Rhodobacteraceae* was significantly higher in coastal than in open-ocean conditions with 6.4% and 4.9% respectively (but with lower significance level) (t-test, n_{Rhodo} = 281, $p<0.05$). The range was 1-32% in coastal areas and 0 to 18% in open-ocean environments.

	Global scale			Coastal			Open-Ocean		
	N of observations	AVERAGE % (±CV)	AVERAGE CELLS ML^{-1} (±SD)	N of observations	AVERAGE % (MIN/MAX)	AVERAGE CELLS ML^{-1} (±CV)	N of observations	AVERAGE % (MIN/MAX)	AVERAGE CELLS ML^{-1} (±CV)
Eubacteria	292	73.5 (±26%)	1.12 ±1.80 x10^{6}	239	73 (4 / 100)	1.24 ± 1.93 x10^{6}	53	74 (12 / 100)	5.62 ± 8.48 x10^{5}
Alphaproteobacteria	351	26 (±53%)	3.91 ± 10.6 x10^{5}	290	25 (0.21 / 85.09)	4.03 ± 11.60 x10^{5}	61	32 (5.13 / 58.97)	3.35 ± 3.89 x10^{5}
SAR11	252	29 (±44%)	1.94 ± 1.87 x10^{5}	106	25 (0 / 59)	1.97 ± 1.43 x10^{5}	146	32 (5.25 / 66.94)	1.91 ± 2.15 x10^{5}
Rhodobacteraceae	281	6 (±95%)	5.04 ± 8.38 x10^{4}	149	6 (0 / 38.32)	6.54 ± 9.84 x10^{4}	132	5 (0.4 / 18)	3.15 ± 5.52 x10^{4}
Gammaproteobacteria	289	11 (±95%)	1.92 ± 8.15x10^{5}	222	12 (0.7 / 95.82)	2.21 ± 9.12 x10^{5}	67	9 (0 / 22.55)	8.02 ± 1.07 x10^{5}
Bacteroidetes	465	21 (±71%)	2.02 ± 3.22 x10^{5}	301	25 (0.26 / 81.14)	2.77 ± 3.82 x10^{5}	164	14 (1 / 51.49)	8.22 ± 1.13 x10^{5}
Betaproteobacteria	174	13 (±84%)	2.08 ±3.18 x10^{5}	152	13 (0 / 51.17)	2.22 ± 3.30 x10^{5}	8	9 (4.40 / 16.23)	7.82 ± 8.20 x10^{4}
Temperature (ºC)	346	17.37 (±40%)		209	17 (-1.3 / 30.1)		137	17.5 (-0.67 / 29.7)	

Table 2. Average and range of environmental parameters (Minimum and Maximum) and bacterial group relative and absolute abundances (% relative to DAPI or flow cytometry counts. Bacterial group abundance expressed in cells ml^{-1}) as measured by the FISH/CARDFISH protocol in different types of ecosystems. Coastal sites are those placed on the continental platform. CV for Coefficient of Variation, SD for Standard Deviation

The *Bacteroidetes* relative abundance and range of variation were significantly higher in coastal than in offshore conditions (t-test, $n_{Bacteroidetes} = 465$, $p<0.001$). At the coast, an average contribution of 25% (range 1-81%) was found, while offshore, *Bacteroidetes* were contributing to an average of 15% (range <1- 46%).

In comparison with open-ocean waters where *Gammaproteobacteria* contribution to BCS averaged 9%, significantly higher relative contribution of *Gammaproteobacteria* was measured in coastal environments, averaging 12% of BCS (t-test, $n_{Gamma} = 289$, $p<0.001$) and with a very high range of variability <1-90% of the DAPI counts. The strong maximum contribution was found in coastal lagoon waters (>90% BCS) with high chlorophyll *a* concentration and high temperature in the southwestern coastal Atlantic (Piccini et al. 2006).

As *Bacteroidetes*, *Gammaproteobacteria* and *Rhodobacteraceae*, higher relative abundance of *Betaproteobacteria* were measured in coastal than offshore with respectively 14% and 9% (t-test, $n_{Beta} = 174$, $p<0.001$) averages. Similarly, a larger range of *Betaproteobacteria* contribution was measured at coastal sites (<1- 48%) than in offshore environments (<4-16%) (Tables 1 and 2). Compared with coastal, the observation's number for *Betaproteobacteria* abundance was very low in offshore conditions, mostly measured during coast to offshore transects in the south China Sea and off the Oregon coasts (Zhang et al. 2006; Longnecker et al. 2006; Table 1).

Patterns in BCS across environmental parameters- We analyzed to what extent the variability measured in the bacterial group contribution to BCS was driven by different environmental parameters such as chlorophyll *a*, temperature and salinity.

We represented with contour plots the relative contribution to BCS as a function of chlorophyll *a* concentration and temperature (Figure 1), and as a function of chlorophyll *a* concentration and salinity (Figure 2). No evident patterns in *Alphaproteobacteria* and *Gammaproteobacteria* relative contribution to BCS could be observed across the range of chlorophyll *a* concentration and temperatures or salinity, the maximum (> 50%) appearing at both low and high temperatures levels. However, strong effects of temperature were seen on Eubacterial relative contribution as revealed by a strong correlation found (Pearson t test, $N=159$, $p<0.005$), the maximum contribution observed at lower chlorophyll *a* and temperatures levels (Figure 1A and Table 3). Furthermore, the highest *Bacteroidetes* contribution (>18%) was observed at high chlorophyll *a* levels but within a large range of temperature from 2ºC to 17ºC (Figure 1C). Even more pronounced patterns were observed when focusing on Alphaproteobacterial groups: SAR11 and *Rhodobacteraceae* (Figure 1B and 1D, Figure 2B). While highest SAR11 relative contribution were observed at

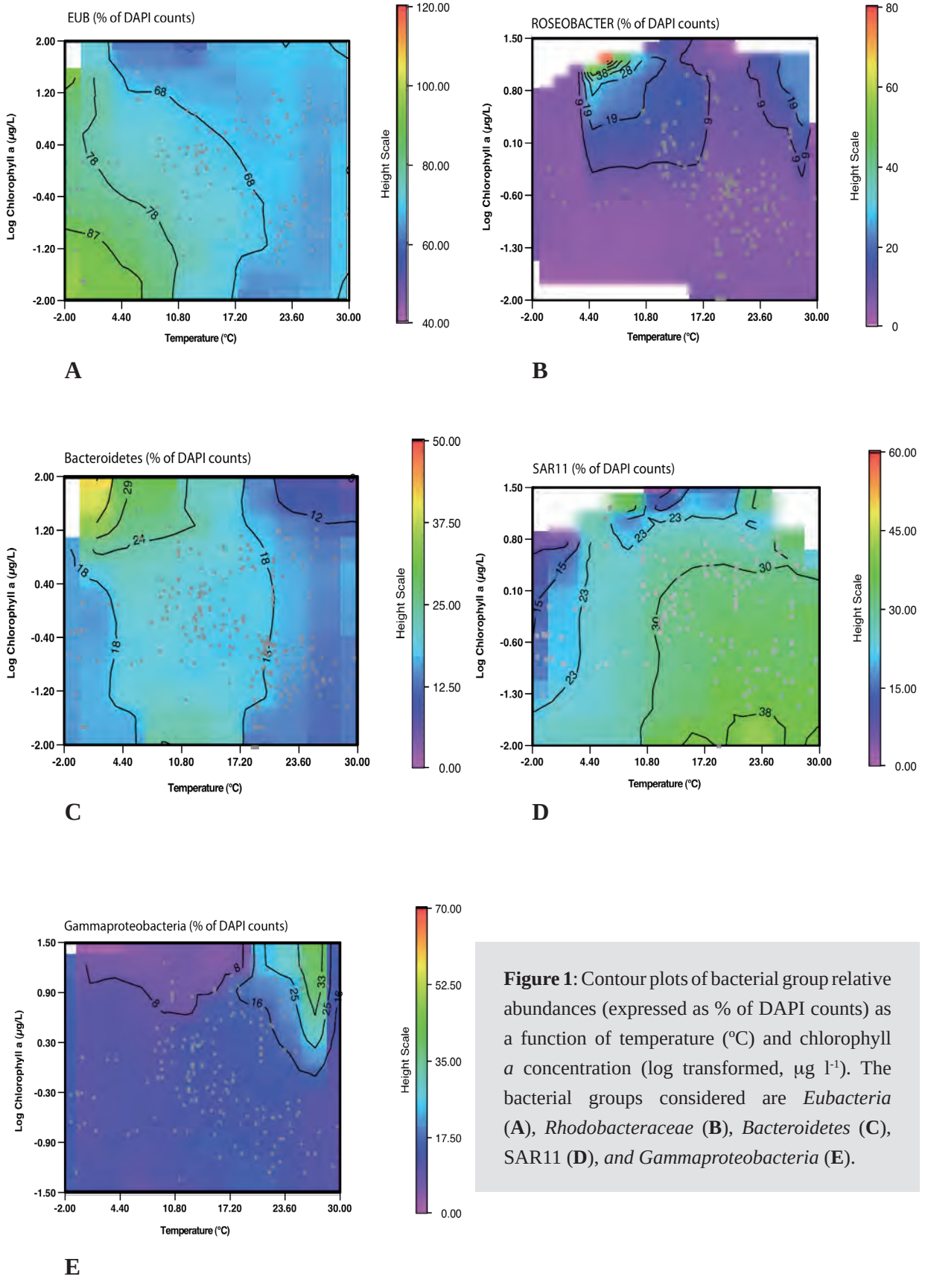


Figure 1: Contour plots of bacterial group relative abundances (expressed as % of DAPI counts) as a function of temperature (ºC) and chlorophyll *a* concentration (log transformed, µg l^{-1}). The bacterial groups considered are *Eubacteria* (**A**), *Rhodobacteraceae* (**B**), *Bacteroidetes* (**C**), SAR11 (**D**), *and Gammaproteobacteria* (**E**).

Bacterial groups	Dependant variable	(**1**) Temperature and Log Chlorophyll *a*					(**2**) Salinity and Log Chlorophyll *a*				
		N_{OBS}	R^2	F ratio	Beta Temperature	Beta Chlorophyll *a*	N_{OBS}	R^2	F ratio	Beta salinity	Beta Chlorophyll *a*
Bacteria total	Log Abs. abundance	254	0.43	96 (p<0.0001)	**0.24***	**0.61***	146	0.22	20 (p<0.0001)	**-0.29***	**0.23***
Eubacteria	Rel. abundance (%)	137	0.20	18 (p<0.0001)	**-0.34***	**-0.22***	97	0.04	3 (n.s)	-0.13	**-0.31***
	Log Abs. abundance	114	0.41	41 (p<0.0001)	**0.17***	**0.58***	81	0.18	10 (p<0.0001)	-0.11	**0.37***
Alphaproteobacteria	Rel. abundance (%)	185	0.03	4 (p<0.05)	0.01	**-0.20***	137	0.09	8 (p<0.0001)	**0.22***	-0.13
	Log Abs. abundance	156	0.06	6 (p<0.005)	0.15	**0.22***	115	0.04	3 (p<0.05)	0.01	**0.22***
SAR11	Rel. abundance (%)	166	0.08	8 (p<0.0005)	**0.20***	-0.07	73	0.10	5 (p<0.05)	0.10	**-0.33***
	Log Abs. abundance	156	0.07	7 (p<0.005)	**0.16***	**0.30***	73	0.01	0.40 (n.s)	0.04	-0.10
Rhodobacteraceae	Rel. abundance (%)	164	0.08	8 (p<0.0005)	0.01	**0.30***	67	0.21	10 (p<0.0001)	**-0.30***	**0.37***
	Log Abs. abundance	158	0.22	24 (p<0.0001)	0.04	**0.50***	67	0.29	14 (p<0.0001)	**-0.35***	**0.42***
Gammaproteobacteria	Rel. abundance (%)	173	0.01	0.07 (n.s)	0.01	0.01	135	0.01	0.17 (n.s)	-0.06	-0.04
	Log Abs. abundance	154	0.03	3 (p<0.05)	0.10	**0.18***	128	0.06	5 (p<0.05)	**-0.21***	0.10
CFB	Rel. abundance (%)	249	0.05	8 (p<0.001)	**-0.15***	**0.18***	143	0.15	13 (p<0.0001)	0.10	**0.45***
	Log Abs. abundance	220	0.34	57 (p<0.0001)	0.04	**0.59***	123	0.22	18 (p<0.0001)	0.11	**0.53***
Betaproteobacteria	Rel. abundance (%)	79	0.08	4 (p<0.05)	-0.01	**0.32***	62	0.56	41 (p<0.0001)	**-0.78***	0.08
	Log Abs. abundance	47	0.24	8 (p<0.001)	0.21	**0.37***	40	0.32	10 (p<0.001)	**-0.37***	**0.31***

***: significant values (p<0.05)**
N_{obs}: Number of observations
n.s: Non significant values

Table 3. Results of Multiple Linear Regression analysis. Bacterial relative abundances (expressed as % of DAPI counts and standardized according to FISH and CARDFISH, see M&M) and Log of absolute abundances (cells ml^{-1}) were considered as a linear function of both: **1**: Temperature (ºC) and Log chlorophyll *a* (µg l^{-1}) , **2**: Salinity (psu) and Log chlorophyll *a* (µg l^{-1}). Beta coefficients (Beta) represent the contribution of each independent variable (Tº, chlorophyll *a* or salinity) to the prediction of the dependant variable (relative or absolute abundances).

high temperatures and low chlorophyll *a* concentration (Figure 1D), *Rhodobacteraceae* relative abundance increased with increasing levels of chlorophyll *a* concentration (Figure 1B and 2B) and was strongly influenced by salinity (Pearson t test, N=70, p<0.05) (Table 3). Similarly, particularly pronounced effects of salinity on *Betaproteobacteria* relative contribution were obvious (Pearson tests, N=66, p<0.005), reaching values of >18% at salinities <6 (Figure 2A).

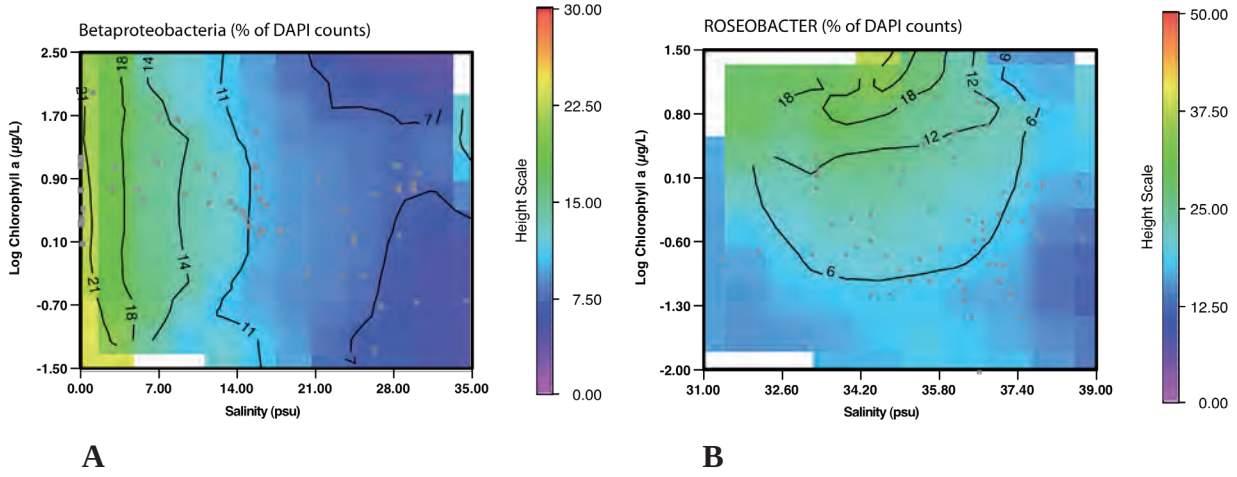
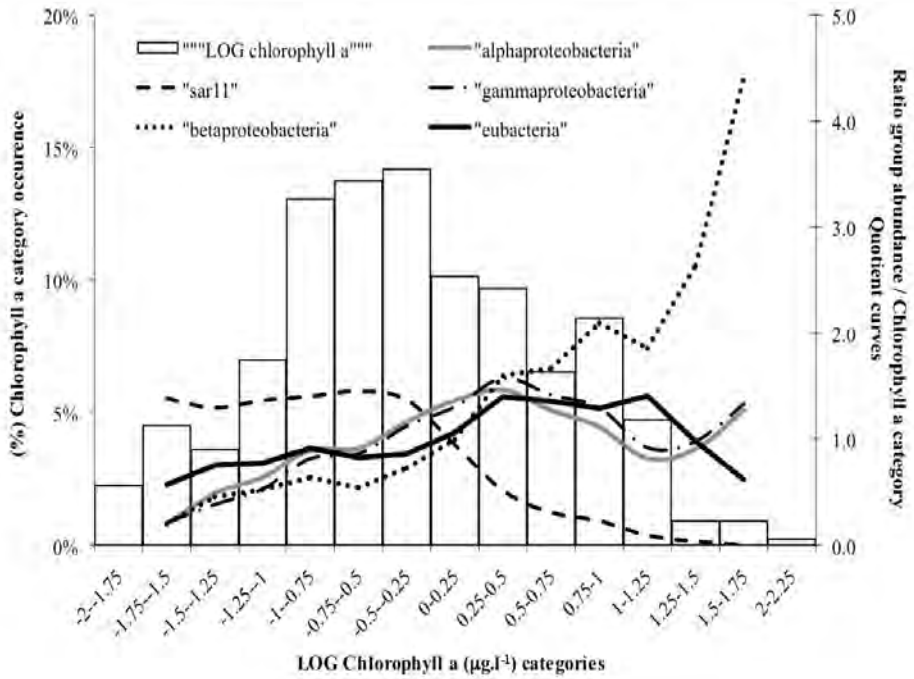


Figure 2: Contour plots of bacterial group relative abundances (expressed as % of DAPI counts) as a function of salinity (psu) and chlorophyll *a* concentration (Log values, µg. l^{-1}). Bacterial groups are *Betaproteobacteria* (**A**) and *Rhodobacteraceae* (**B**).

The distribution of bulk bacteria was not related significantly to the examined environmental parameters (Table 4). However, the study at narrower phylogenetic levels showed that the contribution of the different bacterial groups to community structure did not vary uniformly across the intervals of the different environmental variables (Table 4). While Quotient rule analysis and quotient curve plots showed similar patterns in the preference of *Eubacteria*, *Alphaproteobacteria* and *Gammaproteobacteria* for increasing chlorophyll *a* levels (even more pronounced for *Betaproteobacteria*), SAR11 on the contrary exhibited pronounced ”avoidance” and relatively lower contributions to BCS at increasing chlorophyll *a* levels (Figure 3A, Table 4) with quotient curve values < 1 at intermediate chlorophyll *a* levels. *Gammaproteobacteria*, *Alphaproteobacteria*, and to a lesser extent *Eubacteria* followed similar patterns of preference and avoidance for increasing levels of temperatures (Figure 3B, Table 4). Not one but several intervals of “preference” and “avoidance” were observed for these groups along the gradient of temperature.

A



B

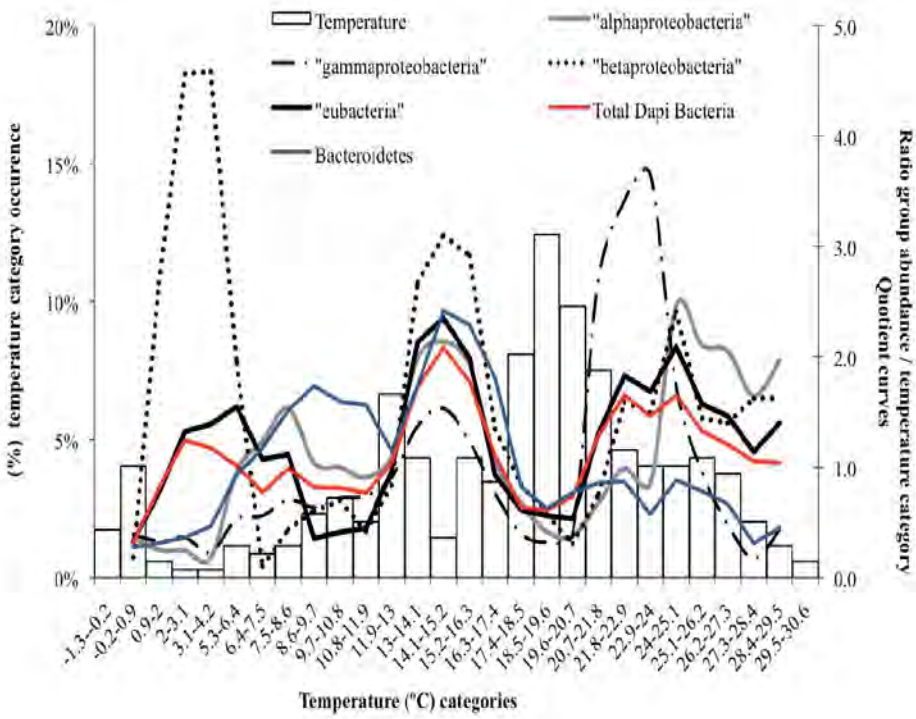
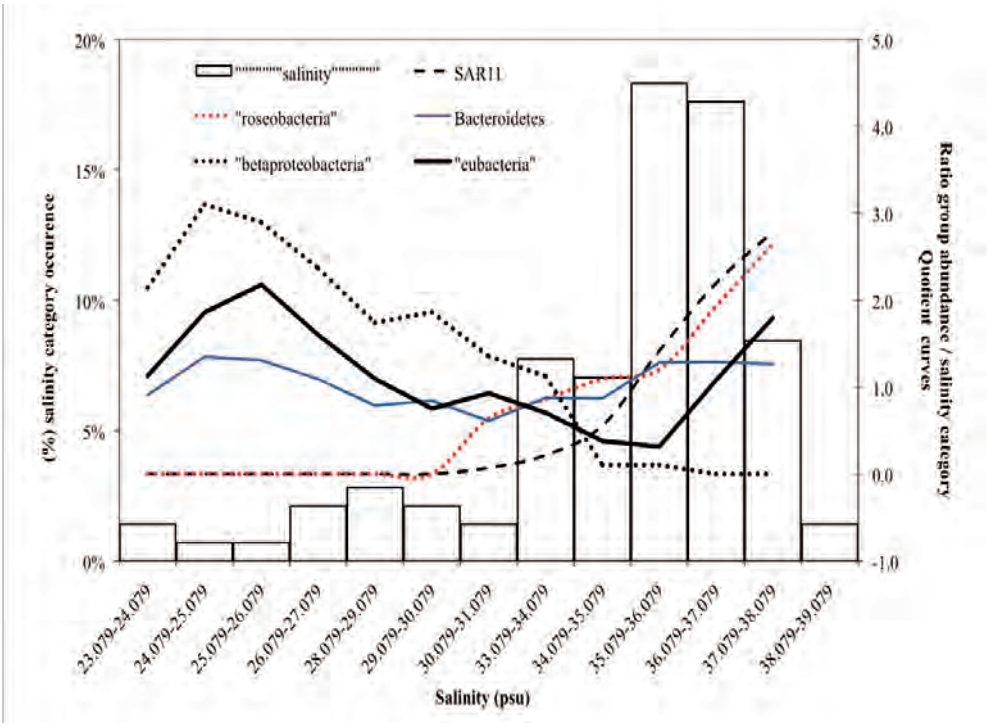


Figure 3: Quotient rule analysis showing the frequency of occurrence of the environmental variables and the occurrence ratio of bacterial group concentration to environmental variables (3 point running means). Environmental variables are chlorophyll *a* (µg l^{-1}) (**A**), temperature (°C) (**B**), Salinity (psu) (**C**). Bacterial group abundances and chlorophyll *a* concentrations were Log transformed.

C



The contribution of *Gammaproteobacteria* to BCS was particularly low from 0 ºC to 7.5 ºC but increased in the temperature interval 11.9 ºC - 17.4 ºC and 19.6 ºC- 26.2ºC. An opposite pattern was observed in the *Betaproteobacteria* quotient curve that showed maximum preference for the low temperature intervals 0ºC - 7.5ºC and increasing avoidance with increasing temperatures (Figure 3B, Table 4). Opposite patterns were also observed with salinity since increasing preference for high salinity was observed for SAR11 and *Rhodobacteraceae*, in opposition to *Betaproteobacteria* that showed progressive avoidance with increasing salinity (Figure 3C, Table 4).

ENV VARIABLES	N	dmax							
		Total bacteria	*Alphaproteobacteria*	SAR11	*Rhodobacteraceae*	*Gammaproteobacteria*	*Bacteroidetes*	*Betaproteobacteria*	EUB
LOG CHLOROPHYLL *A*	20	0.01	**0.13***	**0.26***	0.06	**0.16***	0.04	**0.28***	**0.13***
TEMPERATURE	20	0.02	**0.20***	**0.18***	**0.16***	**0.21***	0.05	**0.23***	**0.15***
SALINITY	13	0.07	0.05	**0.49***	**0.40***	0.07	**0.16***	**0.58***	**0.15***

N: Number of categories

Table 4. Results of the Kolmogorov-Smirnov goodness of fit, testing the normality assumption of the distribution for the different bacterial group relative abundances.

Bacterial group concentrations as a function of chlorophyll *a*- To investigate the relationships between the different bacterial groups and trophic level (as estimated from chlorophyll *a* concentration), the absolute abundances of each bacterial group were regressed against chlorophyll *a* concentrations (log transformed, Figure 4). Parameter details of the 8 different equations are presented in Table 5 and follow the form:

Standardized Log bacterial abundance (cells ml^{-1}) = **intercept** (±SE) + **Slope** (±SE) x log chlorophyll *a* ($\mu g\ l^{-1}$)

We compared the different relationships and tested for the homogeneity of slopes, performing an analysis of covariance that included interaction of covariates. The homogeneity of slopes assumption was rejected (Ancova, n=1980, $p<0.001$) indicating that the slopes between bacterial concentration and chlorophyll *a* differed among bacterial groups. Based on Student's t test for slope comparison (Zar 1999), we observed that the bacterial group abundances increased at different rates with chlorophyll *a* concentration (Figure 2A). All equations were significant (analysis of variance $P<0.0001$).

The slope of 0.35 (±0.02) measured between bulk bacterial concentration (total DAPI counts) and chlorophyll *a* (equation 1A) was significantly different from the different slopes measured at narrower phylogenetic levels (Table 5A). The highest slope was found for *Betaproteobacteria* (equation 8A) followed by *Bacteroidetes* and *Eubacteria* concentration (equations 7A and 2A) with respectively 0.53 (±0.08) and 0.49 (±0.04) and 0.48 (±0.04). Since the lowest slope of 0.13 (±0.04) observed for SAR11 was significantly different than the one measured for *Alphaproteobacteria* at 0.24 (±0.04), significantly different intercepts were also measured, the contribution of SAR11 to the Alphaproteobacteria group decreasing with increasing chlorophyll *a* levels. No significantly different slopes were found between *Alphaproteobacteria*, *Gammaproteobacteria* and *Rhodobacteraceae* (Table 5A). However, significantly different intercepts were found between these groups. *Alphaproteobacteria* and *Bacteroidetes* dominated BCS at low chlorophyll *a* levels but the slopes indicated that the relative contribution of these groups will vary little along the gradient of chlorophyll *a*.

