UNIVERSIDADE FEDERAL DE SÃO CARLOS

CENTRO DE CIÊNCIAS BIOLÓGICAS E DA SAÚDE

PROGRAMA DE PÓS-GRADUAÇÃO EM ECOLOGIA E RECURSOS NATURAIS

BIOTIC FACTORS DRIVE BACTERIOPLANKTON COMMUNITY IN A TROPICAL COASTAL SITE OF THE EQUATORIAL ATLANTIC OCEAN

Vinicius Silva Kavagutti

São Carlos – SP

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Aos
meus familiares e amigos... que também são minha família, então... À minha FAMÍLIA.

“A cura para tudo é sempre água salgada: o suor, as lágrimas ou o mar”

(Isaak Dinesen)

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"Amigos são a família que nos permitiram escolher. " (William Shakespeare)

Resumo

A relação entre a latitude e diversidade microbiana no oceano é controversa. Modelos de nicho preveem maior riqueza em altas latitudes no inverno, enquanto amostragens pontuais indicam uma maior riqueza em latitudes intermediárias, com valores mais baixos para regiões equatoriais e polares. No entanto, dada a natureza dinâmica do ecossistema oceânico, é difícil explicar variações temporais da biodiversidade microbiana nas avaliações empíricas. Nesse trabalho comparamos os componentes da diversidade (riqueza e equitabilidade) e estabilidade das populações microbianas (coeficiente de variação) em dois observatórios oceânicos costeiros com estados tróficos semelhantes, localizados em latitudes contrastantes: um localizado no Oceano Atlântico Equatorial e um em clima temperado localizado no noroeste do Mar Mediterrâneo, a fim de avaliar quais fatores estruturam a dinâmica das comunidades microbianas em cada local. Observamos que tal como animais e plantas, as comunidades microbianas exibem maior (ou pelo menos similar) riqueza no equador pelo menos em águas costeiras. Também encontramos evidências de aumento da estabilidade com o aumento da uniformidade nas comunidades microbianas tropicais, quando comparadas com as de clima temperado. De modo geral, temperatura e silicatos foram as variáveis que condicionaram as comunidades procariotas de vida livre no observatório da região temperada, enquanto que no observatório tropical, fatores estocásticos tais como interações bióticas com eucariotos, foram os fatores que mais influenciaram as comunidades bacterianas. Assim, propomos um quadro conceitual onde a composição da comunidade microbiana seria impulsionada por fatores determinísticos em latitudes mais elevadas, enquanto que em latitudes menores, seriam determinados por fatores mais estocásticos, como interações bióticas. Nosso estudo destaca a importância de estudos comparativos utilizando series temporais Eulerianas em diferentes latitudes para entender os padrões de diversidade das comunidades microbianas no oceano.

Palavras-chave

Diversidade bacteriana, Gradiente latitudinal, Séries temporais, Amostragem Euleriana, Sequenciamento de nova geração.

Abstract

The relationship between latitude and microbial diversity in the ocean is controversial. Niche models predict higher richness at high latitudes in winter, while snapshot field-sampling point towards higher richness at intermediate latitudes, with lower values both towards equatorial and Polar Regions. However, given the dynamic nature of ocean's ecosystem it is difficult to account for temporal variations in empirical assessments of microbial biodiversity. Here, we compared the components of diversity (richness and evenness) and microbial population stability (coefficient of variation) in two coastal ocean observatories with similar trophic state located in contrasting latitudes, one located in the Equatorial Atlantic Ocean, and one temperate located in the Northwestern Mediterranean Sea, to evaluate which factors drive the dynamics of microbial communities in each site. Our observations support the view that, as animals and plants, microbial communities exhibit higher (or at least similar) richness towards the equator, at least in the coastal ocean. We also found evidence of increasing stability with increasing evenness in tropical microbial communities when compared to the temperate ones. Temperature and silicates drove temperate free-living prokaryotic communities, while tropical ones were driven by stochastic factors such as biotic interactions with eukaryotes. We propose a conceptual framework where microbial community composition would be driven by deterministic factors in higher latitudes and once the factor temperature is removed moving towards the equator, more stochastic factors such as biotic interactions would emerge as the main factors shaping microbial communities. This study highlights the importance of comparative studies on Eulerian time-series distributed at different latitudes to fully understand the diversity patterns of microbial communities in the ocean.