The lowest coefficients of determination (R^2) were measured for the equations of *Alphaproteobacteria*, SAR11 and *Gammaproteobacteria* with respectively 0.10, 0.04 and 0.07, indicating that almost no variance was accounted for by the regression model used. However, the highest R^2 were measured for respectively bulk bacterial and *Bacteroidetes*, indicating that 47% and 45% of the variance was explained by the "X" parameter of the regression.

Lastly, individual pairwise comparisons of the least squares means using Student's t-test showed no significant differences between the relationships when including or excluding the

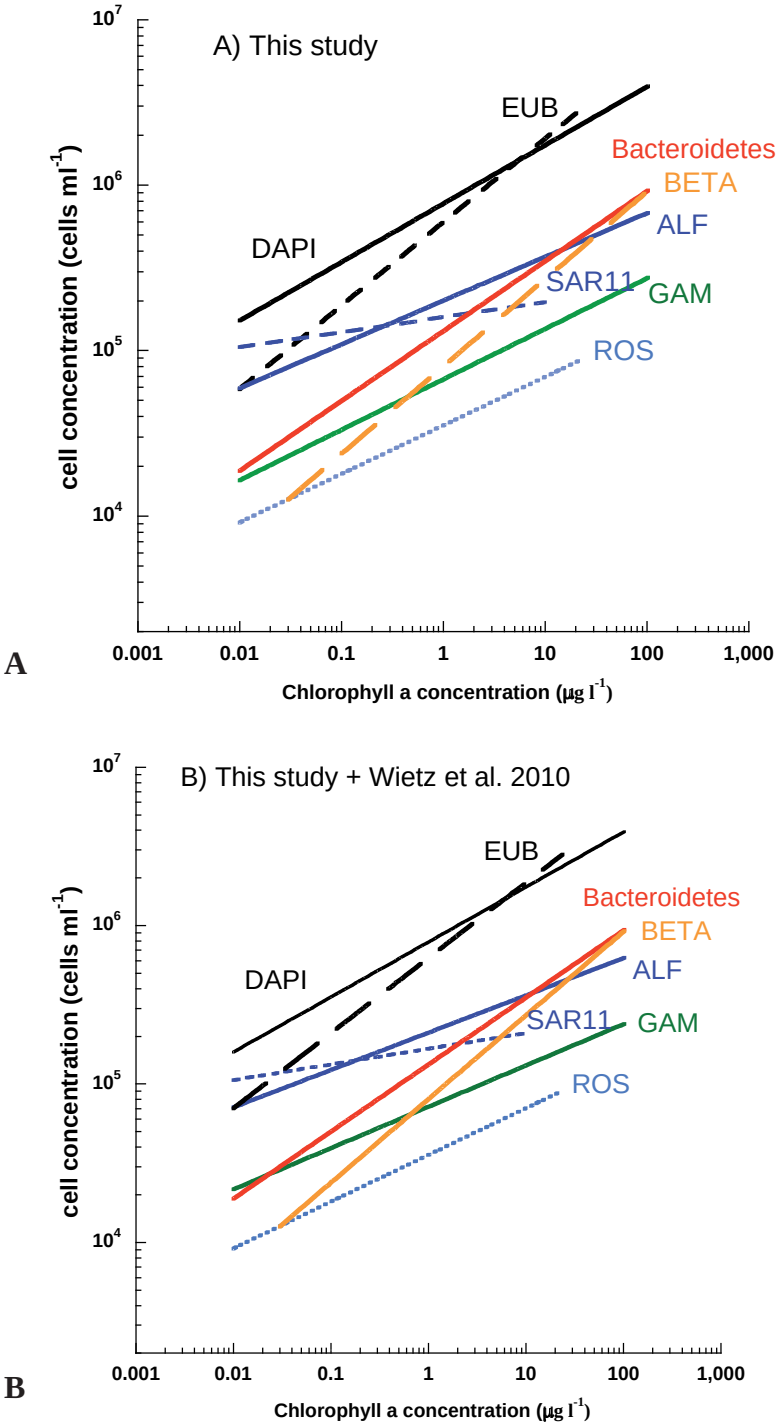


Figure 4: Relationships between log transformed bacterial subgroup absolute abundances and log chlorophyll *a* concentration (µg l^{-1}) with dataset excluding results from Wietz et al. (2010) (**A**) with dataset including results from Wietz et al. (2010) (**B**). "DAPI" corresponded to total bacterial concentration (cells ml^{-1}) as determined by DAPI or Flow Cytometry.

A

LOG CONCENTRATION (CELLS.ML^{-1}) - LOG CHLOROPHYLL *A* (µg. L^{-1})	N $_{obs}$	R^2	Intercept (± SE)	Slope (± SE)	Bulk Bac.	EUB	Alpha	SAR11	Roseo	Gamma	CFB	Beta
BULK BACTERIA (1A)	425	0.39	**5.89*** (±0.02)	**0.35*** (±0.02)	0.00							
EUBACTERIA (2A)	206	0.47	**5.79*** (±0.03)	**0.48*** (±0.04)	**-2.91****	0.00						
ALPHAPROTEOBACTERIA (3A)	245	0.10	**5.32*** (±0.03)	**0.24*** (±0.04)	**2.46****	**4.24****	0.00					
SAR11 (4A)	211	0.04	**5.21*** (±0.04)	**0.13*** (±0.04)	**4.92****	**6.19****	**1.94***	0.00				
RHODOBACTERACEAE (5A)	245	0.23	**4.55*** (±0.03)	**0.29*** (±0.03)	**1.66***	**3.80****	-1.00	**-3.20****	0.00			
GAMMAPROTEOBACTERIA (6A)	233	0.07	**4.84*** (±0.04)	**0.22*** (±0.05)	**2.41****	**4.06****	0.31	-1.41	1.20	0.00		
BACTEROIDETES (7A)	342	0.45	**5.05*** (±0.03)	**0.49*** (±0.03)	**-3.88****	-0.20	**-5.00****	**-7.20****	**-4.71****	**-4.63****	0.00	
BETAPROTEOBACTERIA (8A)	73	0.41	**4.90*** (±0.06)	**0.53*** (±0.08)	**-2.18****	-0.56	**-3.24****	**-4.47****	**-2.81****	**-3.29****	-0.47	0.00

B

LOG CONCENTRATION (CELLS.ML^{-1}) - LOG CHLOROPHYLL *A* (µg. L^{-1})	N $_{obs}$	R^2	Intercept (± SE)	Slope (± SE)	Bulk Bac.	EUB	Alpha	SAR11	Roseo	Gamma	CFB	Beta
BULK BACTERIA (1B)	257	0.33	**5.91*** (±0.02)	**0.38*** (±0.04)	0.00							
EUBACTERIA (2B)	167	0.41	**5.76*** (±0.03)	**0.50*** (±0.05)	-1.87	0.00						
ALPHAPROTEOBACTERIA (3B)	200	0.11	**5.29*** (±0.03)	**0.25*** (±0.05)	2.03	**3.54****	0.00					
SAR11 (4B)	97	0.01	**5.07*** (±0.05)	-0.11 (±0.08)	**5.48****	**6.47****	**3.82****	0.00				
RHODOBACTERACEAE (5B)	144	0.22	**4.53*** (±0.04)	**0.36*** (±0.06)	0.28	1.79	-1.41	**-4.70****	0.00			
GAMMAPROTEOBACTERIA (6B)	183	0.08	**4.87*** (±0.04)	**0.25*** (±0.06)	1.80	**3.20****	0.00	**-3.60****	1.30	0.00		
BACTEROIDETES (7B)	214	0.29	**5.12*** (±0.03)	**0.40*** (±0.04)	-0.35	1.56	**-2.34***	**-5.70****	-0.55	**-2.08****	0.00	
BETAPROTEOBACTERIA (8B)	65	0.38	**4.90*** (±0.07)	**0.52*** (±0.08)	-1.57	-0.21	**-2.86***	**-5.57****	-1.60	**-2.70****	-1.34	0.00

C

LOG CONCENTRATION (CELLS.ML^{-1}) - LOG CHLOROPHYLL *A* (µg. L^{-1})	N $_{obs}$	R^2	Intercept (± SE)	Slope (± SE)	Bulk Bac.	EUB	Alpha	SAR11	Roseo	Gamma	CFB	Beta
BULK BACTERIA (1C)	168	0.21	**5.79*** (±0.04)	**0.25*** (±0.04)	0.00							
EUBACTERIA (2C)	39	0.60	**6.09*** (±0.10)	**0.66*** (±0.09)	**-4.16****	0.00						
ALPHAPROTEOBACTERIA (3C)	45	0.18	**5.51*** (±0.08)	**0.34*** (±0.11)	-0.77	**2.25****	0.00					
SAR11 (4C)	114	0.28	**5.40*** (±0.05)	**0.31*** (±0.05)	-0.94	**3.40****	0.25	0.00				
RHODOBACTERACEAE (5C)	101	0.17	**4.54*** (±0.06)	**0.25*** (±0.06)	0.00	**3.79****	0.72	0.77	0.00			
GAMMAPROTEOBACTERIA (6C)	50	0.00	**4.58*** (±0.09)	-0.06 (±0.12)	**2.45****	**4.80****	**2.46****	**2.85****	**2.31****	0.00		
BACTEROIDETES (7C)	128	0.36	**4.95*** (±0.06)	**0.48*** (±0.06)	**-3.19****	1.66	-1.12	**-2.18****	**-2.71****	**-4.02****	0.00	
BETAPROTEOBACTERIA	8		----	----	-----	-----	-----	-----	-----	-----	-----	-----

*: significant values (p<0.005)
**: significant values (p<0.0025)

Table 5. Regression analyses (left part of the panels) and t tests results (right part of the panel) for slope comparison of log transformed and standardized bacterial group concentration vs log transformed chlorophyll *a* concentration at the global scale (**A**), in coastal (**B**), and in open-ocean conditions (**C**) following the equation:

Standardized Log bacterial group (cells. ml^{-1}) = intercept (±SE) + Slope (±SE) x log chlorophyll *a* (µg. l^{-1})

dataset of Weitz et al. (2010) (t tests, $p<0.05$) (Figure 2B).

Coastal versus open-ocean ecosystems- We investigated how different were the relationships between the different bacterial groups and trophic level among coastal or open-ocean ecosystems. The homogeneity of slopes assumption was rejected (Ancova, $n_{coastal}=1242$, $n_{open}=738$, $p<0.001$) indicating that the slopes between bacterial concentration and chlorophyll *a* differed among bacterial groups in both conditions. Comparing the slopes of the relationships among ecosystems by Student's t test (as previously performed at the global scale), we observed that the bacterial group abundances increased at different rates with chlorophyll *a* concentration (Table 5B and 5C). Except for the SAR11 relationship that was not significant in coastal environments (Table 5, equation 4B), all equations were significant in both ecosystems (analysis of variance $P<0.0001$). While the slopes measured for bulk bacteria and *Gammaproteobacteria* appeared significantly lower in open-ocean than coastal ecosystems, the slope of SAR11 in open-ocean was significantly higher (t tests, $p<0.005$) (Table 5B and C).

In coastal conditions, almost no variance was accounted by the regression equations of SAR11 and *Gammaproteobacteria* with R^2 of respectively 0.01 and 0.08 (Table 5B). Higher R^2 values were measured for the other equations, ranging from 0.22 to 0.41 for *Rhodobacteraceae* and *Eubacteria* respectively, indicating that the regression models were good at explaining the variance. The highest slope was measured for *Betaproteobacteria* (equation 8B) with 0.52 (±0.08), followed by *Eubacteria* and *Bacteroidetes* concentration (equation 2B and 7B) with respectively and 0.50 (±0.05) and 0.40 (±0.04). Compared with the negative non significant slope found for SAR11, significantly higher slopes were measured for *Alphaproteobacteria* and *Gammaproteobacteria* with respectively 0.25 (±0.05) and 0.25 (±0.06) (Table 5B).

In open-ocean sites, the highest slope was found for *Eubacteria* (Table 5C, equation 2C) followed by *Bacteroidetes* (equation 7C) with respectively 0.66 (±0.09) and 0.48 (±0.06). While no significant slope was found for *Gammaproteobacteria* with -0.06 (±0.12) with R^2 close to 0, indicating that no variance was accounted for by the regression, higher R^2 and a significantly different slope of 0.31 (±0.05) was measured for SAR11 (equation 4C). However, neither significantly different slopes nor different elevations (ordinates) were observed between the SAR11 and *Alphaproteobacteria* equations (Table 5C), indicating that most of the *Alphaproteobacteria* relationship was driven by the SAR11 contribution. Since significantly different slopes and intercepts were measured between SAR11 and *Bacteroidetes*, *Bacteroidetes* contribution along the gradient of chlorophyll *a* in open-ocean conditions will tend to increase faster than that of SAR11.

Multiple Linear Regression analysis- We calculated the Standardized Beta (Std Beta) coefficients in order to compare the effect of different independent variables in a multivariate regression. Bacterial group absolute abundances (cells ml^{-1}) were first expressed as a function of temperature and chlorophyll *a* concentration and second, as a function of salinity and chlorophyll *a* concentration (Table 3). From the beta weights we observed that chlorophyll *a* effects on bacterial absolute abundances were stronger than the temperature effects (F test, $p<0.05$). However, total bacterial cell concentration (as counted by DAPI or by flow Cytometry), Eubacteria and SAR11 absolute abundances were significantly controlled by both parameters, indicating that bacterial concentration tended to increase with increasing levels of chlorophyll *a* and with increasing temperatures (F test, $p<0.05$). While for total and Eubacterial cell abundances the model using both explanatory variables had a coefficient of determination (R^2) of 0.43 and 0.41 respectively, indicating that the multiple regression was good for fitting the data, the R^2 corresponding to the SAR11 absolute abundance equation was close to 0, indicating that very small variance was accounted for by the regression, in spite of the high statistical significance of the regression equation as measured by the F-ratio.

When bacterial subgroup absolute abundances (cells ml^{-1}) were expressed as a function of salinity and chlorophyll *a* concentration (Table 3), we observed both a significant negative effect of salinity and a positive effect of chlorophyll *a* concentration on bulk bacterial concentration, *Betaproteobacteria* and *Rhodobacteraceae* absolute abundances (F test, $p<0.05$). For total bacterial abundance and for these two groups, the multiple regression model had a R^2 of respectively 0.22, 0.32 and 0.29 indicating that the multiple regression equation fitted relatively well the data, as confirmed by the high significance of the F-ratio ($p<0.0001$).

While we tried regression models with all three independent variables (and also including total bacterial abundance as an independent variable), these models were not better (i.e. did not have a better F-ratio) than the models with two independent variables enumerated above.

DISCUSSION

Bacterial abundance and biomass measured at the community level have been seen to covary with the trophic status of a variety of ecosystems (Bird and Kalff 1984; Cole et al. 1988; Gasol and Duarte 2000). However, less is known about the relationship at more specific phylogenetic levels. Considering the increasing number of studies about in situ quantification of the major bacterial groups composing the bacterial communities, the global scale variability of BCS and its linkage with ecological and environmental parameters is now identifiable. We intended to establish a phylogeography of the major bacterial groups from in situ estimation by FISH or CARDFISH techniques and to precise the bacterial groups interrelation with phytoplankton. We used in this study a statistical approach based on a comparative analysis of 33 different studies reporting both relative and absolute major bacterial group abundance.

Bacterial group biogeography- Our analysis confirmed the global prevalence of *Alphaproteobacteria* and the SAR11 clade in both coastal and open-ocean conditions (Morris et al. 2002; Wietz et al. 2010). The particularly pronounced contribution of *Bacteroidetes* observed here in coastal conditions (whose average relative abundance was exceeding the one observed for *Alphaproteobacteria*) (Table 2) contrasted with previous global scale surveys of bacterial community structure (Pommier et al. 2007; Wietz et al. 2010). Previous studies described *Bacteroidetes* as great contributors in coastal waters and polar regimes, apparently linked to algal blooms and involved in the degradation of polymers such as polysaccharides and proteins (Cottrell and Kirchman 2000; Eilers et al. 2001).

Although the range and the average relative abundance of the different groups (expressed in terms of % of DAPI counts) was significantly higher in coastal than in offshore conditions (except for SAR11 and *Alphaproteobacteria* for which it was the contrary), we showed that the different bacterial groups exhibited different levels of contribution to BCS across different intervals of chlorophyll *a*, temperature and salinity, indicating the presence of biome-related variations in bacterial group relative distribution (Pommier et al. 2007; Wietz et al. 2010). In particular, the preference of *Betaproteobacteria* for colder waters contrasted with increasing preference patterns in *Gammaproteobacteria* for warmer temperatures (Figure 3) and was similar to the previous findings of Wietz et al. (2010) that measured significant differences in the fraction of *Bacteroidetes* at low or high latitudes.

However, note that the patterns observed among the major bacterial groups could also result from the large coverage of broad bacterial probes, hiding patterns in bacterial community

structure at narrower phylogenetic levels. As an example, different SAR11 ecotypes have been shown to vary through time and depth in response to physical and chemical variability (Field et al. 1997; Morris et al. 2005; Carlson et al. 2009). Similarly, different *Flavobacteria* clades, such as DE2 occurring in polar biomes and the VISION clades preference for Arctic provinces, suggest that distinct Flavobacterial clades have different niches and could present different life strategies (Gómez-Pereira et al. 2010).

Relationships between bacterial community structure and trophic level- The average relationship between bulk bacterial and autotroph biomass is characterized by a slope <1 (Gasol and Duarte 2000). The model 1 regression slope found in this study averaged 0.35 (±0.02) and was lower than the slope average of 0.46 (±0.09) measured by Li et al. (2004) across a variety of biogeochemical provinces or the average of 0.47 (±0.03) reported in a comparative analysis of 33 compiled empirical relationships earlier measured from different aquatic ecosystems (Gasol and Duarte 2000). However, the slopes we measured with *Eubacteria* (0.48-0.66) were larger than the value found with DNA-binding dyes such as DAPI (0.35-0.38), indicating that the bacterial fraction of total cell counts which can be visualized by in situ hybridization increased with trophic level. The capacity to detect bacteria using FISH has been correlated to RNA content and thus to the state of single-cell activity (Karner and Fuhrman 1997; Tolkier-Nielsen et al. 1997) and there are increasing evidences that the hybridization technique preferentially detects the cells with a higher level of activity (Oda et al. 2000; Bouvier and del Giorgio 2003). Since bacterial activity increases with trophic state (e.g. Cole et al. 1988), the increasing proportion of cells that could be hybridized with the Eubacterial probe may have been related to changes in community metabolism as suggested by Bouvier and del Giorgio (2002; 2003).

Here we investigated the coupling between primary producers and bacteria at more detailed phylogenetic levels, focusing on bacterial group absolute abundances as determined by FISH (expressed as cells per ml). Distinct log-log relationships were observed with significantly different regression slopes than the one described between bulk bacterial abundance and chlorophyll *a* relationship, ranging from 0.13 (±0.04) to 0.53 (±0.08) for SAR11 and *Betaproteobacteria* respectively, indicating that the different bacterial groups were differently coupled and interrelated to phytoplankton biomass. The rates at which the different bacterial group abundances increased across the gradient of phytoplankton biomass (as estimated from chlorophyll *a*) could indicate different metabolic aptitudes for utilizing the organic matter originating from phytoplankton release processes and thus, different contribution to the recycling of organic matter in the ocean. In that sense, the bacterial groups SAR11 and *Gammaproteobacteria* were the ones with the lowest slopes to chlorophyll *a* and thus with a weaker relation to phytoplankton, while stronger links were

found for *Betaproteobacteria* and *Bacteroidetes*.

BCS-chlorophyll *a* relationships in coastal and open-ocean conditions- Dividing the dataset according to the type of ecosystem (coastal or offshore), we found different regression slopes across increasing chlorophyll *a* levels (Tables 5B and 5C). The regression slopes between bulk bacterial biomass and chlorophyll *a* are less than 1 (Li et al. 2004; Gasol and Duarte 2000), an thus, the bacterial to phytoplankton biomass ratio tend to decrease across increasing gradient of productivity (Fuhrman et al. 1989; Cho and Azam 1990; Li et al. 1993; Buck et al. 1996). However, the slope of this ratio has been shown to vary amongst ecosystem type such as offshore, coastal waters or lakes (Simon et al. 1992; del Giorgio and Gasol 1995; Gasol et al. 1997). Similarly, evidences of significantly different slopes between bulk bacterial abundance and chlorophyll *a* concentration in coastal or in offshore conditions have also been reported by both Cho and Azam (1990) that measured positive regression slopes in eutrophic systems and slopes not statistically different than 0 in oligotrophic systems (<0.5 μg chlorophyll *a* l^{-1}) or by Buck et al. (1996) that also found lower slopes at lower levels of trophy. In this study, relatively lower regression slopes were measured in open ocean ecosystems than in coastal conditions with 0.25 (±0.04) and 0.38 (±0.02) respectively (Table 5C), indicating a lower response of bacterial abundance to increasing phytoplankton biomass in oligotrophic conditions (Gasol and Duarte 2000; Gasol et al. 2009). Whilst bacterial community structure was not analyzed in these previous studies, one can consider that such low slopes found at the bulk level in offshore systems may indicate that the bacterial groups exhibiting the lowest regression slopes were mainly driving the relationship. Indeed, the relatively low slope averages of 0.24 (±0.04) and 0.22 (±0.05) respectively found for *Alphaproteobacteria* and *Gammaproteobacteria* in comparison with their high contribution to BCS corroborate previous findings by Baltar et al. (2007) showing SAR11 as the main responsible for the surface water variability in bulk prokaryotic abundance during a transect from coastal waters to offshore waters of the Canary coastal transition zone.

Contrarily to the generalist paradigm stating that all bacterial groups use the DOCp in an indiscriminate way (Sarmento and Gasol, submitted and ref therein), evidences of co-occurring shifts in both bacterial and phytoplankton community structures (Pinhassi et al. 2004; Van Hannen et al. 1999; Grossart et al. 2005) have since stimulated the "specialist" paradigm in which bacterial groups are specifically linked to specific phytoplankton groups through the differences of quality of the dissolved organic matter excreted (Sarmento and Gasol, submitted). In agreement with the relatively high slopes found in the present study between *Bacteroidetes* and trophic level, several studies have not only identified *Flavobacteria* (belonging to the *Bacteroidetes* phylum) in the algal phycosphere (Rooney-Varga et al. 2005; Sapp et al. 2007) but also reported of its association

to dinoflagellates (Hold et al. 2001) or diatom blooms either by means of molecular fingerprinting techniques (Riemann et al. 2000), or by determining the number of active *Bacteroidetes* cells by MAR-FISH (Sarmento and Gasol, submitted). Moreover, evidences of concomitant shifts in bacterial metabolism and community structure have been shown during dinoflagellate bloom off the Southern California coast (Fandino et al. 2001). Similarly, Tada et al. (2011) using bromodeoxyuridine immunocytochemistry associated to the FISH technique (BIC-FISH) analyzed the bacterial community structure and activity during phytoplankton blooms in the western North Pacific Ocean and showed that *Betaproteobacteria* was strongly correlated with organic matter supply originating from algae.

Even thought association of *Alphaproteobacteria* and *Gammaproteobacteria* with microalgae has been reported in different studies (Hold et al. 2001; Grossart et al. 2005), only weak relationships as indicated by low R values (Table 5) with low rates of increase across the gradient of chlorophyll *a* were measured at the global scale for SAR11 and *Gammaproteobacteria* with slopes of 0.13 (±0.04) and 0.22 (±0.05) respectively. However, these relationships changed when the type of ecosystem was taken into account. A significantly higher slope of 0.31 (±0.05) was measured for SAR11 in open-ocean, while significantly higher value was found for *Gammaproteobacteria* in coastal conditions, indicating specific links between the different bacterial groups and phytoplankton among each type of ecosystem. SAR11 has been shown by FISH to occur abundantly as free-living cells in surface oceans (Morris et al. 2002). Evidences of proteorhodopsin genes expression in strain *Pelagibacter ubique* (Giovannoni et al. 2005) indicate that SAR11 could produce energy either by assimilating DOCp or by using a light-driven proteorhodopsin proton pump, that may not only confer an advantage in growth rate in nutrient-depleted conditions (Giovannoni et al. 2005), but may also lower the relationships with phytoplankton as suggested by the particularly low slope measured in this study. Note that a relatively low slope of 0.24 (±0.04) also measured for the whole *Alphaproteobacteria* group indicated that the relationship was mostly driven by SAR11 high contribution within the *Alphaproteobacteria* phylum, supporting the idea proposed by Yokokawa and Nagata (2005) that different ecological traits (DOCp uptake, bacterial production and growth, grazing rates) can be detected by the use of broad phylogenetic probes, because an ecological trait of a broad group largely reflects the trait of the dominant subgroup in oceanic communities. Low coupling between SAR11 and chlorophyll *a* might also indicate the use by this dominant bacterial group in oceanic waters of non-contemporaneous primary production, something that was suggested to occur for the bulk community across the central Atlantic gyre (Gasol et al. 2009).

On the other hand, less positive or even a zero slope in the relationship between bacterial biomass and the resource gradient can also indicate strong mortality and top-down control on bacteria (Pace and Cole 1994). Li et al. (2004) found a macro-ecological relationship between bulk

bacterial abundance and chlorophyll *a* and observed a non-linearity from positive at low trophic levels to negative at high trophic levels. This transition was thought to represent a transition from bottom-up to top-down control of bacteria by grazing or viruses (Li et al. 2004). However, it may also be linked to the differences in bacterial community structure and bacterial regression slopes across the different trophic levels. Indeed, evidences for different patterns in control of bacterial community compositions linked to differential growth and mortality rates among the major bacterial group were revealed by Yokokawa and Nagata (2005) in western North Pacific coastal waters and by Ferrera et al. (2011) in Blanes Bay measuring lower growth rates of the more abundant groups such as *Alphaproteobacteria* and SAR11 relative to less abundant *Bacteroidetes* and *Gammaproteobacteria*. Ferrera et al. (2011) experimentally showed that even though SAR11 was the most abundant bacterial group, it was also the slower grower. This links to our findings of weak relationships between *Alphaproteobacteria* and SAR11 abundance and chlorophyll *a* in coastal ecosystems in comparison to their importance in terms of contribution to BCS.

In conclusion, our results indicate that bacterial community structure is not random but follows gradient of environmental parameters at different rates. Our results indicate that the relationships between bacterial group abundance and phytoplankton biomass are also a function of the phylogenetic level at which the bacterial community was studied, also a function of the type of ecosystems in which the relationship was measured.

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General discussion

The goal of this thesis was to identify patterns in picoplankton community structure (PCS) and bacterial phylogenetic community structure (BCS) at different spatio-temporal scales, identifying differences in picoplankton community structure between coastal and offshore ecosystems and describing trends in microbial community structure along a gradient of environmental parameters. Two different methods were used for defining microbial groups. The first one was based on flow cytometry and the discrimination of different groups according to DNA content, chlorophyll *a* fluorescence and scatter properties, which allowed to distinguish heterotrophic bacteria from the three different photosynthetic groups within the picoplankton size fraction (<2-3 μm): *Synechococcus*, *Prochlorococcus* and photosynthetic Picoeukaryotes (pPeuk). The second was based on the division of the bulk bacterial community into different phylogenetic groups as determined by FISH and CARDFISH methods.

The analysis of variability in BCS and PCS performed at different spatial and temporal scales was proposed here as one way for attributing ecological functions to the microbial groups composing marine ecosystems. The analysis of BCS performed at the large scale in **Chapter IV** allowed to distinguish for each bacterial phylogenetic group different trends along gradients of chlorophyll *a*, temperature and salinity, while the PCS was studied with a major focus on the small temporal scale (**Chapter II**) and limited to a relatively small spatial area (Blanes bay and a coast to offshore transect) (**Chapter III**). In this section, we will test to what extent the patterns in community structure identified at the relatively small scales are coherent with those measured at the global scale, by collecting both published and non published data for a secondary analysis of picoplankton group abundance as determined flow cytometrically and applying the carbon conversion factor for pPeuk calculated in **Chapter I.**

pPeuk contribution to the total biomass

The importance of picophytoplankton (<2-3 μm) in marine food webs stems from several observations: In addition to their ubiquity in both marine and freshwater ecosystems, they contribute significantly to total phytoplankton biomass (Stockner 1988), their relative contributions to biomass and primary production tending to increase in oligotrophic regions (Agawin et al. 2000; Bell and Kalff 2001).

However, the accurate assessment of phytoplankton carbon standing stocks can be complicated by the strong variability in individual cell volume (several orders of magnitude) and abundance of different size fractions. The size structure of phytoplankton biomass is fundamental

for determining the fate of assimilated carbon, affecting pathways and rates of growth. Relative to *Synechococcus* and *Prochlorococcus*, pPeuk have been greatly ignored in spite of their large participation in primary production and picoplankton biomass over wide oceanic regions (Li et al. 1994; Worden et al. 2004). Because of their small size relative to large phytoplankton (e.g. diatoms), pPeuk are believed to contribute relatively little to carbon export. However, it is possible that the participation of pPeuk in ocean carbon flux might have been greatly disregarded (Richardson and Jackson 2007)

Several studies have shown that most of the variance measured in the contribution of *Synechococcus* and *Prochlorococcus* to picoplankton biomass could be explained by the variability in their numerical abundance, while both abundance and cell size explained the variance measured in Picoeukaryote contribution to total carbon (Worden et al. 2004; Shalapyonok et al. 2001; Durand et al. 2001). The discrepancies in pPeuk size structure suggest that natural pPeuk communities are not always composed by the same species and strains but rather by different taxonomical groups (Worden et al. 2004; Worden and Not 2007), varying not only in cell size and cell abundance, but possibly also in terms of carbon content per cell. To constrain these uncertainties, we choose in **Chapter I** to focus more particularly on the establishment of a new carbon conversion factor for pPeuk using a set of different cultures ecologically representative of natural pPeuk assemblages.

We found an average carbon content per pPeuk of 1540 fgC $cell^{-1}$ corresponding to a cell volume average of 2.14 μm^3 equivalent to a cell diameter of 1.60 μm, likely representative of the picoplanktonic size category constrained between 0.2 and 2 μm (as defined by Sieburth 1978). While this value was apparently close to the conversion factor estimated at 1500 fgC $cell^{-1}$ by Zubkov et al. (Zubkov et al. 1998; 2000), we showed that the pPeuk cell could contain much more carbon that it was believed before, because when calculating the carbon content per unit of volume (C/V) as a measure of the carbon cellular density (fgC μm^{-3}), we established an average C/V value of 467 fgC μm^{-3}, higher than the value previously thought, (e.g. 220 fgC μm^{-3}). As an example, Zubkov et al. (1998; 2000) estimated this value from the relationship proposed by Booth (1988) for a cell diameter average of 2.35 μm as determined by using image analysis of randomly selected cells stained by DAPI (Sherr et al. 1987). The value we found for the pPeuk is much closer to the one found for *Synechococcus* at 470 fgC μm^{-3} estimated from the relationship described by Verity et al. (1992). Using our overall median value of 467 fgC μm^{-3} instead of the 220 fgC μm^{-3} that has been widely used (e.g. Campbell et al. 1994; Zubkov et al. 1998; 2000…) and assuming the cell size average of 2.35 μm determined by Zubkov et al. (1998) instead of the average cell size of 1.60 μm determined for the mixture of pPeuk in **Chapter I**, the estimation of pPeuk biomass would have increased by more than two-fold and reached an extremely high value of 3176 fgC $cell^{-1}$, meaning that pPeuk should be considered to make a greater contribution to total

biomass. The application of this carbon content per cell (instead of the 1540 fgC cell^{-1}, **Chapter I**) to a database collected from a panel of both published and non published studies containing picoplankton group abundances measured by flow cytometry during different oceanographic cruises, covering a wide range of oceans and marine provinces (Table 1) would have increased pPeuk relative contribution to picophytoplankton by an average of 30% at the global scale. Whether such very high CF value might be or not relevant for characterizing Picoeukaryotes communities will likely depend of how much of the pPeuk community is dominated by the larger cells (e.g. *Ochromonas* or *Chrysochromulina* with 4.38 μm and 5.06 μm respectively, **Chapter I**). Other authors showed also large discrepancies in the cell size average of the pPeuk community. Grob et al. (2007) measured a cell average of 1.74 ± 0.13 μm that ranged from 1.37 to 1.99 μm for Peuk communities from Eastern South Pacific Ocean, close to our value of 1.60 μm but lower than the average size measured for Peuk communities in North and South Atlantic subtropical gyres (2.35 μm, Zubkov et al. 1998) or in tropical Pacific Ocean (2.28 μm, Claustre et al. 1999). For these reasons, a more accurate determination of the cell size of natural pPeuk communities, together with the quantification of the relative contribution of the different taxa to the Peuk community, is

CRUISE	SAMPLE AREA
Latitud-2 (Gasol et al. unpublished)	From NW African coast To southern Atlantic
Inco-I and Inco-II (Gasol et al. unpublished)	NW Iberian Penisula (Ría de Vigo) From NW African coast
Coca-II (Gasol et al. unpublished)	To offshore North Atlantic Subtropical gyre
Dharma (Zabala et al. unpublished) (Diez et al. 2004)	Southern Ocean Weddell sea
Varimed'96 (Gasol et al. unpublished)	NW Mediterranean From coast to offshore transect
Blanes Bay (Gasol and Massana, unpublished)	coastal NW Mediterranean
AMT 3, 4, 6 (Zubkov et al. 2000)	From North Atlantic To Southern Atlantic Ocean
Mater 97 and 98 (Massana et al. 1997) (Diez et al. 2001)	SW Mediterranean Alboran Sea
Wecoma (del Giorgio and Gasol, 2011)	North Pacific From coast to offshore transect
Charpy and Blanchot 1998	Atoll lagoon French Polynesia
Sondergaard et al. 1991	Danish coastal waters (Baltic)
Hall and Vincent 1990	SW Pacific Ocean

Table 1. Geographic locations of the different cruises and studies used for this discussion, regrouping picoplankton group abundances determined by flow cytometry.

crucial for estimating more accurately their contribution to total picoplankton biomass.

This rough estimate of higher pPeuk participation in picophytoplankton biomass might corroborate previous studies. For example, Amacher et al. (2009) showed that most of 18S rDNA sequences retrieved in the eastern subtropical North Atlantic from trap material below the euphotic zone could be assigned to small phytoplankton taxa rather than large ones (such as diatoms) as it was previously thought, suggesting that the contribution of smaller taxa to carbon export was greatly underestimated. Corroborating such observation, Richardson and Jackson (2007) suggested that all primary producers, and not only the large phytoplankton cells, could contribute to the carbon export at rates proportional to their production rates. Among the variety of parameters implied in the sinking dynamics of particles to the deep ocean such as cell size and shape (Smayda 1970), one should also include the relatively high C/V ratio found in small eukaryotes as a factor of primary importance.

In **Chapter I**, no significant differences were found between the C/V in organisms considered representative of coastal or open-ocean environments, although the C/V of open-ocean species was highly variable (with a relatively large distance between the 25^{th} and 75^{th} quartiles, Figure 2B). However, further studies centered on the quantification of the different taxa and the findings of patterns in pPeuk phylogenetic distribution are still required for accurately estimating picophytoplankton carbon standing stocks, particularly if we consider that on the one hand, pPeuk communities are composed of complex assemblages of diverse species with specific contributions that are largely unknown and, on the other hand, that the eukaryotic mean cell size vary among ecosystems. Shalapyonok et al. (2001) showed for instance profound changes in phytoplankton community composition in the Arabian sea during summer South West and fall North East monsoon, that were accompanied by characteristics shifts in phytoplankton size structure, the mean eukaryotic cell size being smaller in the most nutrient-rich well mixed waters, while larger cells were found in more offshore conditions. When the relative abundances of the different picophytoplankton groups were considered, such changes in mean eukaryotic cell size resulted in overall homogeneity of their contribution to carbon biomass.

SMALL VERSUS GLOBAL SCALE PATTERNS IN PICOPLANKTON COMMUNITY STRUCTURE

Several large-scale surveys have demonstrated a link between the composition of the picoplankton community and water mass properties (Li 1995; Buck et al. 1996; Zubkov et al. 2000; Li and Harrison 2001; Tarran et al. 2001; Shalapyonok et al. 2001), showing for example that while *Synechococcus* and pPeuk were more typical of shelf and continental margin waters, *Prochlorococcus* were more important in central ocean gyres (Zubkov et al. 2000). The multi-

scale analysis of variability in PCS performed in **Chapter III** (and in a lesser extent in **Chapter II**) revealed that the different picoplankton group abundances showed different amplitudes of variation, the highest variability being attributed to pPeuk abundance, while the lowest was found for heterotrophic bacteria. Moreover, differing patterns, much more complex than simply co-occurrence, in *Synechococcus* and pPeuk were measured along this gradient of environmental parameters, suggesting that these two groups might have different strategies and functions in the microbial food web.

One particular feature discussed in **Chapter II** was the shift in PCS measured at the short time scale, thought to be provoked by a turbulence event and revealed by a two fold increase, from one day to another, of the ratio between the two major contributors to picophytoplankton biomass at the Blanes Bay station during winter (*Syn*:pPeuk). It pointed out the importance of transient meteorological events in structuring the coastal picoplanktonic communities and showed that significant ecological events often result from episodic physical forcing operating at short time scales (Seymour et al. 2005).

In **Chapter III**, opposite patterns were found between this ratio and chlorophyll *a* concentration levels, at both short (diel scale) and large temporal scales (time series at Blanes coastal station). The ratio was indeed maximum at the lowest daily chlorophyll *a* levels (around 12H00 A.M), as well as during summer. However, the variability of this ratio was only studied at a relatively small spatial scale during the transect (100 km), and an additional objective of this

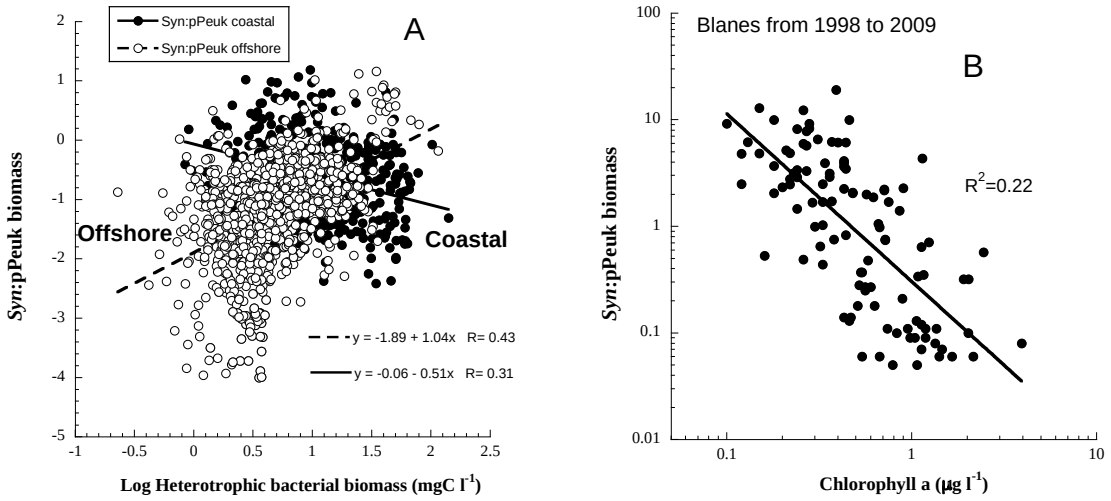


Figure 1. Log transformed *Synechococcus* to pPeuk biomass ratio along the gradient of heterotrophic bacterial biomass in coastal and offshore ecosystems combining all data from Table 1 (**A**) along a gradient of chlorophyll *a* at Blanes coastal station from 1998 to 2009 (**B**).

discussion is to identify patterns in PCS at the global scale, looking more particularly on how the ratio of *Synechococcus* to pPeuk (*Syn*:pPeuk) evolved along a gradient of heterotrophic bacterial biomass and among ecosystems (coastal and open-ocean) (Figure 1).

Two opposite patterns were found: the ratio tended to rise with increasing bulk bacterial biomass in offshore ecosystems, while it decreased in coastal conditions (Figure 1).

These two opposite patterns imply that while the biomass of pPeuk reached its maximum relatively to *Synechococcus* at high heterotrophic biomass in coastal conditions (and high chlorophyll *a* levels if we suppose chlorophyll *a* levels related to bacterial biomass). Inversely, *Synechococcus* biomass reached its maximum relatively to pPeuk in offshore waters (Figure 1A). Studying how this ratio varied on a large temporal scale in a coastal station taking the example of Blanes Bay from 1998 to 2009, we observed a decreasing contribution of *Synechococcus* relatively to pPeuk, for increasing chlorophyll *a* levels (Figure 1B). These large-scale patterns would likely corroborate the findings of **Chapter III**, in which the mesoscale patterns in PCS from coast to offshore waters showed an increase of pPeuk contribution to biomass from coastal station C to the slope CM (from low to high chlorophyll *a* levels), while *Synechococcus* contribution decreased, suggesting that the ratio was driven by the differences between the ecosystem characteristics of coastal station C, slope station CM, or more offshore influenced stations. Evidences of such a link between PCS and water mass properties were reported by Hall and Vincent (1990) in the upwelling regions off the south island of New Zealand, who observed that the abundance of *Synechococcus* and pPeuk increased with the distance towards offshore, reaching a maximum at slope stations, but also by Calvo-Díaz et al. (2004) showing that Picoeukaryote cell abundance exceeded cyanobacterial abundance (mostly *Synechococcus*) in the chlorophyll *a* rich Cantabrian Sea zones, suggesting different PCS between shelf and open-ocean waters. Sherr et al. (2005), however, reported negative relationships between pPeuk abundance and chlorophyll *a*, and low abundance of small picophytoplankton in upwelled waters off the Oregon coast. The same decreasing tendency of the ratio *Syn*:pPeuk for increasing chlorophyll *a* levels was observed when both *Synechococcus* and pPeuk abundances were considered, and this corroborates our results of larger contributions of pPeuk to picophytoplankton biomass relatively to *Synechococcus* at high chlorophyll *a* levels in coastal ecosystems.

Tarran et al. (2006) described latitudinal shifts in standing stocks of pPeuk in the Atlantic Ocean by showing different peaks of eukaryotic group abundance, including one particularly pronounced in the Mauritanian upwelling off the west coast of Africa. For comparison, an additional objective of this discussion was to know to what extent the ratio Syn:pPeuk could also vary with latitude, selecting, for that purpose, data from the Atlantic Ocean (from Table 1, Figure

2). Two clear maxima could be distinguished, indicating that *Synechococcus* biomass contribution clearly exceeded pPeuk biomass in very precise locations. The first peak was observed around the position 20ºN in the Mauritanian upwelling, while the second was found around 40ºS and corresponded to the Argentine shelf-break zone, both regions known as productive systems. Upwelling systems constitute major oceanic processes, representing around 80-90 % of global

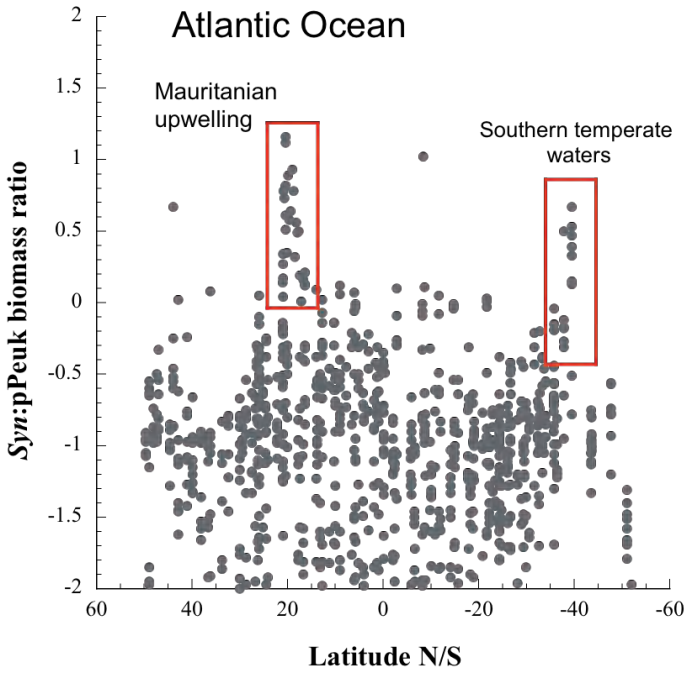


Figure 2. Log transformed *Synechococcus* to pPeuk biomass ratio (*Syn*:pPeuk) along a transect from North to South Atlantic Ocean combining surface data (<40m) from AMT3, AMT4, AMT6 cruises (Zubkov et al. 2000), Latitud-II and Coca-II (Gasol et al. unpublished).

ocean new production (Berger et al. 1989; Brink et al. 1995). To what extent such high ratio value found at high chlorophyll *a* levels likely indicated different controlling factors of the different abundances in such productive environments (e.g. differential grazing rates, etc…) still remains to be elucidated.

Predicting bacterial and picoplankton community structure from environmental parameters

An important goal of this thesis was to describe patterns not only in PCS at different spatial and temporal scales, but also to identify ecological patterns in BCS along gradients

of environmental parameters such as chlorophyll *a*, temperature and salinity. We showed significantly different relationships between bacterial group absolute abundances and chlorophyll *a* concentration, with significantly different log-log slopes ranging at the global scale from 0.13 (±0.04) for SAR11 to 0.53 (±0.08) for *Betaproteobacteria*, differing significantly among coastal or open-ocean ecosystems. We could use the regression equations of Table 5 (**Chapter IV**) to make predictions about community composition in different types of ecosystems and at different levels of chlorophyll *a*. In open-ocean conditions, SAR11 would tend to dominate at both low and high chlorophyll *a* levels (Figure 3), with increasing contribution of *Bacteroidetes* from low to high chlorophyll *a* levels. In coastal ecosystems, SAR11 would still be relevant at low chlorophyll *a* levels but not at higher levels, when they would be replaced progressively by higher contributions of *Betaproteobacteria* and *Bacteroidetes*.

In parallel and by using the large dataset of picoplankton group abundances determined flow cytometry (Table 1), we quantified the large scale patterns in pPeuk, *Synechococcus* and *Prochlorococcus* relative contributions to picophytoplankton biomass along a gradient of temperature among coastal and open-ocean ecosystems (Figure 3), from which we could also predict picoplankton community composition at different levels of temperature. In coastal ecosystems, we observed that pPeuk always tended to dominate picophytoplankton biomass at both low and high temperatures, with an increasing contribution of *Synechococcus,* from low to high temperatures and a systematically little contribution of *Prochlorococcus* (low temperatures being associated with high chlorophyll *a* levels, such gradient would be equivalent from high to low chlorophyll *a*). In open-ocean conditions, pPeuk was dominating picophytoplankton biomass at low temperatures but progressively replaced by *Prochlorococcus* at higher temperatures (low chlorophyll *a* levels) (Figure 3).

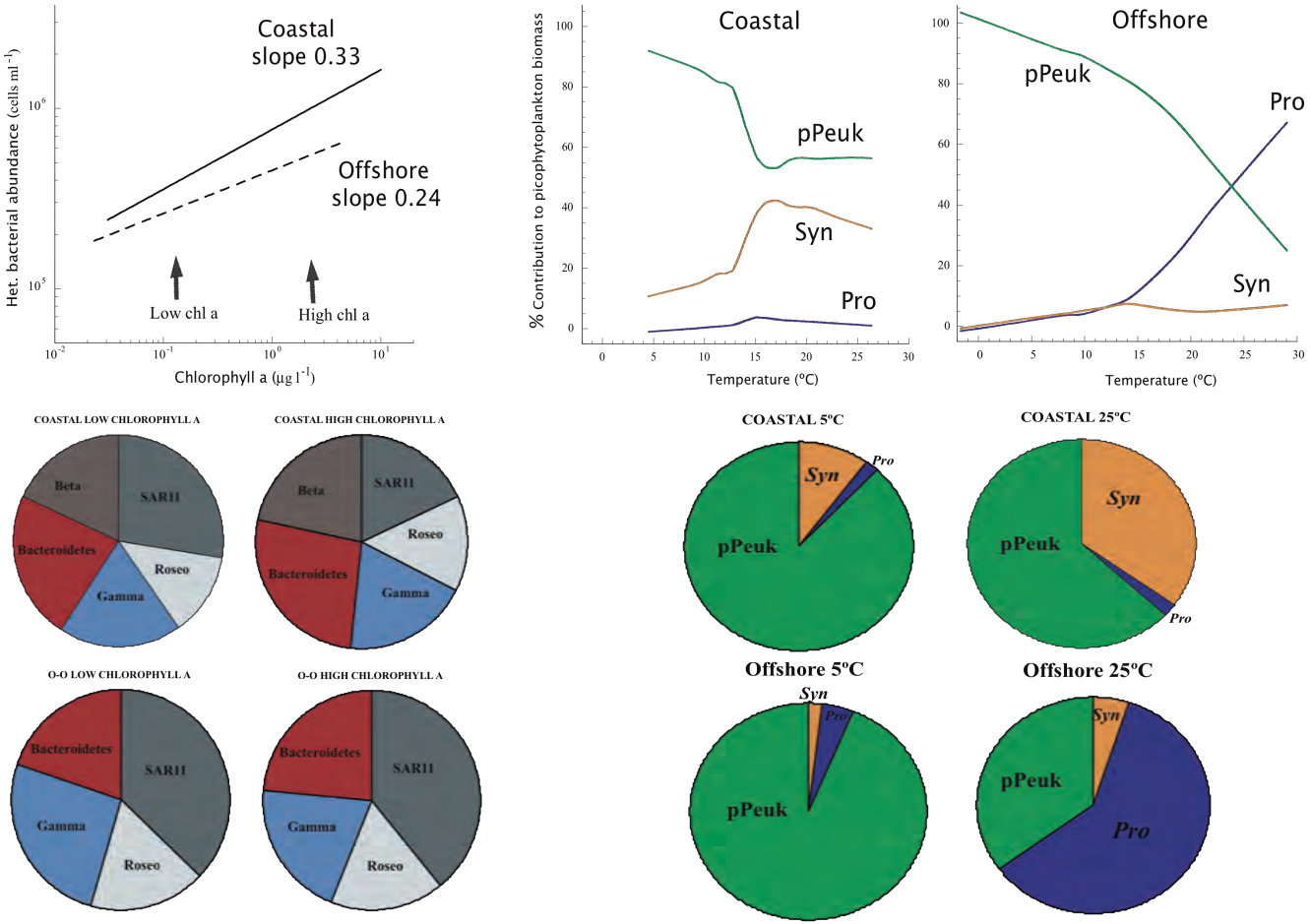


Figure 3. Bacterial community structure at low and high chlorophyll *a* levels predicted from log transformed linear relationships between bacterial group concentration and chlorophyll *a* levels for both coastal and open-ocean conditions (from Table 4B and 4C **Chapter IV**). Percentages contribution of *Synechococcus* (Syn), *Prochlorococcus* (Pro), photosynthetic Picoeukaryotes (pPeuk) to total picophytoplankton biomass along a gradient of temperature in coastal and open-ocean using a smooth fit in software Kaleidagraph vs 3.6.2 (Synergy Software). pPeuk biomass was estimated using our CF of 1540 fgC cell^{-1}, *Synechococcus* and *Prochlorococcus* using standard carbon CFs of 100 and 50 fgC cell-1 respectively (see details Figure 1). Low and high chlorophyll *a* levels corresponded to the 25th and 75th quartile of chlorophyll *a* concentration range in both ecosystems.

MAIN CONCLUSIONS

The main conclusions arising from this thesis are the following:

i) The use of cultures representative of a relatively wide range of genera and classes of pPeuk allowed the description of new relationships between cell volume and C and N content of small Picoeukaryotes for the range of cell size considered (1.38-5.06 μm), and the determination of an average cellular carbon per unit volume (C/V) ratio for global unspecific pPeuk communities of 467 fgC μm^{-3} (±4%), relatively higher than those previously reported to date.

ii) Two different methods of correcting errors in biomass estimation due to the presence of bacteria in cultures were compared. The first one, based on flow cytometric cell sorting, allowed to render axenic 11 of the 16 cultures. The second one, based on image analysis and the application of bacterial standard conversion factors, resulted in decreases of pPeuk cellular C content values by 7 to 33%. Given that no significant differences were found between the pPeuk C/V or N/V relationships, following both correction methods, we conclude that the two of them were appropriate to establish the relationships. However, a limitation was found for the cell sorting method, since it could not be applied for all cultures and was limited to the cultures presenting very high pPeuk abundance.

iii) The study of picoplankton community structure and heterotrophic activity in coastal NW Mediterranean surface waters showed consistent diel variability of all picoplankton populations, including heterotrophic bacteria and HNF. Moreover, we showed differences in the time of duplication and growth of different picophytoplankton groups, and evidences of coupling between picophytoplankton variability and single-cell bacterial activities. We furthermore observed how a relatively small variation in weather patterns could change considerably the structure of the microbial community and disrupt most diel cycles.

iv) The study of the temporal and spatial variability of Picoplankton Community Structure (PCS) by flow cytometry during a cruise performed from coast to offshore showed pronounced differences in picoplankton group distributions, *Synechococcus* dominating in coastal station C, pPeuk peaking at slope station CM, and *Prochlorococcus* in more offshore influenced stations M, MD and D.

v) The analysis of variability of the PCS during this cruise showed that while Picoeukaryote cell numbers exhibited the highest spatio-temporal variability, the lowest was found for bacterial abundance.

vi) The largest source of group abundance variability was observed at the spatial scale, vertically promoted by water column stratification, and horizontally by the differences in trophic level between stations. Coastal stations presented high bacterial abundance and activity but low spatio-temporal variability. On the contrary, offshore waters presented lower bacterial abundances and activities but higher spatio-temporal variability.

vii) Opposite patterns between *Synechococcus* to Picoeukaryotes biomass ratio and chlorophyll *a* levels were observed not only spatially, but also at both the short-term and large temporal scale, representing a variable potentially interesting to act as ecological indicators of the trophic state.

viii) We described global scale patterns between the major groups (*Alpha-*, *Beta-*, *Gammaproteobacteria* and *Bacteroidetes*, as well as *Rhodobacteraceae* and SAR11) and environmental variables such as chlorophyll *a* concentration, salinity and temperature, distinguishing between coastal (i.e. over the continental platform) or open-ocean environments. We found significantly different relationships between bacterial group absolute abundances and chlorophyll *a* concentration, with significantly different log-log slopes among ecosystem types. The different slopes measured for the different groups across trophic levels, in different ecosystem types, suggests different metabolic capabilities for utilizing algal-derived DOC.

ix) Single Parameter Analysis revealed preference of *Gammaproteobacteria* for increasing temperatures and the opposite for *Betaproteobacteria.* Multiple regression analyses showed significant effects of both chlorophyll *a* and temperature on total bacterial and SAR11 absolute abundances (expressed as cells ml^{-1}) and significant effects of chlorophyll *a* and salinity levels on absolute abundances of *Betaproteobacteria* and *Rhodobacteraceae.*

FURTHER PROGRESS

In **Chapter IV**, we identified patterns in bacterial community structure, considering bulk bacterial community as composed by different phylogenetic groups. We showed significantly different relationships between bacterial group absolute abundances and chlorophyll *a* concentration, with significant differences among coastal and open-ocean ecosystems. Significantly different slopes were found among phylogenetic levels. For instance, the slope of 0.25 (±0.05) measured for *Alphaproteobacteria* in coastal sites was significantly different to the one calculated at the clade level for SAR11 at -0.11 (±0.08) suggesting not only that low phylogenetic resolution might smooth out most of the ecological response of particular clades or subgroups along gradients of environmental factor (chlorophyll *a*, temperature, salinity…), but also that phylogenetically close groups can respond very differently to similar environmental conditions. The two questions that emerged from these considerations were the ecological relevance of the phylogenetic level at which the bacterial community was studied, and to what extent increasing the phylogenetic resolution might allow allocating more precise ecological functions to more specific groups.