Keywords

Bacterial diversity, Latitudinal gradient, Time series, Eulerian sampling, Next generation sequencing.

Lista de Abreviações e Siglas

BBMO - Blanes Bay Microbial Observatory

CV – Coefficient of Variation

DNA - Deoxyribonucleic Acid

EAMO - Equatorial Atlantic Microbial Observatory

Euk – Eukaryotes

FL – Free-Living (< 3 µm)

FL3 – Fluorescence Type 3 (Red)

H' – Shannon Index

HNA - High Nucleic Acid

NGS – Next-Generation Sequencing

OTU – Operational Taxonomic Units

PA – Particle-Attached (> 3 µm)

PCR - Polymerase Chain Reaction

Prok – Prokaryotes

rRNA - Ribosomal Ribonucleic Acid

SSC – Side-Scatter

Temp – Temperate

Trop – Tropical

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Capítulo I

Introdução Geral

O que sabemos é uma gota; o que ignoramos é um oceano.
Mas o que seria o oceano se não infinitas gotas?
(Isaac Newton)

Introdução geral

Os microrganismos marinhos foram os primeiros organismos vivos na Terra e únicos durante milhões de anos de evolução. Cada litro de água do mar está repleta de milhões de vírus, bactérias e protistas, ultrapassando todos os metazoários multicelulares em abundância, biomassa, atividade metabólica e diversidade genética e bioquímica (Falkowski *et al.*, 2008; Kirchman, 2008; Hug *et al.*, 2016). Na visão mais atual da árvore da vida (Figura 1.1), os o conjunto de todas as formas de vida eucariótica (como animais e plantas, por exemplo) caberiam em uma pequena ramificação dessa árvore, sendo a esmagadora maioria da diversidade filogenética constituída por bactérias e arqueas (procariotos).

Ao contrário dos animais e plantas, morfologicamente diferenciáveis em sua maioria, apenas uma parte da diversidade microbiana pode ser identificada com base na morfologia. Isso é válido especialmente no caso de protistas e picoeucariotos, dentre os quais a existência de espécies crípticas é uma característica generalizada (de Vargas *et al.*, 2015b). Justamente estes microrganismos constituem o maior conjunto de diversidade genômica e metabólica (funcional) ainda a ser explorada (Yooseph *et al.*, 2007, 2010). Até há bem pouco tempo, as técnicas de biologia molecular disponíveis para estudar a identidade filogenética dos microrganismos se limitavam aos organismos mais abundantes ou a alguns (poucos) cultiváveis, geralmente pouco representativos das comunidades presentes no ambiente (Yooseph *et al.*, 2007, 2010; Pedrós-Alió, 2006) (Figura 1.2). Entretanto, nos últimos anos a ecologia microbiana passou por uma revolução impulsionada pelas tecnologias de sequenciamento de nova geração (*next generation sequencing* - NGS) (Sogin *et al.*, 2006). Esta nova abordagem independente de cultivo transformou a nossa perspectiva sobre a estrutura, evolução e ecologia do mundo microbiano, e têm-se revelado extremamente útil na descoberta de moléculas

bioativas. Recentemente, estas técnicas de NGS combinadas com ferramentas bioinformáticas permitiram a caracterização de comunidades microbianas diretamente de amostras ambientais (Logares *et al.*, 2013; Sunagawa *et al.*, 2015; Venter *et al.*, 2004) .