With the recent development of culture-independent techniques, information about bacterial community composition has been used to test ecological theory (Horner-Devine et al. 2004). As an example of an approach for testing the hypothesis of "everything is everywhere" by Baas-Becking (1934), Pommier et al. (2007) analyzed at large spatial scale whether bacterial communities from coastal waters showed endemism or more likely cosmopolitanism, through the use of clone libraries of 16S ribosomal RNA genes used as Operational Taxonomic Units (OTU). Taking into account the considerable number currently available of marine microbial studies based either on the use of clones library or on DNA high-throughput sequencing techniques (such as pyrosequencing), one possible issue would be to test whether the patterns in bacterial community structure as composed by OTUs are similar to the ones found in **Chapter IV** in which BCS was measured by FISH and CARDFISH.

In order to test this point, we selected different published studies reporting data of clones libraries of 16s RNA genes from different coastal waters (Alonso-Sáez et al. 2003; Pommier et al. 2007). By multiplying the relative abundance of each phylogenetic group (expressed here in percentages of OTUs attributed to each phylogenetic group relatively to the total number of OTUs) by the bacterial abundance (expressed in cell ml^{-1} estimated from DAPI or flow cytometry), it was then possible to translate the number of OTUS into cell abundance. To investigate the relationships between the different bacterial groups and trophic level (as already done in **Chapter IV**), the absolute abundances of each bacterial group were regressed against chlorophyll *a* concentrations (log transformed, Figure 4).

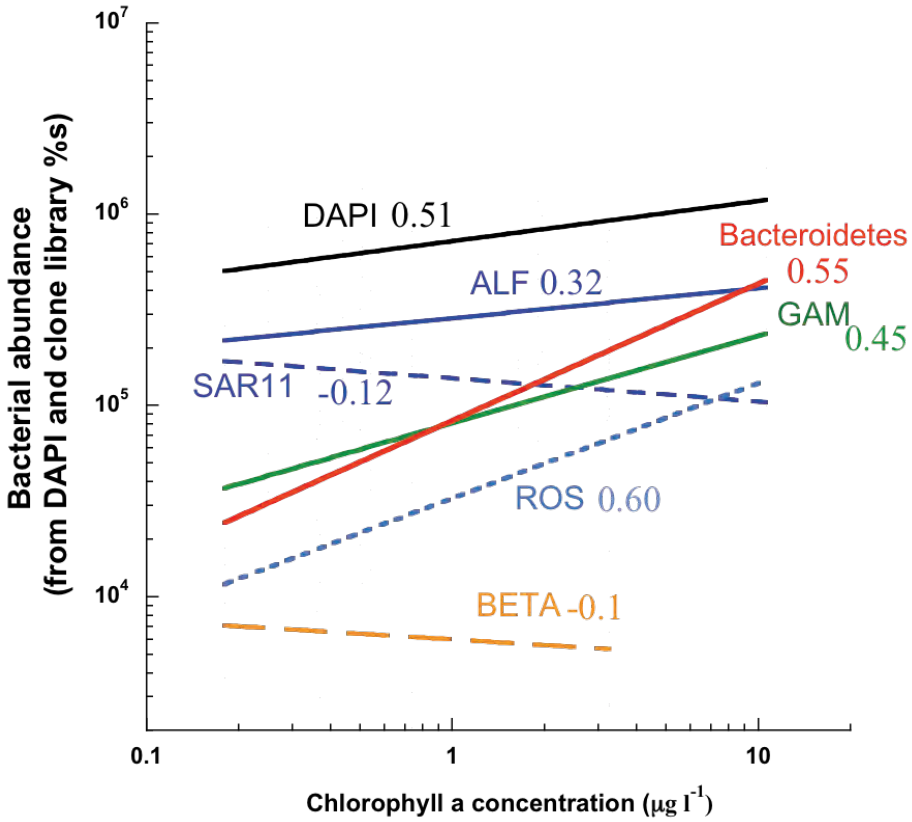


Figure 4. Relationships between log transformed bacterial subgroup absolute abundances and log chlorophyll *a* concentration (µg l^{-1}).

In spite of the fact that the bias generated by the PCR amplification step didn't allow to compare the intercepts of the different regression lines, we could however measure the differences between the slopes, assuming that the bias was constant along chlorophyll *a* levels. The slopes of relationships varied among phylogenetic groups, with *Rhodobacteraceae, Bacteroidetes* and *Gammaproteobacteria* strongly responding to chlorophyll *a* with rates of respectively 0.60 (±0.04) and 0.55 (±0.04) and 0.45 (±), comparatively with *Alphaproteobacteria* with a slope of only 0.32 (±). The differences were even more pronounced with SAR11 that sloped downwards with -0.1 (±). These partial results mostly corroborated the results for coastal ecosystems obtained in **Chapter IV**, in which a strong positive relationship between *Bacteroidetes* and trophic level was described, while opposite patterns were shown for SAR11. However, discrepancies between results obtained with *Betaproteobacteria (*exhibiting a negative trend with chlorophyll *a* levels while it was positive using the FISH approach), would call for a better test of these patterns using a much larger dataset. The recently accumulating pyrosequencing data could be used for that purpose.

CONCLUDING REMARKS

Whether microbes and bacteria exhibit biogeographical patterns has been controversial for much of the last century (Hedlund and Staley 2003). From a broader perspective, the results obtained in this thesis represent an approach for exploring the complexity of microbial community by studying widely observable patterns. Finally, we claim that in the perspective to precise more accurately the roles of microbes as well as their coupling in natural ecosystems, not only one, but different ways of representing the microbial community are needed, spanning from functionality or activity aspects by using flow cytometry, to more genetic perspectives by studying patterns in phylogenetic distribution.

Resumen general

RESUMEN

Contexto

La comprensión de los patrones y procesos que intervienen en la distribución de los organismos constituye el objetivo principal de la biogeografía. Aunque sus orígenes se encuentran en la clasificación taxonómica propuesta por Carl Linneo (1707-1778) basada en las diferencias y similitudes compartidas entre los distintos tipos de plantas y animales (Species Plantarum 1753), la biogeografía se inició con la teoría de George Louis Leclerc (Comte de Buffon) (1707-1788) quien afirmó que ambientes similares pero separados geográficamente presentaban diferentes especies de plantas y animales (Histoire naturelle, générale et particulière, 1749-1788). A finales del siglo XVIII surgieron varias teorías contradictorias, por un lado, el endemismo de las especies defendido por Charles Lyell (1797-1875) se podía explicar por la historia geológica y se oponía a la teoría de la dispersión global postulada por el botánico Alfonso de Condolle (1806-1893) quien formuló que "cuanto menor es la complexidad de organización del organismo, más generalmente se distribuye" implicando que la vida microbiana se encuentre en ambientes variados y que prolifera en condiciones apropiadas. Esta teoría fue considerada rápidamente como la "ley fundamental" de la biogeografía, impulsada por el nacimiento de la microbiología.

Sin embargo, el estudio de los patrones de distribución de los micro-organismos estaba restringido por razones tecnológicas. Al principio, la medición se basaba en el aislamiento de células en cultivos puros y la microscopía óptica (Cierto 1884, Zobell 1946) subestimadaba por varios órdenes de magnitud la abundancia microbiana real (Jannash y Jones 1959). Pero el desarrollo de la microscopía de epifluorescencia durante los años 70 (Hobbie et al. 1977, Zimmerman 1977, Porter y Feig, 1980), así como la introducción del contaje automatizado de células con Coulter y citometría de flujo (Sheldon y Parsons 1967, Sheldon 1978) hizo posible la detección y la enumeración de los diferentes grupos de micro-organismos en trabajos de campo (Olson et al. 1985).

Identificación de los grupos microbianos por citometría de flujo

La identificación de distintos patrones de distribución depende de los criterios utilizados para definir y clasificar los diferentes grupos microbianos. La división de las

comunidades según categorías de tamaño, de funciones y actividades hasta incluso aspectos más filogenéticos no darán sistemáticamente las mismas tendencias de distribución.

La citometría de flujo representa una técnica para dividir la comunidad microbiana en distintos grupos funcionales, aunque relacionados en parte con la clasificación taxonómica. Puede utilizarse de forma rutinaria para identificar, diferenciar y cuantificar los tres grupos de picoplancton: las algas eucariotas, las cianobacterias (*Synechococcus* y *Prochlorococcus*) y las bacterias heterotrófas (Olson et al. 1993; Gasol and del Giorgio 2000). La clorofila *a* y la concentración intra-celular de pigmentos constituye el principal factor que se utiliza para discriminar las células fotosintéticas del fitoplancton de otras partículas (Yentsch and Yentsch 1979; Li et al. 1995), otros foto-pigmentos como la ficoeritrina (de 550 a 590 nm, una vez excitados por la luz azul) se utilizan para distinguir los *Synechococcus* (Johnson y Sieburth 1979; Waterbury et al. 1979) (Wood et al. 1985) de los *Prochlorococcus* (Chisholm et al. 1988). Sin embargo, la mayoría de las bacterias fotosintéticas son demasiado pequeñas para ser detectadas, e incluso la autofluorescencia de los *Prochlorococcus* (en los ecosistemas oligotróficos) es demasiado baja para ser detectada con precisión por los foto-detectores. Por eso, diferentes sondas fluorescentes pueden ser utilizadas para teñir el DNA de las células y enumerar las bacterias así como los virus (Li et al. 1995; Marie et al. 1999) e incluso de distinguir los *Prochlorococcus* de las bacterias no fotosintéticas (Monger and Landry 1993)(Figura 1).

Importancia de las bacterias heterótrofas en la red Microbiana

Representando un total de 10^{29} células en el océano global, las bacterias heterótrofas son consideradas como los organismos más abundantes en la Tierra (generalmente alrededor de 10^9 células L^{-1}) (Whitman et al. 1998), representando actores imprescindibles del ciclo de carbono oceánico a través del bucle microbiano (Azam et al. 1983). Mientras inicialmente la función principal de las bacterias en los ecosistemas acuáticos se limitaba a la descomposición de materia orgánica y la remineralización de nutrientes inorgánicos, Pomeroy en 1974 demostró que sus funciones podían ser más diversas de lo que se pensaba antes. En efecto, Hagström et al. (1979) y Fuhrman y Azam (1980) encontraron que una proporción importante de las bacterias no eran inactivas sino que crecían a través del metabolismo de la materia orgánica oceánica disponible y que la producción heterotrófica bacteriana podía representar en promedio entre el 20 y 30% de la producción primaria (Cole

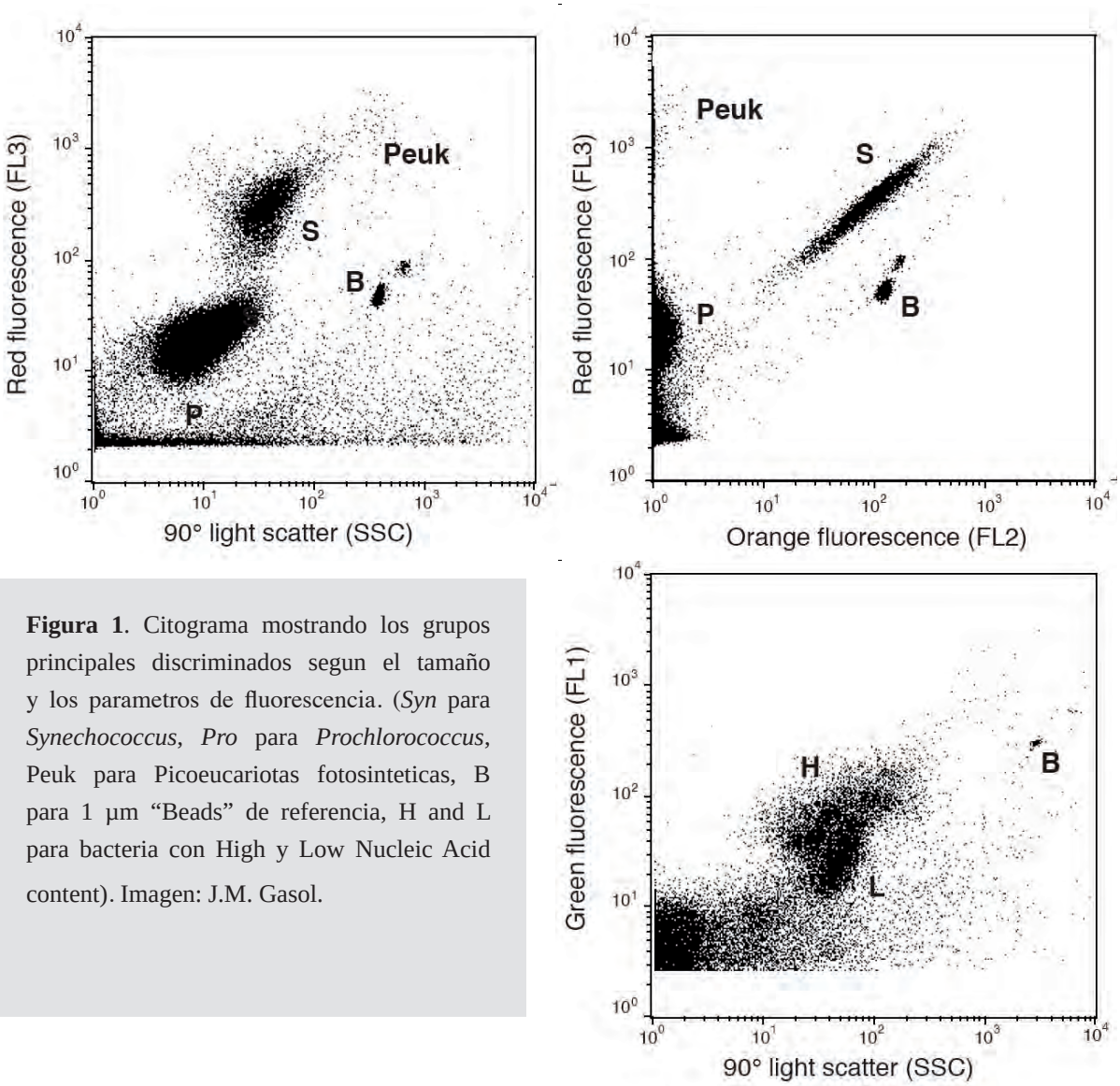


Figura 1. Citograma mostrando los grupos principales discriminados segun el tamaño y los parametros de fluorescencia. (*Syn* para *Synechococcus*, *Pro* para *Prochlorococcus*, Peuk para Picoeucariotas fotosinteticas, B para 1 µm "Beads" de referencia, H and L para bacteria con High y Low Nucleic Acid content). Imagen: J.M. Gasol.

et al. 1988). La red microbiana, que se resume en la figura 2, supone una inter-relación entre las bacterias y el fitoplancton (Fuhrman and Azam 1980; Bird and Kalff 1984; Cole et al. 1988) no sólo controlada por la depredación por parte de ciliados y flagelados (Fenchel 1982, Sherr y Sherr 1984 y 1987), sino también por crustáceos y rotíferos (Pedrós-Alió y Brock, 1983). Este vínculo sugiere que las bacterias utilizan la materia orgánica disuelta producida por el fitoplancton para apoyar el crecimiento y la producción bacteriana (Nagata et al. 2000; Morán et al. 2002). Varios análisis comparativos han demostrado tal relación, describiendo una relación positiva con una pendiente <1 entre las biomasas heterótrofa y autótrofa (Por ejemplo, Gasol et al. 1997) y mostrando que la biomasa bacteriana variaba menos que la biomasa autotrófica a lo largo de un gradiente de clorofila *a*, sugiriendo un papel menos relevante de los heterótrofos en la red microbiana en los sitios más eutróficos.

Actividad a nivel celular

La cuestión de la proporción de células activas dentro de la comunidad bacteriana no es obvia sino que ha constituido una cuestión central de la ecología microbiana.

Las comunidades de bacterias marinas presentan diferentes estados metabólicos. La asignación de una amplia gama de actividades a distintos grupos de bacterias mediante el uso de diferentes técnicas, permite representar la actividad bacteriana como un continuo de diferentes estados fisiológicos (Smith and del Giorgio 2003; del Giorgio y Gasol 2008). La microscopía fue primero utilizada para medir la tasa de división de las bacterias (Hagström et al. 1979) y fue seguida por la micro-autoradiografía que todavía se aplica ampliamente para medir la actividad de asimilación de sustratos por parte de micro-organismos en los sistemas acuáticos (Parsons and Strickland 1961; Wright and Hobbie 1965; Hoppe 1976). Además, la aplicación de sondas moleculares específicas en combinación con la citometría de flujo permite la caracterización rápida de la estructura fisiológica de la comunidad bacteriana (Gasol and del Giorgio 2000). Diferentes sondas fisiológicas pueden ser utilizadas para la detección de una variedad de estados celulares, como por ejemplo comprobar el estado de muerte celular mirando la permeabilidad de las membranas (Grégori et al. 2001, **Capítulo**

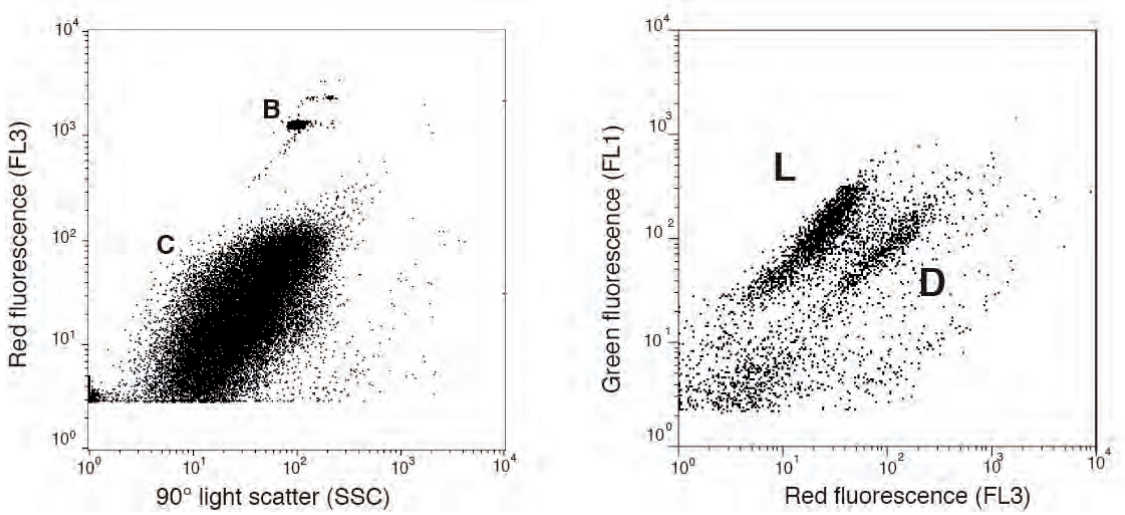


Figura 2. Citogramas mostrando diferentes grupos fisiológicos de bacterias discriminados por la presencia de sal de Tetrazolium CTC indicador de células que respiran activamente **(A)** y por la presencia de sondas SGI y PI que se hibridan al DNA bacteriano para diferenciar las células "muertas" de las "vivas" **(B)**, C para las CTC positivas, B para "beads" de referencia de 1μm, L para células vivas y D para células muertas como definido por el método NADS. Imagen : J.M. Gasol

II) o también cuantificar los diferentes procesos metabólicos tales como el porcentaje de células activas estimadas por la cuantificación de las células respirando (CTC positivas) (por ejemplo, Gasol y Arístegui 2007) (Figura 2). Si la disponibilidad de carbono orgánico en los ecosistemas marinos representa tal vez el factor más importante que influye en la actividad heterotrófica (Azam 1998, del Giorgio and Gasol 2008; Church 2008), los factores que regulan la variedad de actividades celular y estados metabólicos a diferentes escalas espacial y temporal siguen siendo poco estudiados.

Importancia del picofitoplancton en la red Microbiana

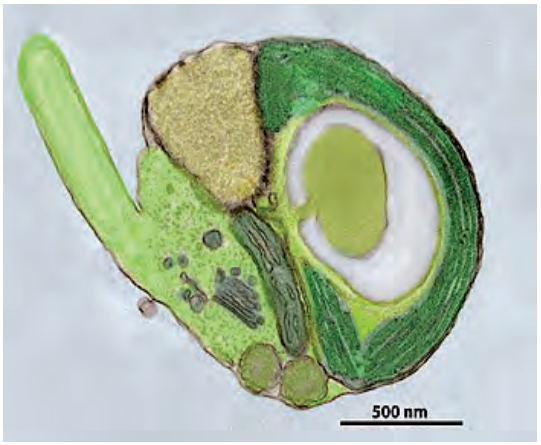
Los primeros modelos oceanográficos de la red microbiana fueron establecidos asumiendo la dominancia de los procesos heterótrofos, subestimando en gran parte el papel del picoplancton fotoautotrófico oxigénico. Richardson and Jackson (2007) postuló que el picofitoplancton constituía una fuente importante de carbono orgánico para el zooplancton, a través de su contribución al flujo de partículas sedimentadas en el océano profundo. Al ser de un rango de tamaño similar que las bacterias heterótrofas, los grupos de picofitoplancton pueden estar sujetos a similares factores (pero no idénticos) que controlan los procesos de crecimiento o de muerte celular. Por tanto, ellos pueden ser consumidos por los nano-protistas (Caron et al. 1991; Dolan and Simek 1998; Guillou et al. 2001), estar sujetos a la lisis viral (Proctor and Fuhrman 1991) y competir por los nutrientes (Hall and Vincent 1990; Li 1994; Vaulot et al. 1996). Recientes estudios basados en la cuantificación por citometría de flujo han revelado que el picoplancton autotrófico esté dominando la actividad fotosintética en ecosistemas de mar abierto (por ejemplo, Partensky et al. 1999). Los *Synechococcus*, generalmente se encuentran alrededor de 10^5-10^6 células ml^{-1} en varios ecosistemas, dominan particularmente en aguas mezcladas y ricas en nutrientes (Partensky et al. 1999) mientras que la prevalencia de *Prochlorococcus* se observa entre las latitudes 40 ° N y 40 ° S, son numéricamente mas importantes en las aguas estratificadas de mar abierto pobres en nutrientes, pero también están presentes en aguas ricas en nutrientes de primavera o de invierno (Campbell et al. 1997; Durand et al. 2001; Partensky et al. 1999) y representan hasta el 30% de la biomasa de los ecosistemas oligotróficos del Pacífico norte (Campbell et al. 1994). Diferentes estudios han mostrado que las abundancias de los Picoeucariotas y los *Synechococcus* covariaban en ecosistemas diversos (Worden et al. 2004; Campbell et al. 1998; Shalapyonok et al. 2001; Durand et al. 2001).

En comparación con las cianobacterias que son generalmente mas abundantes, menos importancia ha sido atribuido a los Picoeucariotas (Figura 3). Sin embargo, el cálculo de su contribución en términos de biomasa o de producción primaria podría revelar un papel en el ciclo del carbono oceánico mucho mas grande de lo que se consideraba antes (Li et al. 1995; Worden et al. 2004).

Factores de conversión de carbono para la estimación de biomasa

La subestimación de la importancia de los Picoeucariotas en comparación con otros miembros del picoplancton podría venir de la falta de factores de conversión de carbono bien definidos. Para convertir la abundancia de diferentes grupos de micro-organismos (mediante por ejemplo de la citometría de flujo) en biomasa, son necesarios dos parámetros: el tamaño de los grupos de células y el contenido celular en carbono.

Figura 3. Imagen de *Micromonas*: T. Deerinck, M. Terada, J. Obiyashi, M. Ellisman (all National Center for Microscopy and Imaging Research) y A. Z. Worden (MBARI).



Hasta la fecha, las estimaciones de contenido celular en carbono se hacían principalmente mediante la conversión del tamaño o del volumen de la célula en carbono utilizando relaciones lineales establecidas a partir del estudio de cultivos de células de fitoplancton (Strathmann 1967; Mullin et al. 1966). Sin embargo, en comparación con las células del fitoplancton grandes, los Picoeucariotas tienen vacuolas de menor tamaño (del mismo modo que las células bacterianas contienen menos cantidad de agua celular (Simon and Azam 1989) y un contenido relativamente más alto de carbono celular por unidad de volumen, revelado por pendientes negativas de las relaciones entre el contenido de carbono celular por unidad de volumen (fgC μm^{-3}) a lo largo del volumen celular (Verity et al. 1992).

Cuando las mediciones directas del tamaño celular de la comunidad no son disponibles, la única posibilidad para estimar el contenido de carbono celular es asumir un tamaño promedio de célula. Dentro de la categoría de tamaño del picoplanctón (0.2 a 3 μm), los tamaños promedios de *Synechococcus* y *Prochlorococcus* tienen poca variabilidad en comparación con los Picoeucariotas (Durand et al. 2001; Worden et al. 2004). *Prochlorococcus* representan los procariotas fotosintéticos más pequeños con un diámetro estimado a 0.7 μm (Shalapyonok et al. 2001; Worden et al. 2004; Durand et al. 2001), seguido de cerca por *Synechococcus* con un tamaño promedio de 0.87 μm (Worden et al. 2004) variando desde 0.5 a 2 μm (Murphy and Haugen, 1985). Las variaciones encontradas en el contenido de carbono de *Synechococcus* y *Prochlorococcus* están determinadas principalmente por cambios en sus abundancias (Durand et al. 2001), consecuencia de esta mayor estabilidad de tamaño. En comparación, las fluctuaciones de contenido de carbono para los Picoeucariotas pueden aparecer como una función tanto, de cambios en la abundancia, como de cambios en el tamaño, que más probablemente refleja cambios en la composición de las especies (Worden et al. 2004). Por lo tanto, la determinación de los factores de conversión de carbono basados en el conocimiento de una biogeografía taxonómica de los Picoeucariotas sigue siendo un objetivo necesario, pero no cumplido.

Filogenia microbiana

Los patrones de distribución observados dependen mucho del método con el cual los organismos fueron clasificados. Si diferencias pronunciadas entre organismos macroscópicos como los mamíferos o las plantas han permitido distinguir fácilmente diferentes especies, la clasificación de las bacterias en distintos grupos filogenéticos bacterianos ha sido posible sólo recientemente.

Las primeras aproximaciones de la filogenia bacteriana surge a finales del siglo XIX, se basaron en el estudio de las similitudes y diferencias morfológicas y metabólicas de bacterias aisladas en placas de agar. Sin embargo, este método no tardó en aparecer como sesgado a causa de los niveles poco realistas de concentración de materia orgánica y de nutrientes utilizados en los medios de cultivo, que falsamente estimulaban los rasgos particulares del metabolismo bacteriano. De los años 50 a 70, la evolución de las técnicas independientes del método basado en los cultivos surgió con las mejoras realizadas en

la extracción de ácidos nucleicos así como en los métodos de secuenciación. Además, la identificación de marcadores genéticos universalmente compartidos por los organismos permitió conceptualizar una nueva clasificación no basada en criterios morfológicos y fisiológicos, sino en la comparación genética de secuencias conservadas de la pequeña subunidad ribosomal de RNA. Siguiendo esta manera de clasificación, los seres vivos podían ser divididos en tres dominios: Arquea, Bacteria y Eucaria (Woese et al. 1977, 1987). Los avances al final de los años 80 de las técnicas de hibridación de fluorescencia *in situ* (FISH) combinado a la microscopía de epifluorescencia, permitió la identificación *in situ* y la cuantificación de los diferentes grupos filogenéticos de bacterias, con una especificidad que varia desde el nivel de especie al nivel de filo y dominio (véase la revisión de Amann y Fuchs, 2008). Entonces, una biogeografía de las poblaciones microbianas al nivel filogenético era posible (Alfreider et al. 1996; Llobet-Brossa et al. 1998; Murray et al. 1998; Simon et al. 1999; Kirchman et al. 2005). Así, se demostró que la clase *Alphaproteobacteria* dominaba el ecosistema costero del Delaware (Kirchman et al. 2005), así como zonas costeras del Mar Mediterráneo noroeste (Alonso-Sáez et al. 2007), en contraste con la clase *Betaproteobacteria* encontrada en abundancia en aguas dulces (Glockner et al. 1999). El grupo SAR11, formando una rama distinta dentro de la clase *Alphaproteobacteria* y probablemente el grupo bacteriano más abundante en la superficie del océano, dominaba en zonas pobres en nutrientes, tales como en el ecosistema oligotrófico del Mar de los Sargazos (Morris et al. 2002) pero también en las zonas costeras del Mar Mediterráneo (Alonso-Sáez et al. 2007). *Rhodobacteraceae*, constituyendo también otro grupo de la clase *Alphaproteobacteria*, ha sido identificado en varios ecosistemas marinos (Buchan et al. 2005), y es generalmente más abundante en las comunidades bacterianas asociadas con algas marinas. *Bacteroidetes* (anteriormente conocido como *Cytophaga-Flavobacteria-Bacteroidetes*) constituye uno de los principales grupos de picoplancton (Glockner et al. 1999; Kirchman 2002) abundantemente representados en una variedad de ecosistemas tales como zonas frías (Simon et al. 1999; Abell and Bowman 2005), y aguas costeras (Eilers et al. 2001; O'Sullivan et al. 2004; Sáez-Alonso et al. 2007), representando hasta la mitad de la comunidad bacteriana enumerada por FISH en muestras de zonas costeras de California (Cottrell and Kirchman 2000), y en condiciones de mar abierto (Simon et al. 1999; Abell and Bowman 2005; Schattenhofer et al. 2009) o generalmente asociados a proliferación de fitoplancton (Simon et al. 1999).

Entonces, la comunidad de picoplancton se puede dividir de diferentes maneras, ya

sea en grupos funcionales (determinados por citometría de flujo), en grupos de actividad (determinados por las sondas fluorescentes de actividad) o en grupos filogenéticos utilizando sondas especificas. La previsibilidad de los patrones de distribución de los grupos microbianos (citometría de flujo, basado en la actividad o determinado filogenéticamente) se puede deducir mediante la adopción de dos enfoques diferentes, ya sea de la caracterización de los diferentes ecosistemas marinos con propiedades específicas biogeoquímicas (Longhurst 1995, 1998) o el supuesto de que las contribuciones relativas de los diferentes grupos varían a lo largo de un continuo de parámetros físicos como la temperatura superficial del mar o de clorofila (Gasol et al, 1997;. Li 1998). Si la primera estrategia supone la existencia de límites bien definidos por la división de los océanos en diferentes provincias marinas (Longhurst, 1995), el contrario, "la hipótesis del continuo" asume el estudio de las estructuras de la comunidad microbiana en una amplia gama de parámetros, suavizando la mayoría de la variabilidad encontrada en escalas más pequeñas. Por ejemplo, las bacterias heterotrófas han sido descritas por aumentar a gran escala la concentración de clorofila y la temperatura (por ejemplo Li et al. (2004)).

Escalas de variabilidad : ecosistema costero o mar abierto

La función ecológica de los diferentes grupos de picoplancton se puede deducir mediante el estudio de sus distribuciones a distintas escalas espaciales y temporales. La estacionalidad en los grupos de picofitoplancton se ha observado a menudo (Campbell et al. 1997; Jacquet et al. 1998; Li 1998; Grégori et al. 2001; Li and Dickie 2001) y varios estudios hecho a grandes escalas espaciales han demostrado que la contribución relativa de cada grupo de picoplancton varía no sólo con el nivel trófico del ecosistema (Zhang et al. 2008), pero también con la temperatura y la estratificación de la columna de agua (Bouman et al. 2011), lo que sugiere que la estructura de la comunidad microbiana no varía al azar sino que puede representar un indicador ecológico de las propiedades de las masas de agua. Del mismo modo, los patrones generales de distribución de las bacterias y de las actividades heterotróficas se han identificado a grandes escalas espaciales a través de una amplia gama de diferentes niveles tróficos (según las estimaciones de la concentración de clorofila *a*) (Cole et al. 1988; Billen et al. 1990; Ducklow and Carlson 1992; Bird and Kalff 1984). Se demostró que el ratio entre la biomasas de las bacterias heterotrófas y del fitoplancton (ratio introducido por Odum 1971) disminuía a lo largo de un gradiente de

clorofila *a*, alcanzando y superando incluso la unidad en zonas poco productivas (Fuhrman et al. 1989; Cho and Azam 1990; Li et al. 1993; Buck et al. 1996), indicando que las bacterias heterótrofas predominaban en los medios más oligotróficos. Diferentes relaciones fueron descritas según los diferentes tipos de ecosistema, con diferentes pendientes en agua dulce, ecosistemas costeros o mas oceánicos (Simon et al. 1992; del Giorgio and Gasol 1995; Gasol et al. 1997) (Figura 5).

La distinción entre los ecosistemas costeros y de mar abierto no es arbitraria sino que las diferencias físicas pueden ser muy marcadas y representar entonces una fuente de variabilidad en los patrones de distribución de microbios. Cloern (1996) definió las zonas costeras como de transición tierra-mar, cuya poca profundidad permite rápidos intercambios entre la superficie del sedimento y la columna de agua por la sedimentación y resuspensión. En zonas costeras, la estabilidad de la columna de agua puede ser alterada debido a la entrada de agua dulce fluvial, mientras que la mezcla vertical en condiciones de mar abierto, está fuertemente influenciada por la estacionalidad, la estratificación térmica de la columna de agua durante el verano. Si bien varios estudios han demostrado que los cambios de estructuras filogenéticas bacterianas a lo largo de zonas de agua dulce a aguas marinas estaban acompañados también por cambios metabólicos, tanto a nivel de la actividad de la célula individual así como al nivel de la comunidad global (por ejemplo, Bouvier and del Giorgio 2002), la cuestión de como diferentes ecosistemas muestran diferentes patrones de estructuras de comunidad bacterianas sigue sin ser determinado.

Variabilidad a corta y larga escala

La identificación de patrones representa un objetivo fundamental de la biogeografía. Una de las herramientas que puede ser utilizada para cumplir este papel es el meta-análisis. Este método produce estimaciones a gran escala de un efecto sobre una variable con una potencia estadística mayor a la que se puede medir con estudios individuales (Lipsey and Wilson 2001). Al contrario, los patrones encontrados a gran escala espacio-temporal pueden suavizar la variabilidad de menor escala. La abundancia de los grupos de picoplancton puede variar drásticamente a muy corto plazo, la medición estacional de las abundancias puede reducir la varianza y suavizar la mayor parte de la variabilidad medida a cortas escalas temporales (Li 2007).

A pesar de que la importancia de la variabilidad día-noche ha sido comúnmente ignorada en comparación con la variabilidad mensual o anual, varios estudios han demostrado que la abundancia de *Synechococcus*, *Prochlorococcus* y Picoeucariotas tienen variaciones diarias: (por ejemplo, Jacquet et al. 1998 y 2002; Vaulot et Marie 1999; **Capítulo II**). Del mismo modo, la abundancia y actividad de bacterias puede variar en períodos muy cortos probablemente debido, por ejemplo, a las variaciones en la liberación de materia orgánica por parte del fitoplancton (Gasol et al. 1998; Ruiz-González et al. 2012; **Capítulo II**). Del mismo modo, las abundancias de los grupos de picoplancton varían drásticamente en distancias muy cortas (distancias menor que 1 Km), indicando que la magnitud de los patrones de variación a corta escala espacial de la estructura de la comunidad de picoplancton podría también estar en gran parte subestimada (Martin et al. 2005).

OBJETIVOS Y ESQUEMA DE LA TESIS

Esta tesis pretende identificar patrones ataxonómicos de distribución de comunidades de picoplancton por medio de la citometría de flujo, y de patrones filogenéticos en la estructura de la comunidad bacteriana utilizando un método basado en al análisis comparativo. Para estudiar estos patrones, en primer lugar re- evaluamos el factor de conversión de carbono para los Picoeucariotas fototróficos de un tamaño menor de 5 μm, con el fin de poder comparar y estimar su contribución relativa a la biomasa del picoplancton en los diferentes capítulos. Hemos tratado de identificar patrones de variabilidad en la distribución de los diferentes grupos de picoplancton a diferentes escalas espacio-temporales durante el invierno y el verano en una estación costera del Mediterráneo noroccidental, centrándonos más concretamente en la variabilidad a corta escala y en el acoplamiento que ocurre entre los grupos de picoplancton y actividades heterotróficas. Por último, se estableció una biogeografía de los grupos filogenéticos bacterianos a lo largo de un continuo de parámetros ambientales, tales como la clorofila *a*, la temperatura y la salinidad, mediante el uso de un análisis comparativo (análisis secundario), tratando de identificar los patrones en la estructura de la comunidad bacteriana en ecosistemas costeros y de mar abierto.

Esta tesis está organizada en cinco capítulos, cada uno conteniendo nuevos datos y resultados, el último capítulo es una discusión general que incluye un análisis poniendo en relieve las conclusiones de los capítulos precedentes. Cada capítulo se basa en varias hipótesis de fondo y tiene como objetivo responder a preguntas específicas:

Capítulo I. Determinación de los factores de conversión de carbono para Picoeucariotas fotosintéticos ecológicamente representativos de los ecosistemas marinos naturales

Contexto Teórico: Los factores de conversión son necesarios para transformar la abundancia de células de picofitoplancton en biomasa de carbono. Si bien estos factores están relativamente bien definidos para *Synechococcus* y *Prochlorococcus*, grandes discrepancias todavía se observan con los Picoeucariotas fotosintéticos. Existen tres razones principales para esta incertidumbre: 1) Pocos cultivos de Picoeucariotas ecológicamente pertinentes están disponibles, 2) los cultivos de Picoeucariotas generalmente se mantienen en condiciones no axenicas, provocando un sesgo en la estimación de carbono celular 3)

la mayoría de las estimaciones entre el tamaño celular y el contenido celular de carbono se han realizado a partir de cultivos de fitoplancton relativamente mas grandes conteniendo vacuolas mas grandes, los cuales no son representativos de la organización que se puede encontrar en células más pequeñas como los Picoeucariotas.

Los objetivos del primer capítulo son: i) comparar dos métodos diferentes para corregir el sesgo generado por la presencia de bacterias en cultivos mantenidos en condiciones no axénicas, por la separación de células o "sorting" por citometría de flujo con el análisis de la imagen, ii) establecer una nueva relación entre el tamaño celular y el contenido de carbono de Picoeucariotas fotosintéticos (tamaño <5 micras) usando un conjunto de 16 diferentes cultivos de Picoeucariotas ecológicamente relevante, y iii) ver si es posible distinguir factores de conversión específicos para zonas costeras y de mar abierto.

Capítulo II. acoplamiento a corta escala temporal de la estructura de la comunidad de picoplancton y de la actividad heterotrófica en una zona costera noroccidental del Mar Mediterráneo en invierno.

La distribución de los *Synechococcus*, *Prochlorococcus* y Picoeucariotas ha sido estudiada principalmente en escalas de tiempo relativamente grandes y sólo unas pocas veces en la escala día-noche. Dado que los acontecimientos de mayor relevancia ecológica provienen a menudo de la perturbación transitoria del medio ambiente (como la fuerza del viento, la turbulencia, alta irradiación solar...), y que la historia de vida microbiana más probablemente opera a corto plazo, es necesario determinar la importancia de la corta escala temporal en la estructuración de las comunidades microbianas. También se ha demostrado que *Synechococcus*, *Prochlorococcus* y Picoeucariotas podían seguir ciclos circadianos con diferentes sincronización de crecimiento. Un estrecho acoplamiento entre el fitoplancton y las bacterias tendrían que resultar en un ciclo circadiano en la abundancia y actividad bacteriana.

Los objetivos del segundo capítulo son: i) seguir las variaciones diarias de la abundancia de picoplancton por citometría de flujo con una alta frecuencia de muestreo en una estación costera del Mediterráneo noroccidental en el invierno de 2007, ii) determinar en qué medida las abundancias de los grupos de picofitoplancton se acoplan con la

abundancia y las actividades heterotróficas, así como con la abundancia de nanoflagelados heterótrofos: y iii) estudiar cómo este acoplamiento puede ser alterado en la corta escala temporal por diferentes eventos físicos, como los cambios de dirección o de fuerza del viento.

Capítulo III. Patrones en la estructura de la comunidad de picoplancton: Análisis de múltiple escala de variabilidad espacial y temporal en el Mar Mediterráneo noroccidental durante el verano

Varios estudios han demostrado que *Synechococcus*, *Prochlorococcus* y Picoeucariotas son distribuidos temporalmente y espacialmente. En comparación con la variabilidad mensual o anual, los factores que contribuyen a la variabilidad de la estructura y actividad heterotrófica de la comunidad de picoplancton en cortas escalas temporales y espaciales han sido relativamente menos estudiados. Algunos de los parámetros ecológicos (por ejemplo, la abundancia o la actividad de los grupos picoplanctónicos) se pueden utilizar para estimar los cambios que se producen en los ecosistemas marinos, la estructura de la comunidad microbiana así como las variables de actividad podrían captar la complejidad del ecosistema.

Los objetivos de este tercer capítulo son: i) identificar los patrones en la distribución grupo y la actividad del picoplancton en diferentes escalas espacio-temporales, ii) cuantificar y comparar la variabilidad de cada parámetro en cada escala espacial o temporal, y iii) determinar los vínculos de la variabilidad de los parámetros con los diferentes factores ambientales y ecológicos, tales como la estratificación y el nivel trófico (según las estimaciones de concentración de clorofila *a*).

Capítulo IV. Patrones de distribución de los grupos bacterianos, medidos por FISH, en relación a la clorofila, la temperatura y la salinidad

Evidencias de un acoplamiento trófico entre el fitoplancton y las bacterias en la mayoría de los ecosistemas marinos se han encontrado empíricamente mediante el análisis de grandes bases de datos, con la observación de correlaciones y relaciones significativas entre la biomasa bacteriana y del fitoplancton, con pendientes log-log <1, indicando que la

biomasa bacteriana varía proporcionalmente menos que la del fitoplancton. Se demostró que esta relación difería entre los tipos de ecosistema, las zonas de mar abierto teniendo más biomasa heterotrófica por unidad de biomasa autotrófica que los ecosistemas costeros. Sin embargo, la existencia de tales relaciones no solo al nivel de la comunidad bacteriana total pero a nivel de grupos filogenéticos mas precisos queda por establecer.

El objetivo de este capítulo fue estudiar como estas relaciones al nivel filogenético variaban de los existentes (que solo fueron estudiados a nivel comunitario) mediante la recopilación (análisis secundario) de los datos publicados de FISH y su combinación con las variables ambientales tales como la concentración de clorofila a, la temperatura y la salinidad.

Síntesis de resultados y discusión general

El objetivo de este último capítulo consiste en aplicar el factor de conversión determinado en el capitulo I a un conjunto de datos publicados y no publicados de abundancias de grupos de picoplancton obtenido mediante citometría de flujo en una amplia gama de diferentes provincias oceánicas con el fin de i) volver a evaluar la contribución relativa de este grupo a la biomasa de picoplancton ii) para identificar los patrones macroecológicos entre la estructura de la comunidad de picoplancton, la estructura de la comunidad bacteriana y los parámetros ambientales como la concentración de clorofila *a* y la temperatura.

METODOLOGÍA

Abundancia de los distintos grupos del picoplancton- Las abundancias de bacterias, cianobacterias y Picoeucariotas fotosintéticos en las muestras se analizaron por citometría de flujo (Gasol y Del Giorgio 2000). Para la cuantificación de las bacterias, se fijaron alícuotas de 1.2 mL con 1% de paraformaldehído + 0.5% de glutaraldehído (conc. final) y se almacenaron congeladas a -80ºC hasta su posterior análisis mediante tinción con SybrGreen I (1:10,000) en un citómetro de flujo FACSCalibur (Becton-Dickinson). Las bacterias se detectaron y se cuantificaron de acuerdo al tamaño de la célula (*side scatter,* SSC) y a la fluorescencia del SyberGreen I (FL1, fluorescencia verde). Por otra parte, las abundancias de *Synechococcus, Prochlorococcus* y Picoeucariotas fotosintéticos se estimaron inmediatamente tras el muestreo a partir de las muestras vivas, analizando alícuotas sin teñir por citometría de flujo. Todos los grupos se detectaron y contaron de acuerdo a su tamaño y a la fluorescencia naranja (FL2) o roja (FL3).

Determinación de la abundancia de los virus- La abundancia viral también fue determinada por citometría de flujo. se fijaron Submuestras (2 ml) con glutaraldehído (0,5% concentración final), que rápidamente se congelaron en nitrógeno líquido y almacenaron a -80 ° C como fue descrito por (Marie et al. 1999). Las muestras fueron teñidas con SYBRGreen I usando una velocidad de flujo medio siguiendo los protocolos estándares (Brussaard, 2004).

Actividades de las bacterias heterótrofas- la medición del estado fisiológico de las bacterias se hizo de dos maneras: i) los procariotas altamente activos, como aquellos capaces de reducir el 5-ciano-2 ,3-diotolyl cloruro de tetrazolio (CTC; Polysciences), convierte el CTC en un sal de formazán fluorescente rojo que es detectado por citometría de flujo y por microscopía de epifluorescencia (Sherr et al, 1999;.. Sieracki et al, 1999). Se añadió una solución de CTC a alícuotas de muestra a una concentración final de 5 mM (0,4 ml) (de una solución madre fresca a 50 mM) inmediatamente después de la recogida, se incubaron durante 90 minutos en la oscuridad a temperatura ambiente. Las células CTC positivas (CTC+) fueron enumeradas por citometría de flujo utilizando el parámetro FL2 contra FL3 (Gasol y Arístegui, 2007). Para estos análisis, se utilizó el flujo

de alta velocidad (aprox. 100 μl m^{-1}) ii) Las células con membranas intactas se enumeraban utilizando el protocolo de viabilidad NADS, basado en la combinación de un marcador permeable de ácido nucleico SybrGreen I (Molecular Probes, Eugene, OR) y de sonda fluorescente de ioduro de propidio PI (Sigma Chemical Co.). Se utilizó un 10x de SG1 y 10 μg ml^{-1} de concentración para PI. Después de la adición simultánea de cada sonda, las muestras fueron incubadas durante 20 minutos en la oscuridad a temperatura ambiente y analizadas después por citometría de flujo. La fluorescencia de SG1 y de PI se detectaron en los canales de citometría verde (FL1) y rojo (FL3) respectivamente. Una gráfica del parámetro FL3 contra el FL1 permitió distinguir las células "vivas" (es decir, las células con membranas intactas y con el ADN presente) de las "muertas" (es decir, con membranas comprometidas) (Grégori et al. 2001; Falcioni et al. 2008). Los parámetros de fluorescencia y dispersión lateral se estandarizaron con "beads" Polysciences de referencia de 1 μm.

Separación de células por citometría de flujo- La citometría de flujo se utilizó para corregir los errores en la estimación de la biomasa debido a la presencia de bacterias en 11 de los cultivos de Picoeucariotas (**Capítulo I**). Se usó un citómetro de flujo FACSAria (Becton Dickinson), usando como fluido de vaina agua de mar artificial recién preparada y filtrada con filtros de 0.2 micras esterilizadas por filtración para separar las células de algas de las células bacterianas dibujando un eje SSC (dispersión lateral) frente a FL1 (fluorescencia verde) después de teñir el DNA con SYTO13 (Molecular Probes, Eugene, EE.UU., 5 mM) y separando en el modo de pureza. El análisis y la separación de células se hicieron usando una boquilla de 70 μm, con una presión de 70 psi y el flujo de la muestra adaptado para mantener la tasa de partícula por debajo de 1000. Un alícuota de 1 ml de cada una de las muestras después del "sorting" fue preservada con glutaraldehído (0,25% de concentración final) y se almacenó en nitrógeno líquido para la determinación subsiguiente de la abundancia de algas y bacterias por citometría de flujo.

Actividad heterotrófica bacteriana- La actividad heterotrófica bacteriana se estimó a partir de la incorporación de ^{3}H-leucina y también se utilizó ^{3}H-timidina. Para la ^{3}H-leucina empleamos el método descrito por Kirchman et al. (1985) con las modificaciones de Smith y Azam (1992). Brevemente, 4 alícuotas de 1.2 mL y 2 mL fijados con ácido tricloroacético (TCA) al 50 % se incubaron con leucina radioactiva (40 nmol l^{-1} conc. final, 160 Ci $mmol^{-}$

[1]) durante unas dos horas en oscuridad y a temperatura *in situ*. La incorporación se detenía añadiendo 120 μl de TCA frío al 50% a las muestras vivas, que se almacenaban a -20ºC hasta su procesado por el método de centrifugación descrito por Smith y Azam (1992). Por otra parte, para el método de la timidina se siguió el protocolo descrito por Fuhrman y Azam (1980) con las modificaciones de Smith y Azam (1992). Las muestras se incubaron con 10 nmol L^{-1} de ^{3}H-timidina (conc. final) y fueron procesadas igual que las muestras de ^{3}H-leucina.

Determinación del tamaño y del volumen celular- Los triplicados de 16 cultivos fueron muestreados en fase exponencial de crecimiento. El diámetro promedio de células se determinó con un citómetro de flujo de la célula-Lab-Quanta SC (Beckman Coulter) calibrado antes de cada determinación del tamaño con perlas de 3 micras (Polysciences) diluidas en agua MilliQ. Los recuentos de células y el análisis de diámetro se realizaron después de dibujar los parámetros FL3 (fluorescencia roja) contra EV (volumen electrónico). Se suponía una distribución normal del tamaño celular y el pico de distribución se tomó como representativo del promedio aritmético del diámetro celular. Los biovolumes se calcularon posteriormente asumiendo las células como esféricas.

Análisis estadísticos usados en esta tesis- Los Análisis de regresión lineal y de co-varianza fueron utilizados para comparar las pendientes de las relaciones logarítmicas transformadas entre la abundancias absolutas estandarizadas de cada grupo de bacterias y la variable independiente (por ejemplo clorofila *a*). Las ecuaciones de las regresiones se presentan como log (Y) = a + b log (X) siendo Y las células por mililitro; a el intercepto, b la pendiente; X la variable independiente (por ejemplo, clorofila *a*). Con el fin de comprobar si las pendientes e interceptos de las relaciones fueron significativamente diferentes, se realizaron test de Student se llevaron a cabo después de aplicar el modelo I de análisis de regresión aplicando los métodos descritos por Zar (1999). Para comparar las regresiones y comprobar la heterogeneidad de las pendientes, se realizaron tests de ANCOVA.

Análisis multivariante- Para evitar las diferencias generadas por las escalas de las variables independientes (la clorofila *a*, temperatura, salinidad) y para permitir la comparación del

impacto relativo de cada variable independiente en los modelos multivariantes, se utilizaron los coeficientes estandarizados beta en los modelos de regresión múltiple.

Análisis de distribución de los grupos bacterianos- Los datos de abundancias relativas de los grupos de bacteria y los parámetros ambientales como la concentración de clorofila *a*, temperatura y salinidad fueron utilizados para estimar el intervalo de "preferencia" de cada grupo de bacterias para intervalos de parámetros ambientales con un análisis estadístico QRA (Quotient Rules Analysis, Somarakis et al 2006). Cada variable ambiental se dividió en intervalos regulares y se expresaron las frecuencias de ocurrencia en porcentaje. El número de intervalos en todas las variables del medio ambiente fue establecido para asegurar que la ocurrencia máxima por intervalo no supere el 20% del total de las mediciones. Un test non paramétrico de Kolmogorov-Smirnov de bondad de ajuste (Zar 1999) se utilizó para comparar las distribuciones de los grupos bacterianos en función de cada categoría de variable ambiental. Nuestra hipótesis nula (H0) suponía que los grupos de bacterias no seguían una distribución particular a lo largo de la variable ambiental.

Variabilidad de los parametros- Para estimar la variabilidad de cada parámetro de abundancia o de actividad, se calculó el coeficiente variación (CV) expresado como la desviación estándar de los valores integrados divididos por el promedio integrado. Los CV medidos durante los ciclos día noche se compararon con los CV medidos a gran escala temporal. Se hicieron correlaciones de Pearson para resumir la fuerza de las relaciones lineales entre cada par de variables de respuesta.

RESULTADOS

Capítulo I. Determinación de los factores de conversión de carbono para los Picoeucariotas fotosintéticos ecológicamente representativos

Con el fin de analizar los factores de conversión utilizados para estimar la biomasa a partir de los valores de abundancia, se determinó el tamaño y el contenido celular en carbono (C) y nitrógeno (N) de 16 diferentes cultivos de Picoeucariotas mono-específicos (Tabla 1).

STRAIN	DIVISION	CLASS	Genus species	Area of isolation	Ecosystem assignment
RCC 245	**Archeaplastida**	Prasinophyceae	*Pycnococcus sp.*	Mediterranean Sea	C
RCC 287			Clade VIIA	Pacific ocean	O-O
RCC 419			*Bathycoccus prasinos*	English channel	C
RCC 422			*Ostreococcus sp.*	English channel	C
RCC 299			*Micromonas pusilla* Clade A	Pacific ocean	C
RCC 497			Micromonas *pusilla* Clade C	Mediterranean Sea	C
RCC 927			*Prasinoderma singularis*	Pacific ocean	O-O
RCC 656	**Haptophyta**	Prymnesiophyceae	*Chrysochromulina sp.*	Atlantic ocean	C
RCC 361			*Imantonia rotunda*	English channel	C
RCC 703	**Heterokontophyta**	Bacillariophyceae	*Minutocellus sp.*	Indian Ocean	O-O
RCC 101		Pelagophyceae	*Pelagomonas calceolata*	Atlantic ocean	O-O
RCC 480		Chrysophyseae	*Ochromonas sp.*	Indian Ocean	C
RCC 446		Dictyophyceae	*Florenciella parvula*	English channel	O-O
RCC 239		Bolidophyceae	*Bolidomonas mediterranea*	Mediterranean Sea	C
RCC 503		Pinguiophyceae	*Phaeomonas* sp.	Mediterranean Sea	C
RCC 504		Eustigmatophyceae	*Nannochloropsis gatidana*	Mediterranean Sea	

Tabla 1. Cultivos de Picoeucariotas fototróficos utilizados en este estudio y origen geográfica de cada cepa. Todos provienen de la biblioteca de cultivos de Roscoff (RCC). Asignación del ecosistema: C para costero y OO para mar abierto determinado según la literatura.