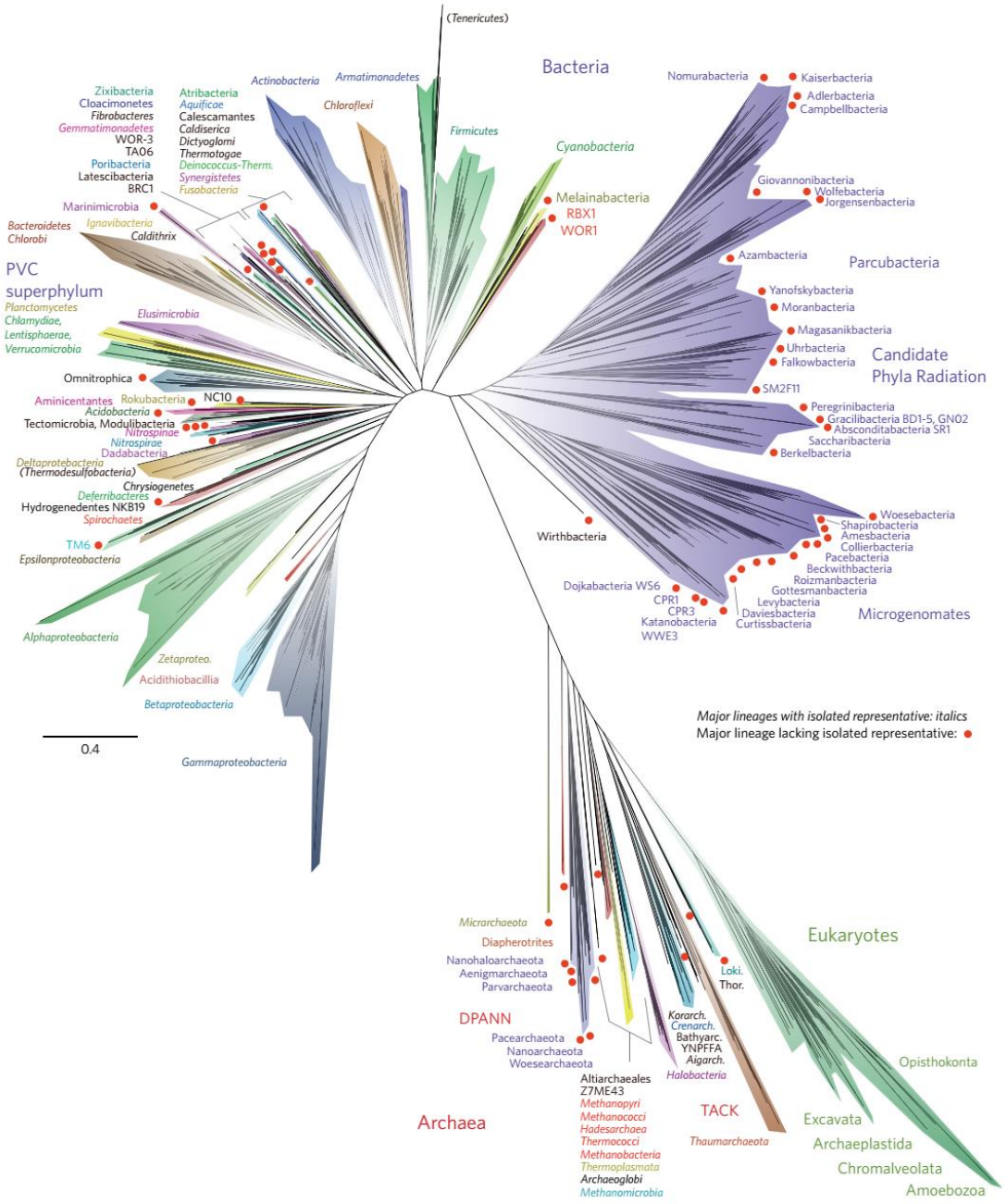

Figura 1. 1. Visão atual da árvore da vida, adaptado de Hug *et al*. (2016), considerando genomas inteiros já sequenciados. A árvore inclui 92 filos bacterianos, 26 filos de Archaea e todos os cinco dos supergrupos eucarióticos.