Los contenidos celulares de carbono y nitrógeno variaron desde 230 fgC $cell^{-1}$ (±1.21%) y 38.8 fgN $cell^{-1}$ (±2.73%) para *Ostreococcus* a 21800 fgC $cell^{-1}$ (±23.61%) y 4920 fgN $cell^{-1}$ (±14.11 %) para *Pycnococcus* (Tabla 2).

Genus species (Strain)	**Algal concentrations (cells ml^{-1})** (±SD)	**Cell diameter (μm)** (±CV%)	**Per group cell diameter average (μm)** (±CV%)	**Per cell Carbon (not corrected) fgC cell^{-1}** (±CV%)	**Per cell Nitrogen (not corrected) fgN cell^{-1}** (±CV%)
Pycnococcus sp. (RCC245)	9.48 ± 2.08 10^4	2.61 (±3.31%)		**21,800** (±23.61%)	4,450 (±4.65%)
Clade VIIA (RCC287)	1.31 ± 0.05 10^6	2.28 (±0.48%)		2,990 (±8.96%)	520 (±16.50%)
Bathycoccus prasinos (RCC419)	1.75 ± 0.09 10^7	1.38 (±0.71%)		349 (±21.43%)	77.2 (±40.06%)
Ostreococcus sp. (RCC422)	3.39 ± 0.11 10^7	1.44 (±1.24%)	2.01 (±34.47%)	**230** (±1.21%)	**38.8** (±2.73%)
Micromonas pusilla Clade A (RCC299)	1.68 ± 0.08 10^7	1.66 (±16.87%)		811 (±5.27%)	128 (±3.43%)
Micromonas pusilla Clade C (RCC497)	1.25 ± 0.06 10^7	**1.51** (±0.66%)		857 (±9.07%)	105 (±9.12%)
Prasinoderma singularis (RCC927)	2.08 ± 0.74 10^5	3.17 (±1.69%)		15,300 (±22.56%)	2,670 (±25.28%)
Chrysochromulina sp. (RCC656)	8.42 ± 0.09 10^5	**5.06** (±2.53%)	3.78 (±47.89%)	14,700 (±1.95%)	1,830 (±1.94%)
Imantonia rotunda (RCC361)	1.76 ± 0.07 10^6	2.5 *		5,180 (±4.32%)	819 (±7.42%)
Minutocellus sp. (RCC703)	4.86 ± 0.57 10^5	3.26 (±0.33%)		8,810 (±18.25%)	1,420 (±13.19%)
Pelagomonas calceolata (RCC101)	6.13 ± 2.40 10^4	2.31 (±3.55%)		20,500 (±14.04%)	**4,920** (±14.11%)
Ochromonas sp. (RCC480)	1.22 ± 0.14 10^5	4.38 (±0.53%)		18,200 (±28.64%)	1,050 (±64.78%)
Florenciella parvula (RCC446)	4.54 ± 0.06 10^5	3.56 (±30.14%)*	3.16 (±23.81%)	19,000 (±8.98%)	2,480 (±8.19%)
Bolidomonas mediterranea (RCC239)	2.09 ± 0.06 10^5	2.18 (±0.59%)		8,800 (±5.17%)	1,530 (±4.35%)
Phaeomonas sp. (RCC503)	7.33 ± 3.19 10^5	3.38 (±1.61%)		11,000 (±32.59%)	1,660 (±29.94%)
Nannochloropsis gatidana (RCC504)	1.94 ± 0.14 10^6	3.06 (±1.34%)		5,090 (±5.38%)	633 (±8.74%)
MIX	4.07 ± 0.02 10^6	----------	**1.60** (±66.54%)**	1,590 (±11.63%)	268 (±12.68%)

Tabla 2. Contenido celular en carbono y nitrógeno y tamaño celular promedio determinado en crecimiento exponencial. Concentración algal y bacteriana al momento de la filtración y de la determinación del tamaño (detalles ver **Capitulo I**)

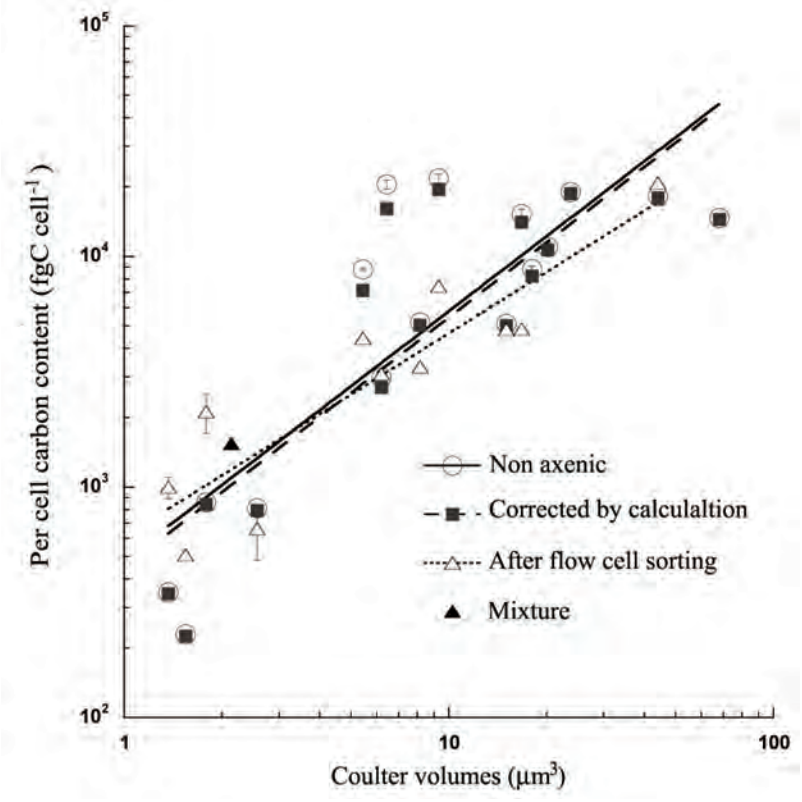


Figura 4. Relación entre el contenido celular en carbono y el volumen determinado por "coulter". Los ejes X i Y son transformados logaritmicamente.

Dado que los cultivos no eran axénicos, se utilizaron dos protocolos diferentes para corregir la presencia de bacterias:

1) por estimación del carbono bacteriano y del contenido en nitrógeno en cada cultivo por análisis de imagen y usando factores estándar de conversión bacterianos,

2) mediante el uso de la separación de células por citometría de flujo para eliminar las bacterias antes del análisis de contenido celular.

La corrección de carbono bacteriana resultó en disminuciones de los valores de contenido en C de los Picoeucariotas del 7% a 33%. La eficiencia de eliminación de bacterias por el método de discriminación de células fue siempre superior a 74%.

Relacion entre el contenido per celula y el biovolumen

Se identificaron nuevas relaciones entre el volumen celular y el contenido en carbono y nitrogeno para el rango de tamaño de célula considerado (1.38-5.06 μm) (Figura 1). Además, se calculó un promedio de densidad celular en carbono (cantidad de carbono por unidad de volumen celular (C/V)) de 467 fgC μm^{-3} (± 4%) como factor de conversión de comunidades inespecíficas de Picoeucariotas. Por otro lado, se estimó un factor de conversión promedio de 1540 fgC por célula (± 12.01%) correspondiendo a un biovolumen de 2,14 μm^3 calculado a partir de una mezcla de cultivos de Picoeucariotas. También sugerimos que factores de conversión más específicos pueden ser elegidos según el tipo de ecosistema y la composición conocida de las comunidades de Picoeucariotas (Figura 4).

Capítulo II. Acoplamiento a corta escala temporal de la estructura de la comunidad de picoplancton y de la actividad heterotrófica en una zona costera noroccidental del Mar Mediterráneo en invierno.

Se utilizó la citometría de flujo para seguir las variaciones diarias de la estructura de la comunidad de picoplancton así como la actividad heterotrófica en aguas costeras del mar Mediterráneo noroccidental durante dos períodos sucesivos de 72 horas en invierno de 2007. Mientras se observó que la estructura de la comunidad de picoplancton estuvo dominada numéricamente por las bacterias heterótrofas durante los dos ciclos, los Picoeucariotas fotosintéticos dominaban la fracción fotosintética del picoplancton durante el primer ciclo, *Synechococcus* dominaban durante el segundo (Figure 5).

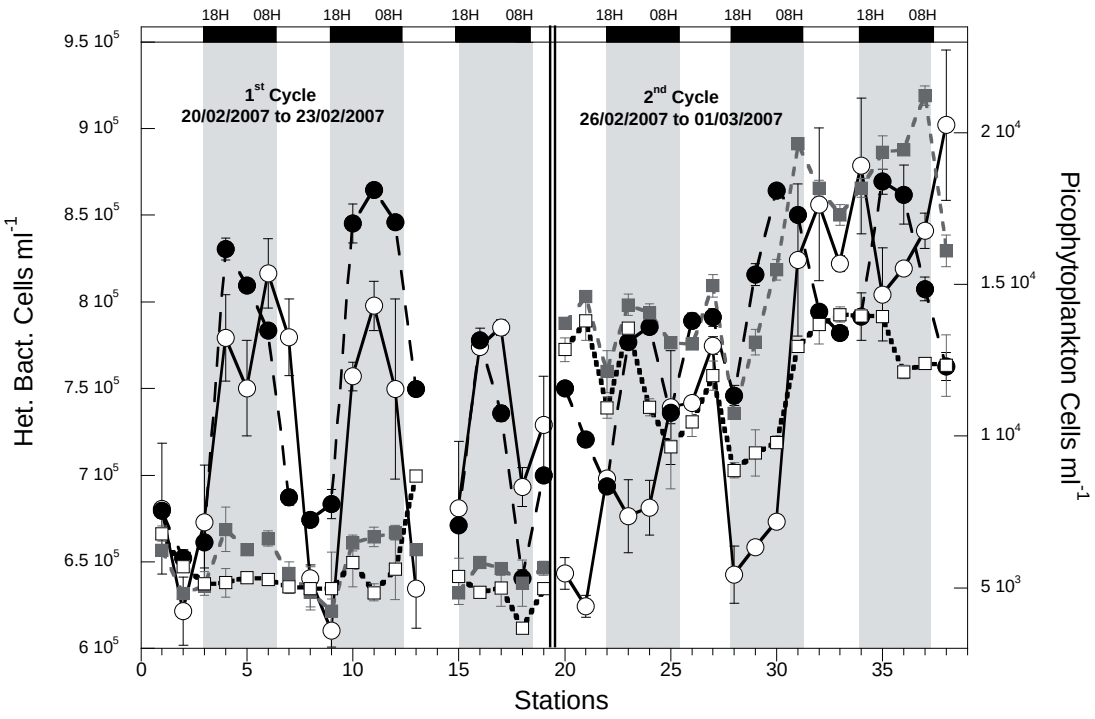


Figura 5. Variaciones circadianas de las abundancias de los grupos de picoplancton medido por citometría de flujo. El Y de izquierda corresponde con la concentración (❍) de bacterias heterotrofas i el Y de derecha, a las abundancias de los grupos de picofitoplancton: (●) para Picoeucariotas, (■) para *Synechococcus*, (❒) para *Prochlorococcus.* Las zonas grisadas corresponden a los periodos de noche.

Para cada grupo de picofitoplancton, se observaron pronunciados patrones circadianos con una periodicidad significativa de 24 horas en los parámetros celulares como el tamaño (SSC) o la fluorescencia. Los *Synechococcus* y Picoeucariotas crecían durante el período de luz y se dividían durante la noche. Sin embargo, patrones opuestos se observaron con los *Prochlorococcus*, ya que su crecimiento se produjo durante la noche (Figure 6).

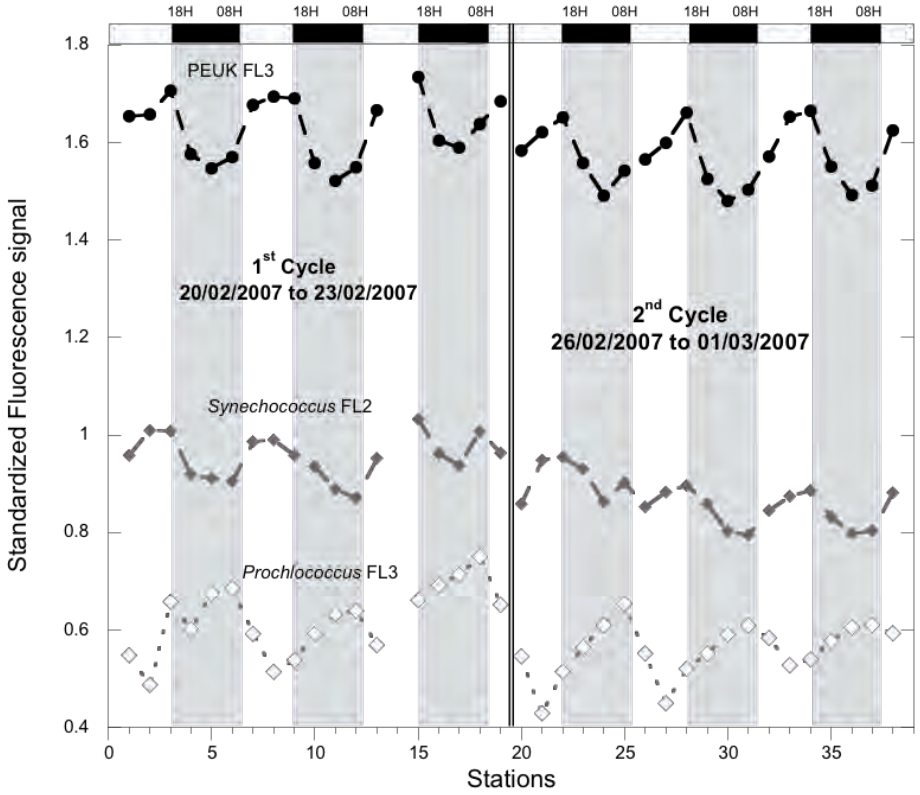


Figura 6. Fluorescencia roja FL3 (unidad relativa) de los Picoeucariotas y *Prochlorococcus,* fluorescencia FL2 (unidad relativa) de los *Synechococcus.*

Los patrones día y noche de los diferentes grupos se vieron afectados fuertemente antes del segundo ciclo por un evento de cambio de viento con precipitaciones y turbulencia asociadas, lo que sugiere que el cambio observado en la estructura de la comunidad es el resultado de los desequilibrios entre el crecimiento y los procesos de perdida.

Se constató una fuerte asociación entre la estructura de la comunidad picofitoplanctonica y la actividad heterotrófica. Durante el primer ciclo, la actividad fue mayor durante la noche que durante el período de luz, lo que indica que el crecimiento de bacterias fue acoplado con las variaciones diarias de la comunidad de picoplancton (Figure 7).

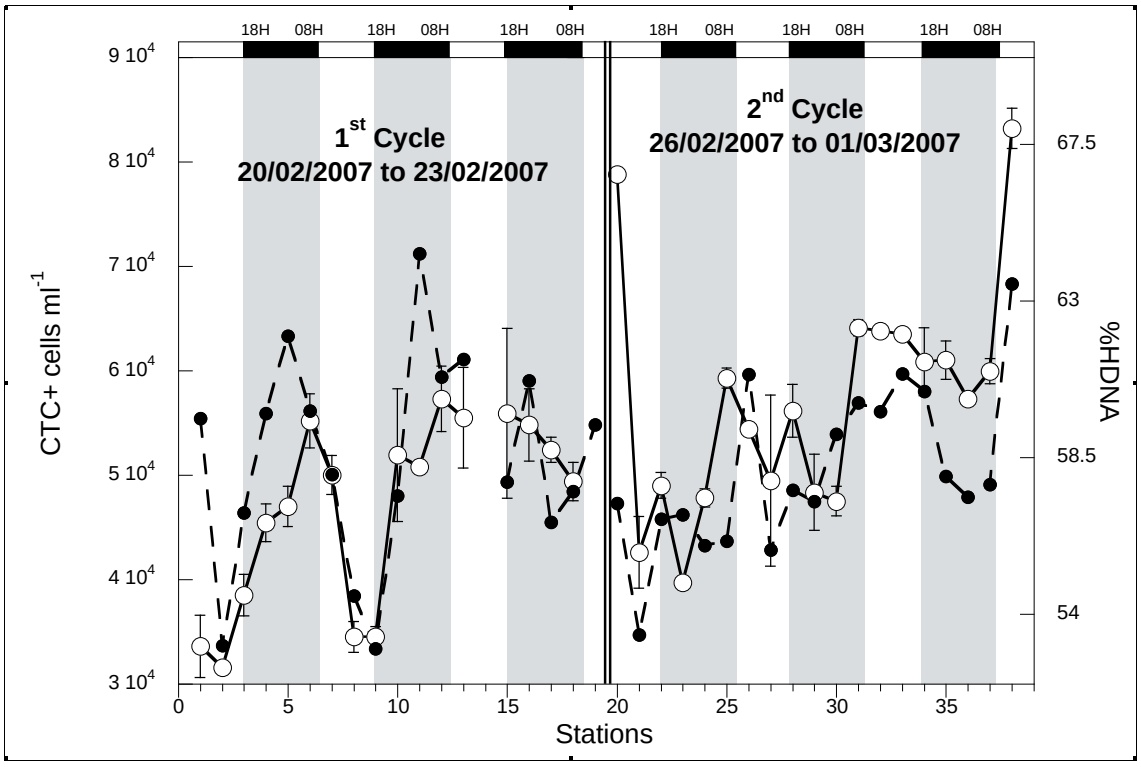


Figura 7. Concentración de bacterias CTC positivas (●) al eje Y de la izquierda, y percentage de celulas HNA (linea discontinua negra) al eje Y de la derecha.

Sin embargo, no se observaron patrones circadianos en la abundancia ni en la actividad bacteriana durante el segundo ciclo, sólo se midieron tendencias similares entre la actividad bacteriana y la abundancia de heteronanoflagelados sugiriendo que la actividad de depredación era una posible fuente de materia orgánica disuelta soportando la actividad bacteriana.

Capítulo III. Análisis de variabilidad a múltiple escala de los patrones de estructura de la comunidad de picoplancton del Mar Mediterráneo noroccidental durante el verano

La variabilidad temporal y espacial de la estructura de la comunidad fitoplanctonica así como la actividad heterotrófica fue estudiada por citometría de flujo y con trazadores radiactivos durante una campaña realizada en el Mar Mediterráneo en frente de la costa catalana (Figura 8). La variabilidad se midió a corta escala temporal durante dos ciclos dia-noche, el primero fue realizado en zona costera y el segundo en una estación de mar abierto y se comparó con la variabilidad a gran escala temporal estimada a partir de dos años de estudio en la bahía de Blanes (la estación costera) y la variabilidad espacial descrita durante un transecto de costa a mar abierto.

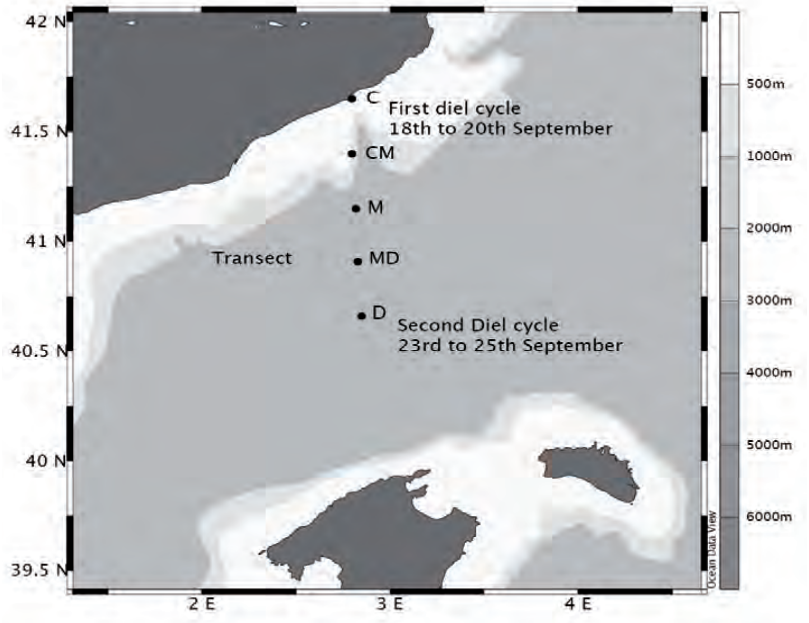


Figura 8. Mapa batimétrica de las diferentes estaciones de la campaña oceanográfica MODIVUS, la estación costera (C) de la Baya de Blanes fue la primera estación del transecto, la (D) fue la última. Los ciclos circadianos fueron hecho a la estaciones (C) Y (D).

Synechococcus dominaba numéricamente en las zonas costeras y fue el principal contribuyente a la biomasa picofitoplanctonica en todas las estaciones. Los Picoeucariotas participaban mas en las estaciones costeras y la pendiente continental. La contribución máxima de *Prochlorococcus* se limitaba a las zonas oceánicas bien estratificadas (Figure 9).

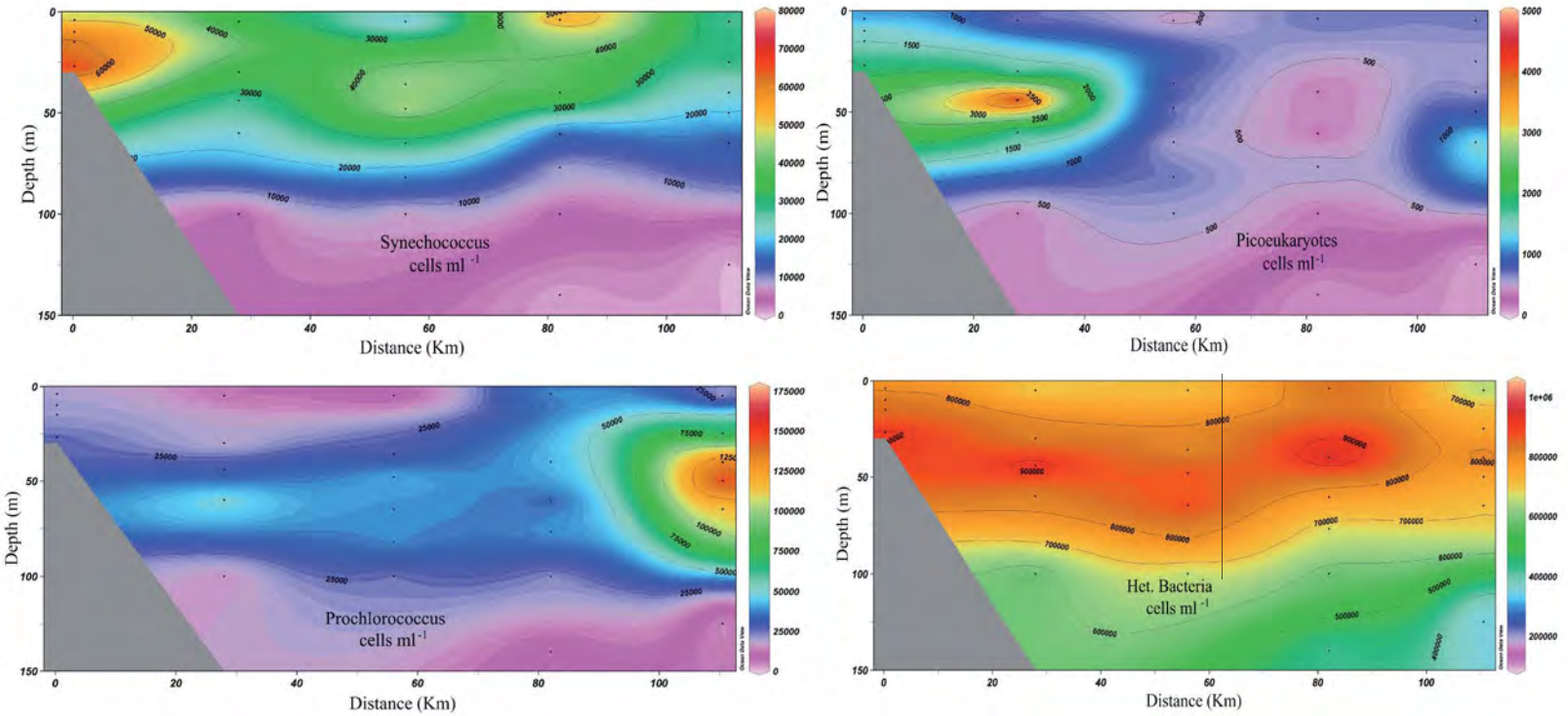


Figura 9. Abundancias de los grupos de picoplancton durante el transecto desde la estación (C) hasta la estación (D). Concentración de *Synechococcus* (cells ml^{-1}) **(A)**, concentración de *Prochlorococcus* (cells ml^{-1}) **(B)**, concentración de Picoeucariotas (cells ml^{-1}) **(C)**, concentración de bacterias heterótrofas (cells ml^{-1}) **(D)**.

PARAMETER VARAIBILITY (±%CV)	STATION C			STATION CM	STATION M	STATION MD	STATION D		ST C, CM, M, MD, D	AVERAGE
	DIEL CYCLE	LONG TERM SURVEY	VERTICAL PROFILE	VERTICAL PROFILE	VERTICAL PROFILE	VERTICAL PROFILE	VERTICAL PROFILE	DIEL CYCLE	TRANSECT	VARIABILITY
SYNECHOCOCCUS	14%	97%	31%	61%	79%	137%	60%	16%	68%	63%
PROCHLOROCOCCUS	40%	114%	20%	140%	72%	61%	97%	18%	**57%**	69%
PICOEUKARYOTES	72%	92%	31%	158%	66%	82%	150%	18%	62%	81%
HET. BACTERIA	13%	27%	19%	30%	31%	46%	49%	13%	12%	27%
CTC+	23%	---	23%	33%	70%	82%	66%	33%	44%	47%
BACTERIAL ACTIVITY TDR	70%	---	66%	36%	62%	38%	42%	56%	51%	53%
BACTERIAL ACTIVITY Leu	46%	87%	38%	64%	38%	57%	32%	46%	53%	43%
VIRUS ABUNDANCE	76%	N.A	10%	57%	102%	57%	69%	28%	16%	52%
SYN:PPEUK BIOMASS RATIO	35%	125%	11%	106%	100%	125%	58%	17%	64%	71%
CHLOROPHYLL A	66%	77%	37%	101%	54%	47%	122%	85%	37%	70%
SCALE VARIABILITY AVERAGE	46%	88%	29%	79%	67%	73%	75%	33%	46%	

Tabla 3. Variabilidad (coeficiente de variación) de los diferentes grupos de picoplancton así como de las actividades.

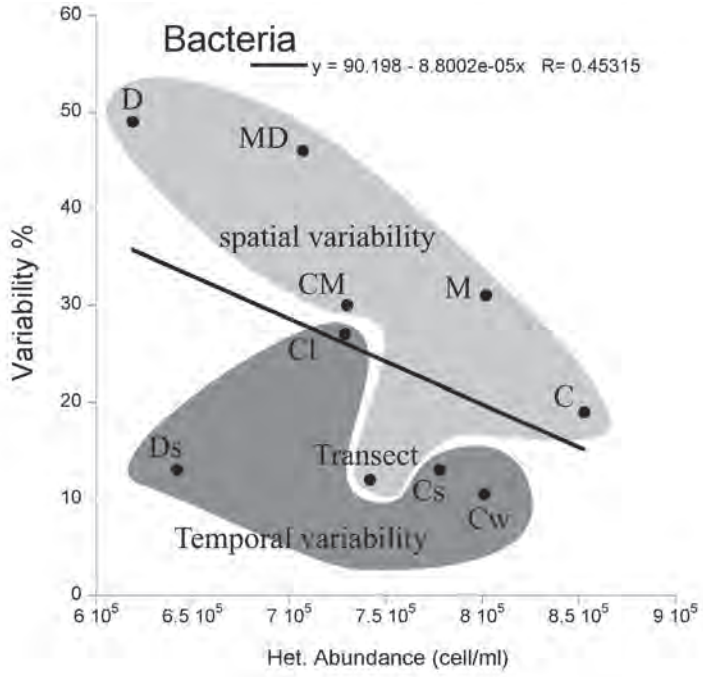
Mientras la variabilidad espacio-temporal más alta se debía a la abundancia de Picoeucariotas, la más baja se encontraba con la abundancia bacteriana (Table 3). Cuando se compararon las diferentes fuentes de variabilidad, se mostró que la más importante se observaba en la escala espacial promovida por la estratificación vertical de la columna de agua, y horizontalmente por las diferencias de nivel trofico entre las estaciones.

Variabilidad de la abundancia y actividad heterotrofica

Comparado con las zonas de mar abierto donde la abundancia y las actividades bacteriana fueron bajas pero con gran variabilidad espacio-temporal, las estaciones costeras presentaban mayor abundancia y actividad bacteriana, pero menor variabilidad espacio-temporal (Figura 10).

Finalmente, se observaron patrones opuestos espaciales y temporales entre el ratio entre las biomasas de *Synechococcus* y de Picoeucariotas, y los niveles de clorofila *a*, sugiriendo que la comunidad de picoplancton puede ser un indicador ecológico del estado trófico.

A



B

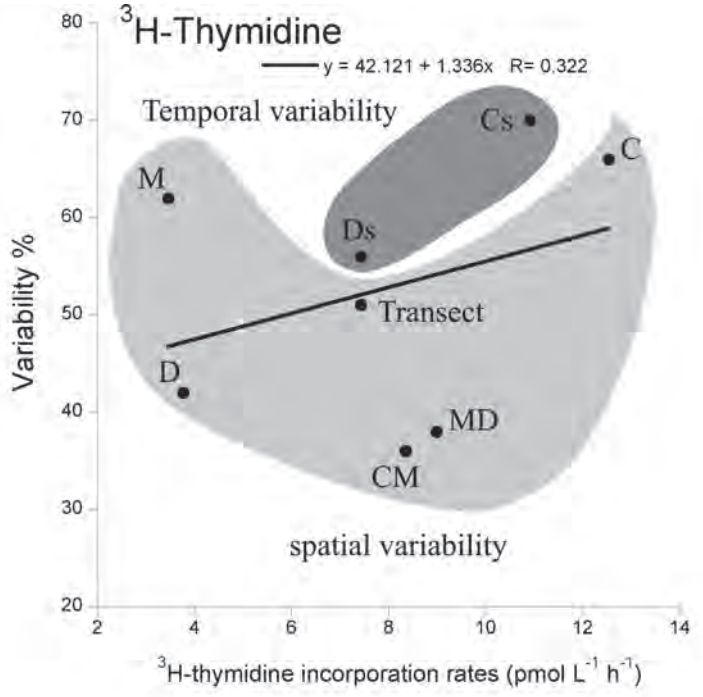


Figura 10. Relación entre la variabilidad espacial e temporal de la abundancia de bacterias heterótrofas **(A)** y actividad heterotrófica medido mediante la tasa de incorporación de ^{3}H-timidina **(B)**. (Detalles ver Capitulo III – Figura 5)

Capítulo IV. Patrones de distribución de los grupos bacterianos, medidos por FISH, en relación a la clorofila *a*, la temperatura y la salinidad

Utilizando datos de la literatura de abundancia de grupos filogenéticos de bacterias determinados mediante la técnica de hibridación fluorescencia *in situ* (FISH o CARDFISH) (Tabla 4), se estudió si la variabilidad de los diferentes subgrupos bacterianos era similar a la de la comunidad bacteriana total. Diferentes patrones fueron descritos entre los principales grupos (alfa, beta-, *Gammaproteobacteria* y *Bacteroidetes*, así como *Rhodobacteraceae* y SAR11) y las variables ambientales como la concentración de clorofila *a*, la salinidad y la temperatura, distinguiendo entre la costa (es decir, sobre la plataforma continental) o el ecosistema mar abierto.

Diferentes relaciones se encontraron entre las abundancias absolutas de los grupos bacterianos y la concentración de clorofila *a*, con diferentes pendientes que variaban desde 0.13 (±0.04) para SAR11 hasta 0.53 (±0.08) para *Betaproteobacteria* a escala global, desde -0.11 (±0.08) para SAR11 a 0.52 (±0.08) para *Betaproteobacteria* en ecosistema costero y de -0.06 (±0.12) para *Gammaproteobacteria* hasta 0.48 (±0.06) para los *Bacteroidetes* en mar abierto (Figura 11). Estas diferentes relaciones sugieren diferentes aptitudes metabólicas por parte de los grupos bacterianos para la utilización del carbono de origen algal.

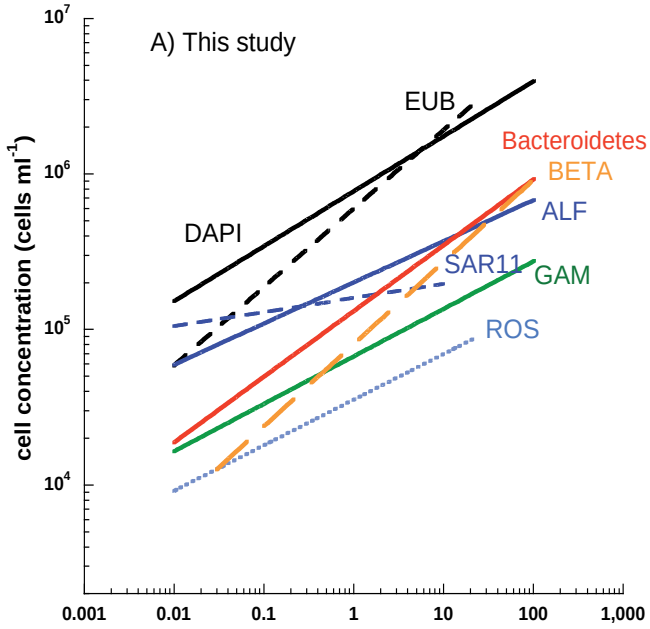


Figura 11. Relaciones transformadas en logaritmo entre las abundancias absolutas de los subgrupos bacterianos y la concentración en clorofila *a* (μg l^{-1}). DAPI corresponde a la concentración bacteriana total (cells ml^{-1}). Las abundancias de grupos bacterianos fueron estandarizados según las diferencias entre el método FISH y CARD-FISH.

REFERENCES	TECHNIQUE TYPE	SAMPLING SITES	PROBES CONSIDERED
Alderkamp et al. 2006	CARD-FISH	Coastal North Sea	CF319, Ros537
Wietz et al. 2010		Global survey	EUB338, ALF968, BET42a, GAM42a, CF319
Alonso Sáez et al. 2007		NW Mediterranean coastal waters (Blanes Bay)	EUB338, ALF968, BET42a, GAM42a, SAR11-441R, ROS537
Obernosterer et al. 2007		South Pacific Ocean	EUB338, ALF968, GAM42a, CF319A
Piccini et al. 2006		Southwestern Atlantic	EUBI-III, ALF968, GAM42a. BET42a, CF319, SAR11-441
Stoica and Herndl 2007		NW Black Sea	EUB338, ALF968, BET42a, GAM42a, ROS537 AND ROS1029
Garcés et al. 2007		NW Mediterranean coastal waters	EUB338, ALF968, BET42a, GAM42a, ROS538
Mary et al. 2006		English Channel	EUB338 I-II-III, ALF968, SAR11-152R, SAR11-542R, RSB67, GAM42a, CF319a
Garneau et al. 2006		Canada Arctic shelf	EUB338, ALF968, BET42a, GAM42a, CF319a
Alonso Sáez et al. 2006		NW Mediterranean coastal waters (Blanes Bay)	EUB338, ALF968, BET42a, GAM42a, SAR11-441R, ROS537
Ruiz-González et al. (unpublished)		NW Mediterranean coastal waters (Blanes Bay)	EUB338-II-III, ALF968, BETA42a, CF319, SAR11-441R, ROS537, GAM42a
Alonso Sáez et al. 2008		Western Arctic	EUB338-II-III, ALF968, BETA42a, CF319, SAR11-441R, ROS537, GAM42a
Baltar et al. 2007		NW Africa upwelling to Canary Coastal Transition zone	EUBI-II-III, SAR11-441R, CF319a, ROS537, GAM42a
Teira et al. 2008		Ría de Vigo, Atlantic	EUB338, ALF968, BET42a, GAM42a, CF319a, SAR11-441R, ROS537
Schattenhofer et al. 2009		Atlantic	EUB338 I-III, SAR11-441R, GAM42a, CF319a
Topping et al. 2006		Scotia Sea, Antarctica	EUB338, ALF968, SAR11-486, SAR11-542R, ROS537, GAM42a, CF319a
Lin et al. 2006	FISH/CARD-FISH	Eastern sub-basin of the Cariaco system and Black Sea	EUB338, ALF968, BET42a, GAM42a, CF319
Lin et al. 2008		Cariaco system	EUB338, ALF968, BET42a, GAM42a, CF319
Zhang et al. 2006	FISH	South China Sea	EUB338, ALF968, BET42a, GAM42a, CF319
Simon et al. 1999		Southern Ocean	EUB338, ALF968, BET42a, GAM42a, CF319
Wells et al. 2003		Canadian archipelago	EUB338, CF319
Longnecker et al. 2006		Oregon, Newport (West US), North Pacific	EUB338, ALF968, BET42a, CF319, GAM42a
Vila et al. 2004		Northern gulf of Mexico	EUB338, ALF968, ROS536, GAM42a, CFB319
Kirchman et al. 2003		Delaware estuary	EUB338, ALF968, BET42a, GAM42a, CF319a
Agawin et al. 2006		NE Subarctic Pacific	EUBI-II-III, ALF968, ALF1B, BETA42a, GAM42a, CF319a
Yokokawa and Nagata 2005		North Pacific coast	EUB338, ALF968, BET42a, GAM42a, CF319
Zubkov et al. 2002		Northern north Sea	EUB338, ALF968, GAM42a, CF319a, RSB67
Cottrell and Kirchman. 2000		California Coast	EUB338, ALF968, BET42a, GAM42a, CF319a, SAR11-A1
Lin et al. 2007		Eastern sub-basin of the Cariaco system	EUB338, ALF968, BET42a
Castle and Kirchman 2004		Delaware estuary and Chesapeake Bay	EUB338, ALF968, BET42a, GAM42a, CF319
Yokokawa et al. 2004		Delaware estuary	EUB338, ALF968, BET42a, GAM42a, CF319
Cottrell et al. 2006		Mid Atlantic bight and North Pacific gyre	EUB338, ALF968, SAR11, CF319, ROS537
Kirchman et al. 2005		Delaware Estuary	Eub338, Alfa968, ROS537, Beta42, Gam42a, CF319
Carlson et al. (unpublished)*		BATS. Sargasso Sea	SAR11, CF319, ROS537

The data from BATS station by Carlson et al. were extracted from icomm.mbl.edu/oocs_summaries/OOCS_**Carlson**/OOCS_**Carlson**_final.ppt

Tabla 4. Datos utilizados en este estudio así como las sondas oligonucleotidas y el tipo de método (FISH o CARD-FISH).

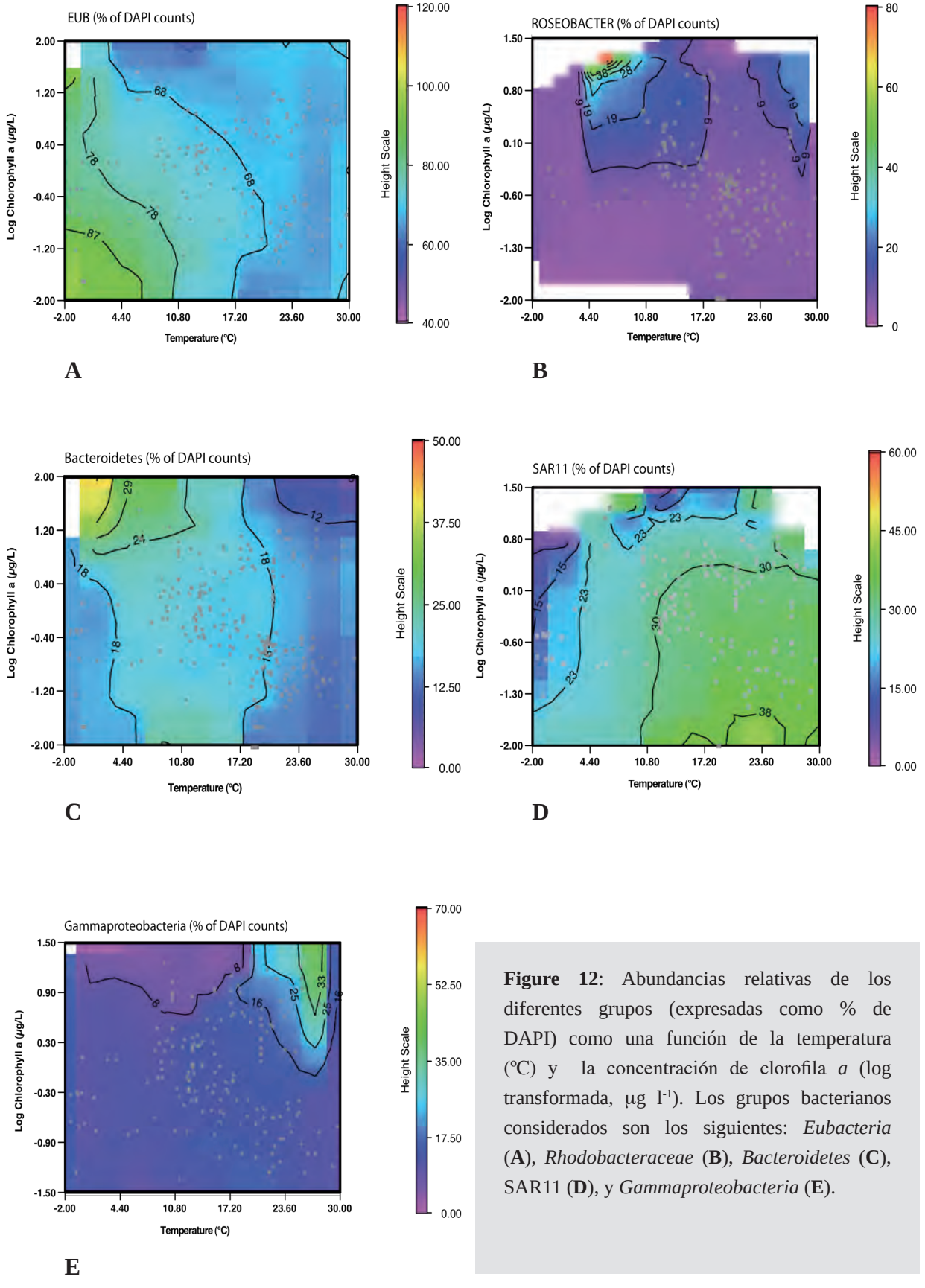


Figure 12: Abundancias relativas de los diferentes grupos (expresadas como % de DAPI) como una función de la temperatura (ºC) y la concentración de clorofila *a* (log transformada, µg l^{-1}). Los grupos bacterianos considerados son los siguientes: *Eubacteria* (**A**), *Rhodobacteraceae* (**B**), *Bacteroidetes* (**C**), SAR11 (**D**), y *Gammaproteobacteria* (**E**).

Bacteroidetes (25%), beta-(14%) *Gammaproteobacteria* (12%) y *Rhodobacteraceae* (6%) tuvieron mayores abundancias relativas(% de los contajes de DAPI) en los ecosistemas costeros, mientras que las contribuciones de *Alphaproteobacteria* y SAR11 (32%) eran significativamente más altas en mar abierto.

El análisis de regresión múltiple reveló ambos efectos significativos de la clorofila *a* y la temperatura en la concentración bacteriana total y la abundancia absoluta de SAR11 y efectos significativos de la clorofila y la salinidad en la abundancia absoluta de *Betaproteobacteria* y *Rhodobacteraceae* (Figure 12).

El análisis estadístico permitió también evaluar el rango de "preferencia" de los diferentes grupos de bacterias por diferentes parámetros ambientales y reveló la "preferencia" de *Gammaproteobacteria* por altas temperaturas y la preferencia de *Betaproteobacteria* por bajas temperaturas.

SÍNTESIS DE LOS RESULTADOS Y DISCUSIÓN GENERAL

El objetivo de esta tesis fue el de identificar patrones en la estructura de la comunidad de picoplancton (PCS) y en la estructura filogenética de la comunidad bacteriana (BCS) a diferentes escalas espacio-temporales, identificando diferencias de estructuras entre los ecosistemas costeros y de mar abierto, y describiendo tendencias entre la estructura de la comunidad microbiana a lo largo de un gradiente de parámetros ambientales. Dos métodos diferentes fueron utilizados para definir los grupos microbianos. El primero se basó en la citometría de flujo y la discriminación de diferentes grupos según el contenido de DNA, fluorescencia y las propiedades de dispersión de la luz, lo que permitió distinguir las bacterias heterótrofas de los tres diferentes grupos fotosintéticos dentro de la fracción de tamaño picoplancton <3 μm: *Synechococcus*, *Prochlorococcus* y los Picoeucariotas fotosintéticos (pPeuk). El segundo se basaba en la división de la comunidad bacteriana en diferentes grupos filogenéticos según los métodos FISH y CARDFISH.

El análisis de la variabilidad en BCS y PCS realizado a diferentes escalas espaciales y temporales fue propuesto aquí para atribuir diferentes funciones ecológicas a los grupos microbianos. Mientras el análisis de la comunidad bacteriana estudiada a larga escala en el **Capítulo IV** permitió distinguir diferentes tendencias al nivel de los grupos filogenéticos bacterianos a lo largo de gradientes de clorofila a, temperatura y salinidad, el análisis de PCS se hizo con un enfoque a corta escala temporal (**Capítulo II**) y se limitó al área de la bahía de Blanes y a meso-escala espacial con el transecto (**Capítulo III**). Uno de los objetivos de la discusión de la tesis era probar en qué medida los patrones de estructura de la comunidad identificados a corta escala fueron coherentes con los que pudieron ser medidos en la escala global, mediante la recopilación y el análisis secundario de datos tanto publicados como no publicados de abundancia de grupos de picoplancton, aplicando el factor de conversión de carbono para los Picoeucariotas calculado en el **Capítulo I.**

Contribución de los Picoeucariotas a la biomasa de picoplancton

La importancia del picofitoplancton (de 0.2 a 2-3 μm dependiendo del autor) en las redes troficas marinas se puede explicar por varias razones:

Además de su ubicuidad en los ecosistemas marinos y de aguas dulces, contribuyendo

significativamente a la biomasa total de fitoplancton (Stockner 1988), la contribución relativa del picofitoplancton a la biomasa y la producción primaria tiende a aumentar en las regiones oligotróficas (Agawin et al. 2000; Bell and Kalff 2001).Sin embargo, la estimación precisa de las poblaciones de fitoplancton en equivalente de carbono puede aparecer como complicada a causa de la variabilidad medida en el volumen celular (varios órdenes de magnitud) y la abundancia de cada fracción de células con distintos tamaños celulares. La estructura del tamaño de comunidad de fitoplancton es fundamental para saber cual será el destino del carbono, porque afecta a las vías metabólicas así como a las tasas de crecimiento. En comparación con los *Synechococcus* y *Prochlorococcus*, los Picoeucariotas han sido en gran medida ignorados, a pesar de su gran participación en la producción primaria y la biomasa de picoplancton en amplias regiones oceánicas (Li et al. 1994; Worden et al. 2004). Debido a su pequeño tamaño comparado con las células de fitoplancton de mayor tamaño (por ejemplo: los diatomeas), se pensaba que los Picoeucariotas contribuían relativamente poco a la exportación de carbono. Sin embargo, es posible que la participación de Picoeucariotas en el ciclo de carbono oceánico pueda haber sido grandemente ignorada (Richardson and Jackson 2007).

Varios estudios han demostrado que mientras la mayor parte de la varianza encontrada en la contribución de *Synechococcus* y *Prochlorococcus* a la biomasa de picoplancton podía explicarse por la variabilidad medida en sus abundancias, la de los Picoeucariotas se podía explicar tanto por la variabilidad en su abundancia tanto por la variabilidad en el tamaño celular (Worden et al. 2004; Shalapyonok et al. 2001; Durand et al. 2001). Las discrepancias en la estructuras de tamaño de la comunidad de Picoeucariotas sugerían que las comunidades naturales de Picoeucariotas no están siempre compuestas por las mismas especies y grupos (Worden et al. 2004; Worden and Not 2007), sino por diferentes grupos taxonómicos con distintos tamaños celulares con diferentes frecuencias de ocurrencia y posiblemente con diferentes contenidos de carbono. Para limitar estas incertidumbres, queríamos que el Capítulo I se centrase en el establecimiento de un factor de conversión de carbono para Picoeucariotas utilizando diferentes cultivos que fueran ecológicamente representativos de las comunidades naturales.

Se encontró un contenido de carbono promedio por Picoeucariotas de 1540 fgC $cell^{-1}$ correspondiendo a un volumen promedio de 2,14 μm^3, (equivalente a un diámetro de 1.60 μm), representativo de la categoría de tamaño del picoplancton restringido entre 0.2 y 2

μm (como definido por Sieburth 1978). Si este valor estuvo aparentemente cerca del factor de conversión estimado de 1500 fgC $cell^{-1}$ por Zubkov et al. (1998; 2000), mostramos que los Picoeucariotas podían contener mucho más carbono del que se creía anteriormente. Calculando el contenido de carbono por unidad de volumen (C/V) como una medida de la densidad celular de carbono (fgC μm^{-3}), se estableció un promedio de C/V de 467 fgC μm^{-3}, mayor que el valor que se pensaba, (por ejemplo, 220 fgC μm^{-3}). Zubkov et al. (1998, 2000) estimaba un CF a partir de la relación propuesta por Booth (1988), eligiendo un promedio de diámetro de células de 2.35 μm determinado mediante el análisis de imágenes de células seleccionadas al azar y marcadas con DAPI (Sherr et al. 1987). El valor que se encontró para los Picoeucariotas fue mucho más cercano a lo encontrado para *Synechococcus* (a 470 fgC μm^{-3}) que fue estimado a partir de la relación descrita por Verity et al. (1992). Utilizando el valor de 467 en lugar del 220 μm fgC μm^{-3} que ha sido ampliamente utilizado (por ejemplo, Campbell et al. 1994; Zubkov et al. 1998; 2000 ...) y suponiendo un tamaño promedio de célula de 2.35 μm determinado por Zubkov et al. (1998) en lugar del tamaño promedio de 1.60 μm calculado en el capítulo I, la estimación de la biomasa de Picoeucariotas se habría incrementado más de dos veces y alcanzado un valor extremadamente alto de 3176 fgC por célula, significando una contribución mayor a la biomasa total.

La aplicación de este contenido de carbono por célula (en lugar del 1540 fgC $cell^{-1}$, Capítulo I) a una base de datos de abundancia de picoplancton determinada por citometría de flujo representativa de una amplia gama de provincias oceánicas (Tabla 5) habría incrementado la contribución de Picoeucariotas a la biomasa de picofitoplancton en promedio de 30% a escala global. Sin embargo, que este valor sea o no relevante para caracterizar la contribución de carbono de las comunidades de Picoeucariotas dependerá en gran parte de la dominancia de células grandes en la comunidad de Picoeucariotas (por ejemplo, con una proporción mas alta de *Ochromonas* o *Chrysochromulina* con 4.38 μm y 5.06 μm, respectivamente, Capítulo I). Otros autores mostraron también grandes diferencias en el tamaño celular promedio de de los Picoeucariotas. Grob et al. (2007) midieron un promedio de células de 1.74 ± 0.13 μm, que osciló entre 1.37 a 1.99 μm para las comunidades de Picoeucariotas del Este del Océano Pacífico Sur, más cerca de nuestro valor de 1.60 μm, pero inferior al tamaño promedio medido para las comunidades de Picoeucariotas en los giros subtropicales del Oceano Norte Atlántico y Atlántico Sur (2.35 μm, Zubkov et al. 1998) así como en la zona tropical del Océano Pacífico (2.28 μm,

CRUISE	SAMPLE AREA
	From NW African coast
Latitud-2 (Gasol et al. unpublished)	To southern Atlantic
Inco-I and Inco-II (Gasol et al. unpublished)	NW Iberian Penisula (Ría de Vigo)
	From NW African coast
Coca-II (Gasol et al. unpublished)	To offshore North Atlantic Subtropical gyre
Dharma	Southern Ocean
(Zabala et al. unpublished) (Diez et al. 2004)	Weddell sea
Varimed'96	NW Mediterranean
(Gasol et al. unpublished)	From coast to offshore transect
Blanes Bay (Gasol and Massana, unpublished)	coastal NW Mediterranean
AMT 3, 4, 6	From North Atlantic
(Zubkov et al. 2000)	To Southern Atlantic Ocean
Mater 97 and 98	SW Mediterranean
(Massana et al. 1997) (Diez et al. 2001)	Alboran Sea
Wecoma	North Pacific
(del Giorgio and Gasol, 2011)	From coast to offshore transect
Charpy and Blanchot 1998	Atoll lagoon French Polynesia
Sondergaard et al. 1991	Danish coastal waters (Baltic)
Hall and Vincent 1990	SW Pacific Ocean

Tabla 5. Posición geográfica de las diferentes campañas y estudios de abundancia de los grupos de picoplancton, determinada por citometría de flujo.

Claustre et al. 1999). Por estas razones, una determinación más precisa de la estructura del tamaño de las comunidades de Picoeucariotas naturales, junto con la cuantificación de la contribución relativa de los diferentes taxones a la comunidad de Picoeucariotas es crucial para estimar con mayor precisión su contribución a la biomasa de picoplancton.