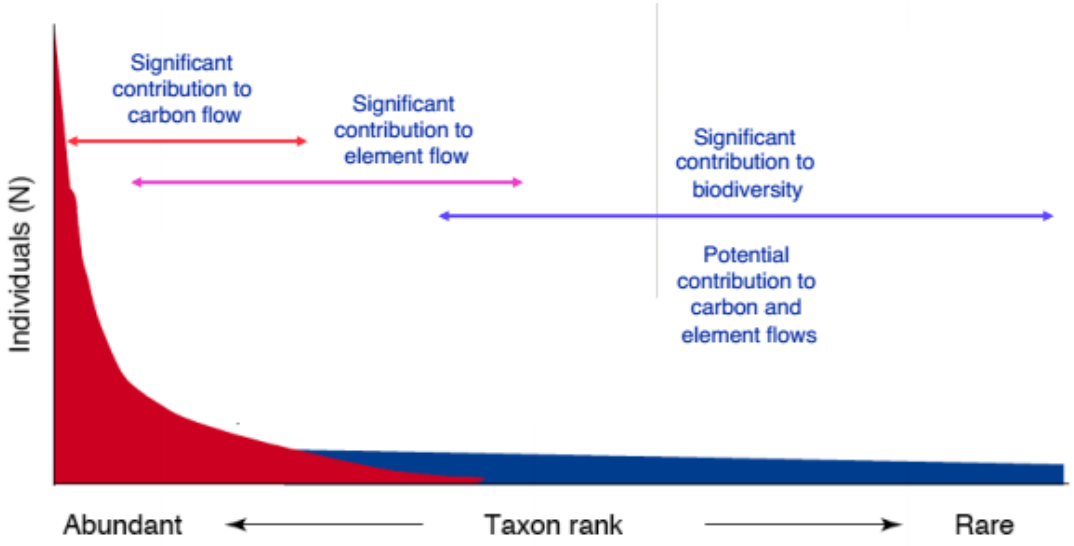

Figura 1. 2. Relação entre a abundância de indivíduos e os taxa ranqueados do mais abundante para o menos abundante (rank-abundance) adaptado de Pedrós-Alió (2006). A área vermelha representa organismos mais abundantes, enquanto a área azul representa a biosfera rara (organismos menos abundantes). A seta em vermelho indica os organismos que contribuem para o fluxo de carbono, em rosa, os que contribuem para o fluxo de outros elementos, em azul, os que contribuem para a real diversidade microbiana e que têm potencial para contribuir para o fluxo de carbono e outros elementos, ao se tornarem abundantes.

Modelos macro-ecológicos predizem maior riqueza em regiões tropicais, onde a estabilidade do ambiente favorece a complexidade e um aumento da riqueza (e.g. Schipper *et al.*, 2008; Olson *et al.*, 2001; Kreft and Jetz, 2007) (Figuras 1.3 e 1.4). Em contrapartida, no que se refere a microrganismos não existe ainda um consenso. Observações empíricas realizadas em expedições oceanográficas que cobrem largas escalas espaciais indicam maior diversidade em latitudes intermediárias (Sunagawa *et al.*, 2015) (Figura 1.5), enquanto modelos de nicho predizem uma maior riqueza em latitudes mais elevadas durante o inverno (Ladau *et al.*, 2013) (Figura1.6).

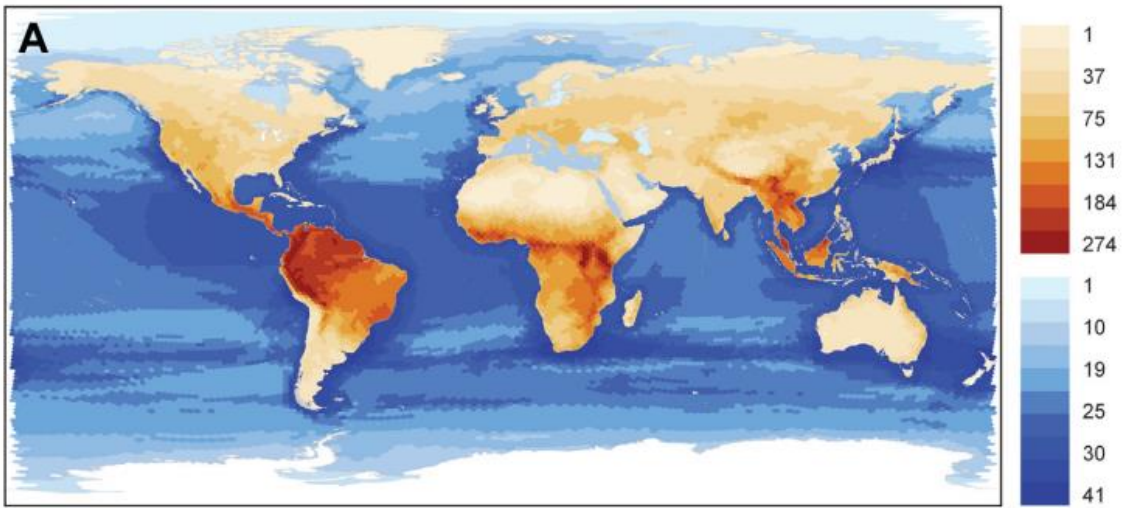

Figura 1. 3. Padrões globais de distribuição da riqueza de mamíferos, em ambientes terrestres e de água doce (em marrom) e no oceano (em azul), adaptado de Schipper, *et al* (2008).

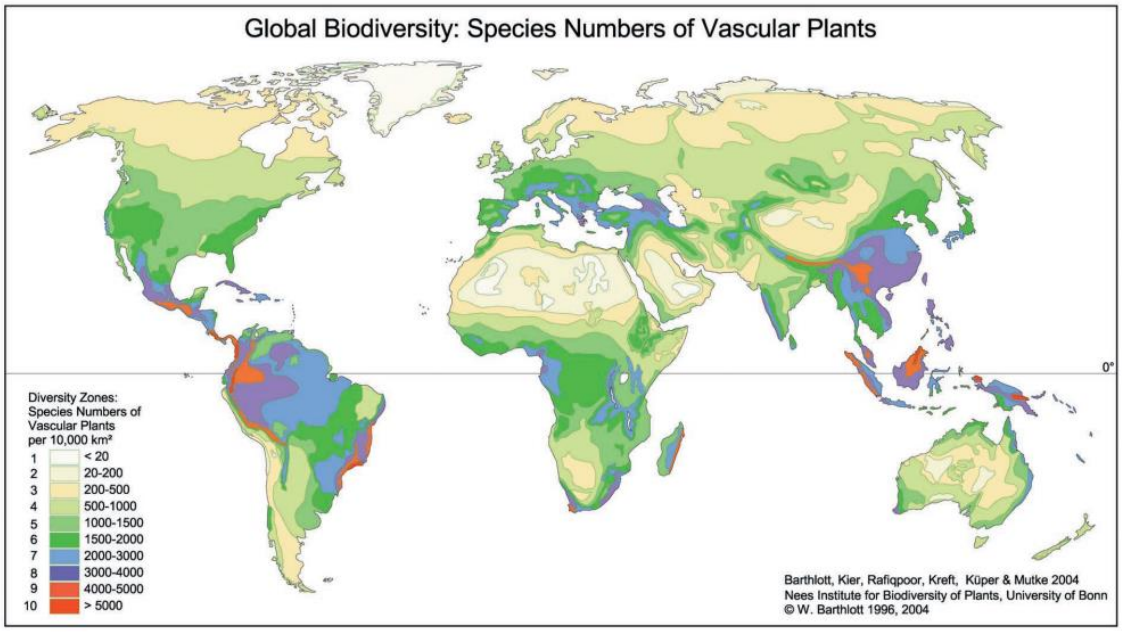

Figura 1. 4. Padrão de distribuição geográfica da riqueza de plantas vasculares, adaptado de Barthlott *et al* (2007).

O movimento das massas de água no oceano dificultam o rastreamento das mesmas por longos períodos (Fuhrman *et al.*, 2015). Assim, a maioria dos estudos de comunidades microbianas marinhas são feitos em sistemas fechados ou em amostragens

em transectos (e.g. Milici *et al.*, 2016), que tentam seguir as correntes e massas de água tanto quanto possível (amostragem Lagrangiana). No entanto, esta estratégia de amostragem não tem uma boa cobertura das variações temporais que ocorrem ao longo do ano. Deste modo, estudos de séries temporais, coletadas em um mesmo ponto durante um intervalo de tempo determinado (amostragem Euleriana), deveriam ser mais comuns para a compreensão da dinâmica microbiana em ecossistemas marinhos (Fuhrman *et al.*, 2015). No entanto, existem poucos observatórios microbianos que monitoram comunidades microbianas marinhas ao longo do tempo, e os que existem estão predominantemente concentrados em regiões temperadas (Fuhrman *et al.*, 2015).