Sin embargo, esta estimación aproximada de la participación más alta de Picoeucariotas a la biomasa picofitoplanctonica podría ser corroborado por varios estudios anteriores. Por ejemplo, Amacher et al. (2009) mostró que la mayoría de las secuencias de 18S rDNA obtenidas en el este del Atlántico Norte subtropical a partir de material genético recogido por debajo de la zona eufótica podrían ser asignados a taxones de fitoplancton de pequeño tamaño en lugar de a los mas grandes (como diatomeas) lo que sugiere que la contribución a la exportación de carbono de los taxones más pequeños puede haber sido subestimada. Corroborando esta observación, Richardson y Jackson (2007) sugirió que todos los productores primarios, y no sólo las células mas grandes de fitoplancton podrían participar en el exporte de carbono en proporción a sus tasas de producción. Entre la variedad de los parámetros implicados en la dinámica del sedimento de las partículas en el océano, como el tamaño y la geometría celular (Smayda 1970), los datos obtenidos en

el capitulo 5 sugieren que se tendría de incluir la densidad de carbono como un factor de primera importancia.

En el Capítulo I, no se encontraron diferencias significativas en los factores de conversión de los Picoeucariotas entre los ambientes costeros o de mar abierto, a pesar de que la densidad en carbono de las especies de mar abierto fue muy variable (Figura 13). Sin embargo, son necesarios futuros estudios centrados en la cuantificación de los diferentes taxones de Picoeucariotas para estimar con precisión su contribución en el carbono oceánico, sobre todo si tenemos en cuenta que las comunidades Picoeucariotas se componen de diversas especies con aportaciones específicas en gran parte desconocidas y que el tamaño promedio de las células varía entre los ecosistemas. Por ejemplo, Shalapyonok et al. (2001) mostró cambios profundos en la composición de la comunidad de fitoplancton en el mar de Arabia acompañados por cambios en la estructura del tamaño de la comunidad, cuyo tamaño promedio fue más pequeño en aguas más ricas en nutrientes, mientras que las células grandes se encontraban en condiciones de mar abierto.

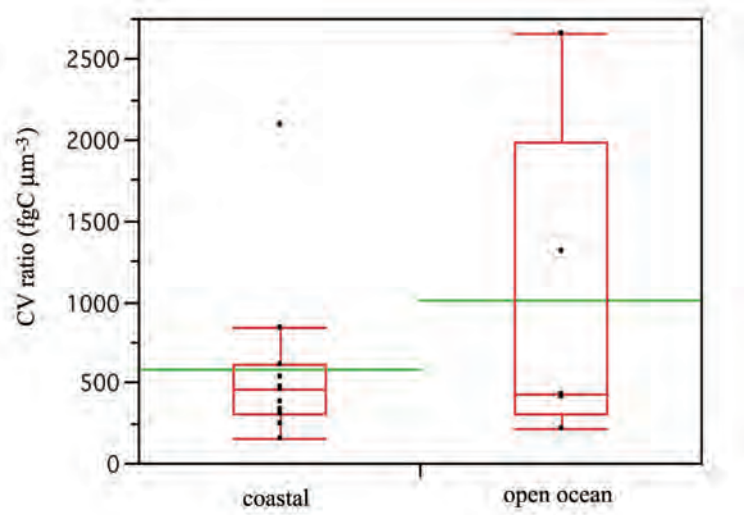


Figura 13. Comparación del contenido en carbono por unidad de volumen (fgC μm^{-3}) de Picoeucariotas entre ecosistemas costeros o de mar abierto.

Patrones a corta o gran escala en la estructura de la comunidad del picoplancton

Varios análisis a larga escala han demostrado un vínculo entre la composición de la comunidad de picoplancton y la propiedades de las masas de agua (Li 1995; Buck et al. 1996; Zubkov et al. 2000; Li y Harrison 2001; Tarran et al. 2001; Shalapyonok et al. 2001), mostrando por ejemplo que, si *Synechococcus* y Picoeucariotas son típicas de la

zonas costeras y de la pendiente continental, *Prochlorococcus* estaba más representado en el centro de los giros oceánicos (Zubkov et al., 2000). El análisis de multi-escala de la variabilidad de PCS realizada en el Capítulo III (y en menor medida en el Capítulo II), reveló que las abundancias de los diferentes grupos de picoplancton mostraron diferentes amplitudes de variación. La mayor variabilidad se atribuyó a la abundancia de los Picoeucariotas, mientras que menor variabilidad fue encontrada en las bacterias. Por otra parte, diferentes patrones de distribución de *Synechococcus* y de Picoeucariotas más complejos que la simple ocurrencia se midieron a lo largo de gradientes de parámetros ambientales, lo que sugiere que estos dos grupos podían tener diferentes estrategias y funciones en la red trófica microbiana.

Una parte relevante del Capítulo II fue el cambio observado en la estructura de la comunidad de picoplancton a corta escala de tiempo. Este cambio en la PCS puede haber sido provocado por turbulencia y se mostró como el incremento de un día para otro del ratio entre los dos principales contribuyentes a la biomasa de picofitoplancton (*Syn*:pPeuk), señalando la importancia de los fenómenos meteorológicos transitorios en la estructuración de las comunidades costeras de picoplancton y mostrando que los cambios ecológicos suelen ser consecuencia de episodios físicos operando a escalas de tiempo muy cortas (Seymour et al. 2005). En el Capítulo III, patrones opuestos se encontraron entre el ratio Syn:pPeuk y la concentración en clorofila *a*, tanto a corta como larga escala temporal (series de tiempo en la estación costera de Blanes), la proporción era de hecho máxima al menor nivel de clorofila a diario (alrededor de 12h00), así como durante el verano. Sin embargo, la variabilidad de esta relación se ha estudiado sólo en una escala espacial relativamente pequeña durante el transecto (100 Km), un objetivo adicional de la discusión fue identificar los patrones de PCS a una escala más global, buscando sobre todo cómo el ratio *Syn*:pPeuk variaba a lo largo de un gradiente de biomasa bacteriana heterótrofa en diferentes ecosistemas: costero y mar abierto. Se encontraron dos modelos opuestos: el ratio aumentaba con la biomasa bacteriana en los ecosistemas mar abierto, mientras que disminuía en condiciones costeras (Figura 14).

Estos patrones opuestos implicaban que mientras la biomasa de los Picoeucariotas fue mayor relativamente a la de *Synechococcus* a altos niveles de biomasa heterótrofa en condición costera, la biomasa de *Synechococcus* fue mayor relativamente a la de Picoeucariotas en mar abierto (Figura 14A). Estudiando cómo esta relación varía a larga

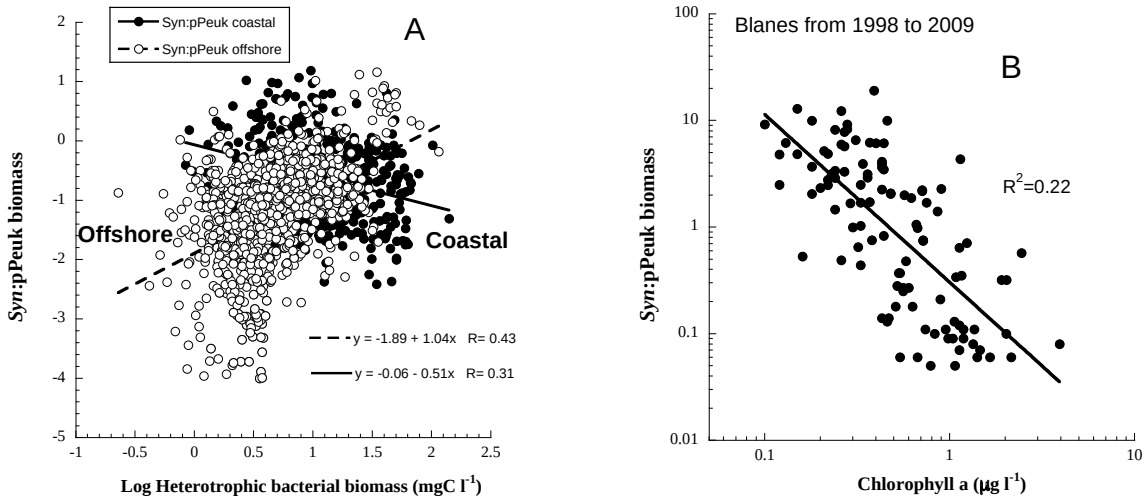


Figura 14. Ratio entre las biomasas de *Synechococcus* y de Picoeucariotas a lo largo de un gradiente de biomasa heterotrófica en zona costera y mar abierto utilizando los datos de la Tabla 5 **(A)**, a lo largo de un gradiente de clorofila *a* en la estación de Blanes desde el 1998 hasta el 2009 **(B)**.

escala temporal en una estación costera tomando el ejemplo de la bahía de Blanes desde 1998 hasta 2009, se observó una disminución de la contribución de *Synechococcus* en relación a Picoeucariotas, a lo largo del gradiente de clorofila *a* (Figure 14B). Estos patrones encontrados a larga escala pueden corroborar las conclusiones del Capítulo III, en el que los patrones medidos a meso-escala desde la costa a mar abierto mostraron un aumento de la contribución de Picoeucariotas a la biomasa de picoplancton, de la estación costera (C) hasta de la estación (CM) en la pendiente continental, mientras que la contribución de *Synechococcus* disminuyó, lo que sugería que la relación se debía a diferentes niveles troficos de ambos ecosistemas (Figura 15).

Varias evidencias del vínculo entre el PCS y las propiedades de las masas de agua se mostraron, Hall and Vincent (1990) observaron que la abundancias de *Synechococcus* y de Picoeucariotas en las regiones de afloramiento al sur de Nueva Zelanda aumentaban con la distancia hacia la costa, llegando a un máximo en las estaciones de la pendiente continental. Calvo-Díaz et al. (2004) mostraron que la abundancia de Picoeucariotas superaba la abundancia de cianobacterias (en su mayoría *Synechococcus*) en zonas ricas en nutrientes con altos niveles de clorofila *a* del mar Cantábrico, sugiriendo también diferentes estructuras de la comunidad de picoplancton en aguas costeras y mar abierto.

Al contrario, Sherr et al. (2005), observaron una relación negativa entre la abundancia de Picoeucariotas y la clorofila *a* así como una menor abundancia de picofitoplancton en zonas de afloramiento frente a la costa de Oregon. Sin embargo, cuando ambos *Synechococcus* y Picoeucariotas fueron considerados, se observó la misma tendencia negativa de la relación entre el ratio *Syn*:pPeuk a lo largo del gradiente de clorofila *a*, lo que corrobora nuestros resultados de mayor contribución de Picoeucariotas a la biomasa picofitoplanctonica en relación a *Synechococcus* a altos niveles de clorofila *a* en los ecosistemas costeros.

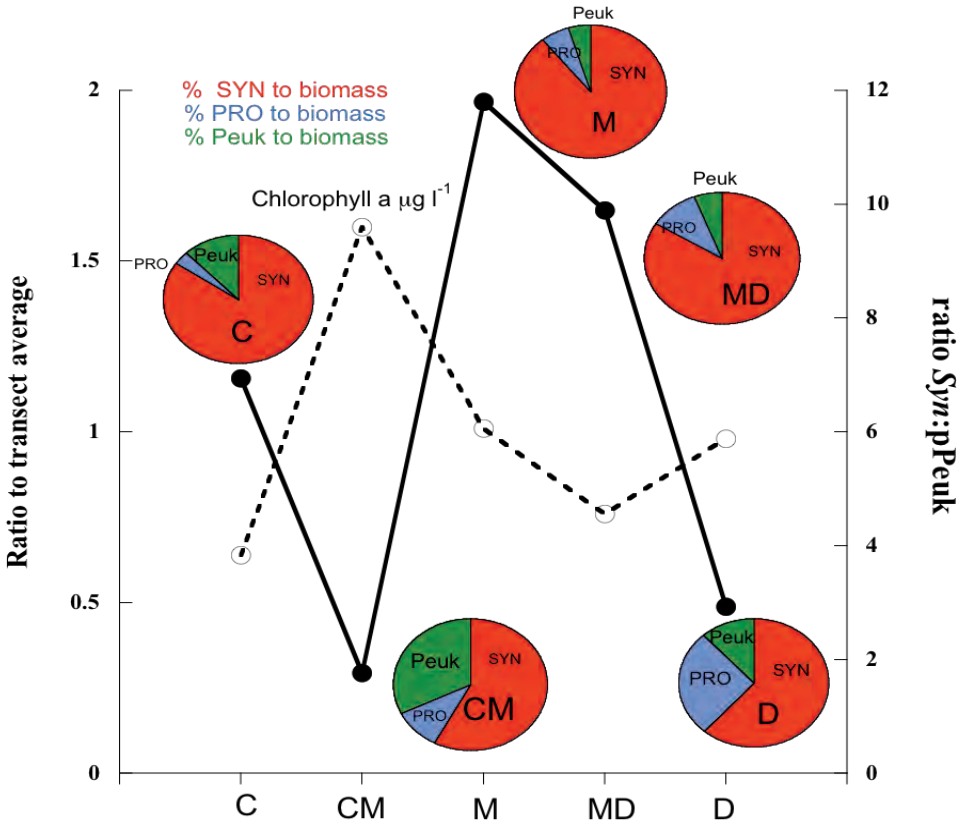


Figure 15. Ratio entre la concentración de clorofila *a* de cada estación y la concentración de clorofila *a* promedio del transecto. Ratio entre las biomasas de *Synechococcus* y Picoeucariotas, así como los porcentajes de contribución de cada grupo de picofitoplancton a la biomasa a lo largo de las estaciones del transecto.

Predicción de la estructura de la comunidad bacteriana y de picoplancton desde factores ambientales

Un objetivo importante de esta tesis fue describir los patrones no sólo en el PCS a diferentes escalas espaciales y temporales, sino también identificar los patrones en el BCS a lo largo de gradientes de parámetros ambientales, tales como la clorofila a, temperatura y salinidad. Se mostró una relación significativa entre la abundancia absoluta de los diferentes grupos bacterianos y la concentración de clorofila *a*, con diferentes pendientes que varían a la escala global de 0.13 (± 0.04) para SAR11 hasta 0.53 (± 0.08) para *Betaproteobacteria*, con diferencias significativas entre las zonas costeras y mar abierto.

Utilizando las ecuaciones de regresión de la Tabla 6 (Capítulo IV) para hacer predicciones acerca de la composición de la comunidad en diferentes tipos de ecosistemas y a diferentes niveles de clorofila *a*, se observó que SAR11 tendían a dominar a alta y baja clorofila *a* en condiciones de mar abierto (Figura 16), con una contribución cada vez mayor de los *Bacteroidetes* con el incremento de clorofila. En los ecosistemas costeros, SAR11 seguía siendo relevante a bajos niveles de clorofila *a*, pero rea reemplazado progresivamente por el aumento de las contribuciones de *Betaproteobacteria* y *Bacteroidetes* a mayor niveles de clorofila *a*.

B

LOG CONCENTRATION (CELLS.ML^{-1}) - LOG CHLOROPHYLL *A* (µg. L^{-1})	N_{obs}	R^2	Intercept (± SE)	Slope (± SE)
BULK BACTERIA (1B)	257	0.33	**5.91*** (±0.02)	**0.38*** (±0.04)
EUBACTERIA (2B)	167	0.41	**5.76*** (±0.03)	**0.50*** (±0.05)
ALPHAPROTEOBACTERIA (3B)	200	0.11	**5.29*** (±0.03)	**0.25*** (±0.05)
SAR11 (4B)	97	0.01	**5.07*** (±0.05)	-0.11 (±0.08)
RHODOBACTERACEAE (5B)	144	0.22	**4.53*** (±0.04)	**0.36*** (±0.06)
GAMMAPROTEOBACTERIA (6B)	183	0.08	**4.87*** (±0.04)	**0.25*** (±0.06)
BACTEROIDETES (7B)	214	0.29	**5.12*** (±0.03)	**0.40*** (±0.04)
BETAPROTEOBACTERIA (8B)	65	0.38	**4.90*** (±0.07)	**0.52*** (±0.08)

C

LOG CONCENTRATION (CELLS.ML^{-1}) - LOG CHLOROPHYLL *A* (µg. L^{-1})	N_{obs}	R^2	Intercept (± SE)	Slope (± SE)
BULK BACTERIA (1C)	168	0.21	**5.79*** (±0.04)	**0.25*** (±0.04)
EUBACTERIA (2C)	39	0.60	**6.09*** (±0.10)	**0.66*** (±0.09)
ALPHAPROTEOBACTERIA (3C)	45	0.18	**5.51*** (±0.08)	**0.34*** (±0.11)
SAR11 (4C)	114	0.28	**5.40*** (±0.05)	**0.31*** (±0.05)
RHODOBACTERACEAE (5C)	101	0.17	**4.54*** (±0.06)	**0.25*** (±0.06)
GAMMAPROTEOBACTERIA (6C)	50	0.00	**4.58*** (±0.09)	-0.06 (±0.12)
BACTEROIDETES (7C)	128	0.36	**4.95*** (±0.06)	**0.48*** (±0.06)
BETAPROTEOBACTERIA	8		----	----

Tabla 6. Análisis de regresión de las concentraciones de grupos bacterianos (estandarizados) vs la concentración de clorofila *a*, en zona costera (A) y en mar abierto (B) siguiendo la formula siguiente:
Log conc. bact. estandarizada (cells ml^{-1}) = intercept (±SE) + slope (±SE) x Log clorofila *a* (µg l^{-1})

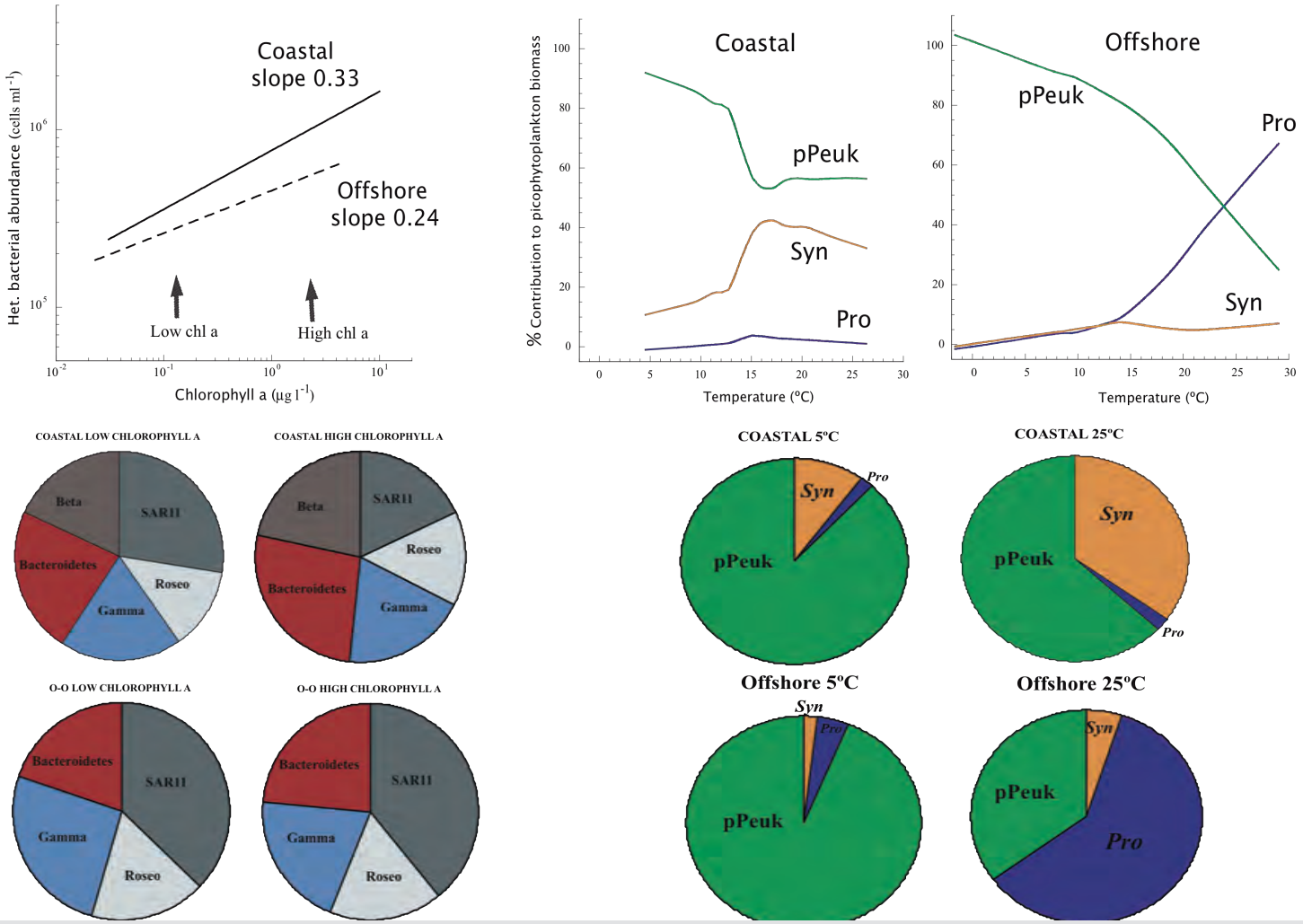


Figure 16. Estructura de la comunidad bacteriana a bajo y alto nivel de clorofila *a* según las relaciones lineales de la Tabla 6 (Log transformada) en condición costera y mar abierto. Porcentajes de contribución de *Synechococcus, Prochlorococcus* y pPeuk a la biomasa de picofitoplancton a lo largo de un gradiente de temperatura en zona costera y mar abierto (Detalles ver Discusión).

Utilizando la base de datos de abundancias de picoplancton (Tabla 5), se cuantificó a gran escala las contribuciones relativas de Picoeucariotas, *Synechococcus* y *Prochlorococcus* a la biomasa del picofitoplancton a lo largo de gradientes de temperatura en los ecosistemas costeros y de mar abierto (Figura 16). En ecosistemas costeros, se observó que los Picoeucariotas dominaban la biomasa de picofitoplancton a baja y alta temperatura, con una contribución de *Synechococcus* cada vez mayor de baja a altas temperaturas y *Prochlorococcus* contribuyendo siempre poco. Mientras que los Picoeucariotas dominaban la biomasa de picofitoplancton a bajas temperaturas en el mar abierto, la contribución de los *Prochlorococcus* fue mayor a temperaturas más altas (Figura 16).

PRINCIPALES CONCLUSIONES

Las principales conclusiones de esta tesis son las siguientes:

i) El uso de cultivos representativos de una amplia gama de géneros y clases de Picoeucariotas permitió describir nuevas relaciones entre el volumen celular y el contenido en carbono y nitrógeno para un rango de tamaño celular desde 1.38 μm hasta 5.06 μm y determinar un promedio de carbono por unidad de volumen celular (C/V) de 467 fgC μm^{-3} (±4%) característico de las comunidades naturales de Picoeucariotas, pero relativamente más altos que los factores de conversión anteriormente medidos.

ii) Se compararon dos métodos para corregir los errores en la estimación de la biomasa debido a la presencia de bacterias en los cultivos. El primero se basó en la separación de células mediante la citometría de flujo que permitió hacer 11 cultivos axénicos de los 16 cultivos totales. El segundo fue basado en el análisis de imagen y la aplicación de factores de conversión estándares para bacteria, resultando en disminuciones de los valores de contenido celular en carbono de los Picoeucariotas de 7% a 33%. Dado que no se encontraron diferencias significativas entre las relaciones (C/V) o (N/V) después de los dos métodos de corrección, se concluyó que ambas técnicas eran adecuadas para estimar el error debido a la presencia de bacterias en los cultivos. Sin embargo, el método basado en la separación de células por citometría fue restringuido ya que no podía ser aplicado a todos los cultivos, limitándose a los que presentaban una abundancia de Picoeucariotas muy alta.

iii) El estudio de la estructura de la comunidad de picoplancton y de la actividad heterotrófica en una estación costera del Mediterráneo mostró que los diferentes grupos seguían patrones de variabilidad en la escala día-noche, incluyendo las bacterias heterótrofas y los heteronanoflagelados. Por otra parte, evidencias de desfases se mostraron entre la duplicación y el crecimiento de los grupos de picofitoplancton así como evidencias de acoplamiento entre la variabilidad de la estructura de la comunidad de picofitoplancton y las actividades bacterianas. Además, se observó que una variación relativamente pequeña de los patrones de climatología cambiaba considerablemente la estructura de la comunidad microbiana perturbando los ciclos día y noche.

iv) Se mostraron marcadas diferencias en la distribución de los diferentes grupos por el estudio de la variabilidad temporal y espacial de la estructura de la comunidad de picoplancton por citometría de flujo durante una campaña oceanográfica de costa a mar abierto. *Synechococcus* dominaba la zona costera, los Picoeucariotas alcanzando un máximo a la pendiente continental, y *Prochlorococcus* dominando en mar abierto.

v) El análisis de la variabilidad de la PCS durante esta campaña demostró que mientras la variabilidad espacio-temporal más alta se encontró con la abundancia de los Picoeucariotas, la más baja se encontró con la abundancia bacteriana.

vi) La mayor fuente de variabilidad de abundancia se observó en la escala espacial, promovida por la estratificación vertical de la columna de agua, y horizontalmente por las diferencias tróficas entre las estaciones. Las estaciones costeras presentaban mayor niveles de abundancia y de actividad bacteriana, pero menor variabilidad espacio-temporal de estos parámetros. Al contrario, las zonas de mar abierto presentaban niveles bajos de abundancia y de actividad bacteriana pero mayor variabilidad espacio-temporal.

vii) Patrones opuestos entre el ratio de biomasas de *Synechococcus* y Picoeucariotas y los niveles de clorofila *a* se observaron no sólo espacialmente, sino también, a corta y larga escala temporal, haciendo de este ratio un indicador potencial del estado trófico de los ecosistemas.

viii) Se describieron patrones de escala global entre los principales grupos (alfa, beta-, *Gammaproteobacteria* y *Bacteroidetes*, así como *Rhodobacteraceae* y SAR11) y las variables ambientales tales como la concentración de clorofila *a*, la salinidad y la temperatura distinguiendo entre los ecosistemas costeros (es decir, sobre la plataforma continental) y los de mar abierto. Se encontraron diferentes relaciones entre las abundancias absolutas de los grupos bacterianos y la concentración de clorofila *a*, con muy diferentes pendientes logarítmico entre los tipos de ecosistemas. Las diferentes tendencias encontradas para los distintos grupos a través de los niveles tróficos, en diferentes tipos de ecosistemas, sugieren diferentes capacidades metabólicas de cada grupo para la utilización del carbono orgánico derivado de la algas.

ix) El análisis estadístico reveló la preferencia de *Gammaproteobacteria* por el aumento

de temperatura y de para *Betaproteobacteria* por la disminución de temperaturas. Además, el análisis de regresión múltiple mostró efectos significativos de la clorofila *a* junto a la temperatura en la concentración bacteriana total así como en la abundancia absoluta de SAR11 y finalmente efectos significativos de la clorofila *a* y de salinidad en la abundancia absoluta de *Betaproteobacteria* y *Rhodobacteraceae*.

Consideraciones finales

Desde una perspectiva más amplia, los métodos usados en esta tesis representan una alternativa para explorar la complejidad de la comunidad microbiana mediante el estudio de los patrones observables. Afirmamos que no sólo una, sino diferentes formas de representación de la comunidad microbiana, que abarca desde los aspectos de funcionalidad o de actividad mediante el uso de citometría de flujo hasta perspectivas más genéticas al estudiar los patrones de distribución filogenética son necesarias para precisar el papel ecológico de los grupos de picoplancton, así como su acoplamiento en los ecosistemas naturales.

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