Vários trabalhos abordando diferentes aspetos da diversidade microbiana têm sido publicados em diversos ambientes marinhos, por exemplo, zonas costeiras (Thompson *et al.*, 2011), gradientes costa - oceano aberto (Pommier *et al.*, 2010), oceano aberto (Delong, 2005), oceano profundo (Delong *et al.*, 2006; Salazar *et al.*, 2015; Sogin *et al.*, 2006) e até mesmo em regiões polares (Ghiglione *et al.*, 2012; Logares *et al.*, 2013). Na maioria dos casos, os fatores ambientais que determinam a estrutura microbiana são a temperatura (Sunagawa *et al.*, 2015) ou a duração do dia (horas de luz) (Gilbert *et al.*, 2012), evidenciando um papel secundário das interações bióticas entre componentes da teia microbiana (por exemplo entre eucariontes e procariontes), na estrutura das comunidades.

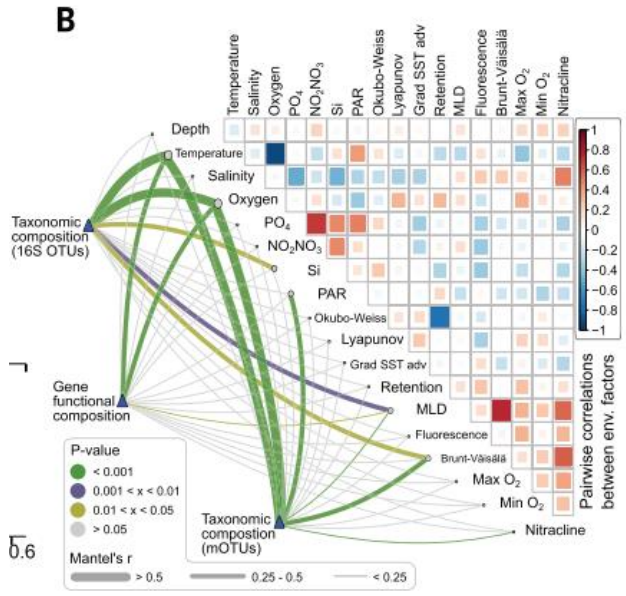

Figura 1. 5. Fatores ambientais que determinam a composição da comunidade microbiana de superfície, adaptado de Sunagawa *et al* (2015). O gradiente de cor nas comparações de variáveis ambientais par a par indicam os coeficientes de correlação de Spearman.

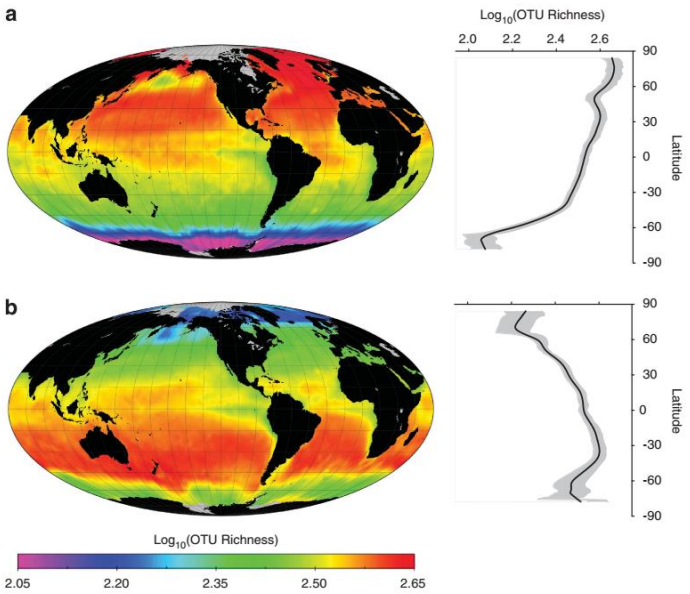

Figura 1. 6. Modelo da diversidade bacteriana global, adaptado de Ladau *et al* (2013). A escala de cores mostra a riqueza relativa de águas superficiais no oceano. A - Em dezembro, maior riqueza em latitudes elevadas do hemisfério norte. B - Em junho, maior riqueza em latitudes elevadas do hemisfério Sul.

Porém, estudos deste tipo em oceano tropical onde variações da temperatura e das horas de luz ao longo do ano são pouco significativas, são ainda incipientes. Considerando que a maior parte da superfície do oceano global é constituída por regiões quentes de oceano tropical, esta poderia ser uma lacuna importante no esclarecimento dos mecanismos que determinam os padrões de diversidade microbiana no oceano. Essas regiões tropicais do oceano são geralmente oligotróficas, e a produção orgânica concentra-se na subsuperfície, sendo dominada por procariotos unicelulares dos gêneros *Synechoccocus* e *Prochloroccocus* e eucariotos de tamanho inferior a 2µm (picoplâncton). A área do oceano com estas características (estratificado e dominadas por picoplâncton) representa cerca de 74% da superfície total do oceano global (Behrenfeld *et al.*, 2006). Por cobrir uma área tão grande do oceano e por apresentar um funcionamento ecológico diferenciado, o estudo das comunidades planctônicas que ocorrem no oceano quente e oligotrófico são de particular interesse para a interpretação de fenômenos de impacto global. Apesar dos esforços e avanços recentes, a diversidade microbiana e sua dinâmica no oceano tropical ainda representa uma lacuna importante.

Dessa forma, esse estudo realizou uma análise comparativa da estabilidade e dos componentes da diversidade (riqueza e equitabilidade) das comunidades microbianas em dois observatórios microbianos situados em latitudes contrastantes, além de determinar quais fatores (ambientais, biológicos ou neutros) dirigem a estrutura dessas comunidades em cada local. Para isso, aplicamos técnicas de NGS, sequenciando *amplicons* do gene rRNA, 16S (para procariotos) e 18S (para eucariotos) para descrever a dinâmica das comunidades microbianas ao longo do tempo, bem como um conjunto de parâmetros ambientais em dois observatórios microbianos, um localizado em águas costeiras oceânicas temperadas (no noroeste do mar Mediterrâneo) e outro em águas costeiras oceânicas tropicais (no litoral de Natal – RN, nordeste brasileiro).

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Capítulo II

Biotic Factors Drive Bacterioplankton Community in a Tropical Coastal Site of the Equatorial Atlantic Ocean

"Tenho a impressão de ter sido uma criança brincando à beira-mar, divertindo-me em descobrir uma pedrinha mais lisa ou uma concha mais bonita que as outras, enquanto o imenso oceano da verdade continua misterioso diante de meus olhos".

(Isaac Newton)

Introduction

Marine microbial communities play a crucial role in most global biogeochemical cycles (Falkowski *et al.*, 2008) and a bear an important share of Earth's biodiversity (Sunagawa *et al.*, 2015). Understanding the factors that drive the dynamics of marine microbial communities at a global scale is an important step to predict the consequences of the growing impacts (Doney *et al.*, 2002). In recent years, aquatic microbial ecology has suffered an authentic revolution driven by high throughput sequencing technologies (Venter *et al.*, 2004). Combined with bioinformatics tools, these new sequencing technologies allowed a characterization of microbial populations directly from environmental samples, and brought a new perspective about the structure, evolution and ecology of the microbial world (e.g. de Vargas *et al.*, 2015; Sunagawa *et al.*, 2015).

Microbial diversity surveys showed that communities are dominated by a relatively small number of some OTUs (operational taxonomic units), but thousands of other less abundant populations (the rare biosphere) are responsible for most of the phylogenetic diversity observed (e. g. Galand *et al.*, 2009; Connolly *et al.*, 2014; Logares, Audic, *et al.*, 2014). Recent large-scale oceanographic cruises focused on how OTU (operational taxonomic units) richness is distributed across latitudinal gradients suggesting that, differently from animals and plants, microorganisms present maximal richness at intermediate latitudes (Milici *et al.*, 2016; Sunagawa *et al.*, 2015). However, ocean's ecosystem is extremely dynamic and seasonal variations are difficult to capture in such cruises covering large spatial scales (Fuhrman *et al.*, 2015). Niche models, a way to overcome that logistical/sampling problem, predicted seasonal fluctuations of microbial diversity in the global ocean, forecasting higher richness at high latitudes in winter (Ladau et al., 2013), which seems contradictory to snapshot field observations.

In a different approach, microbial observatories provided valuable information on microbial community dynamics, where different populations responded to environmental changes revealing recurrent patterns year after year (Fuhrman *et al.*, 2015; Giovannoni and Vergin, 2012; Gilbert *et al.*, 2012). However, the existing microbial observatories have a restricted distribution in mid-latitudes (~22º to 50º) northern hemisphere (Fuhrman *et al.*, 2015) and we lack of comprehensive comparative studies in ocean time-series using the same methods to access microbial diversity, and covering a wide range of latitudes.

Most of the world's ocean surface consists of warm tropical conditions. These regions are usually oligotrophic, where primary production is usually dominated by single-celled prokaryotes *Synechococcus*, *Prochlorococcus* and small eukaryotes (picoplankton) (Alvain *et al.*, 2008). Considering that warm oligotrophic tropical waters represent about 74% of the total area of the global ocean (Behrenfeld et al., 2006), we can not aim to fully understand the global dynamics of microbial communities without studying this part of the world's ocean in detail.

A critical point to decipher large-scale biodiversity patterns is which proxy of diversity one should look at. Most studies have focused on richness and this may provide a restricted view on this question. Other proxies of diversity, independent from richness, such as evenness (Soininen *et al.*, 2012) may provide new insights into this topic. When time-series are available, one can also evaluate stability, and determine which factors (environmental, biological or neutral) drive microbial communities through time (Giovannoni and Vergin, 2012), thus, understand the mechanisms of macro-ecological patterns.

The difficulty of studying stability is the myriad of definitions that can be found in the literature. Most definitions fit in two major categories, those that are based on the

ability of the system to resist or recover from changes (resistance and resilience) and those based on the system's dynamic stability (McCann, 2000). Here, we used the definition of dynamic stability, expressed as the variance of population relative abundance through time (McCann, 2000).

Hypotheses

Given that temperature is the most important factor shaping bacterial community composition (Sunagawa et al., 2015) and the lack of knowledge on the dynamics of marine bacterial communities in oligotrophic tropical regions, we wondered which factors would act in places where temperature remains nearly constant throughout the year, such as the equatorial Atlantic Ocean. We hypothesize that tropical microbial communities are more stable through time, and when the factor temperature is removed, biological factors arise as the structuring factor of microbial communities.

Objective

The aim of this study was to compare the stability and components of diversity (richness and evenness) of microbial communities in two coastal ocean observatories located in contrasting latitudes, and to determine which factors (environmental, biological or neutral) shape bacterial community structure in each site. In order to do that, we sequenced 16S and 18S amplicons from the rRNA gene to cover microbial communities dynamics over time, as well as a set of environmental parameters in two microbial observatories, one located in the Equatorial Atlantic, and one temperate located in the Northwestern Mediterranean Sea.

Material and methods

Sampling and study sites

Seawater samples were collected in two coastal ocean observatories, one tropical site located in the Equatorial Atlantic Ocean (-5.991306°, -35.087361°) 30 km from the city of Natal (Brazil) in the Equatorial Atlantic Microbial Observatory (EAMO), and a temperate site (41.666667°, 2.800000°) in the Mediterranean Sea in the Blanes Bay Microbial Observatory (BBMO), both points had similar total depth (approximately 20 m). Air temperature, precipitation and wind speed at each site can be seen in Figure 2.1., as well as the sampling dates. Six samples were taken during the years 2013 and 2014 in the same months excepting one sample in April 2014, which was not available in BBMO and was replaced by a sample on May 2013 in the temperate site, for comparison purposes (Fig. 2.1.). In order to capture contrasted seasonal conditions in the two sites, the delay between sample dates ranged between at least 20 days and up to 90 days. In the two sites, the same sampling protocols were used.

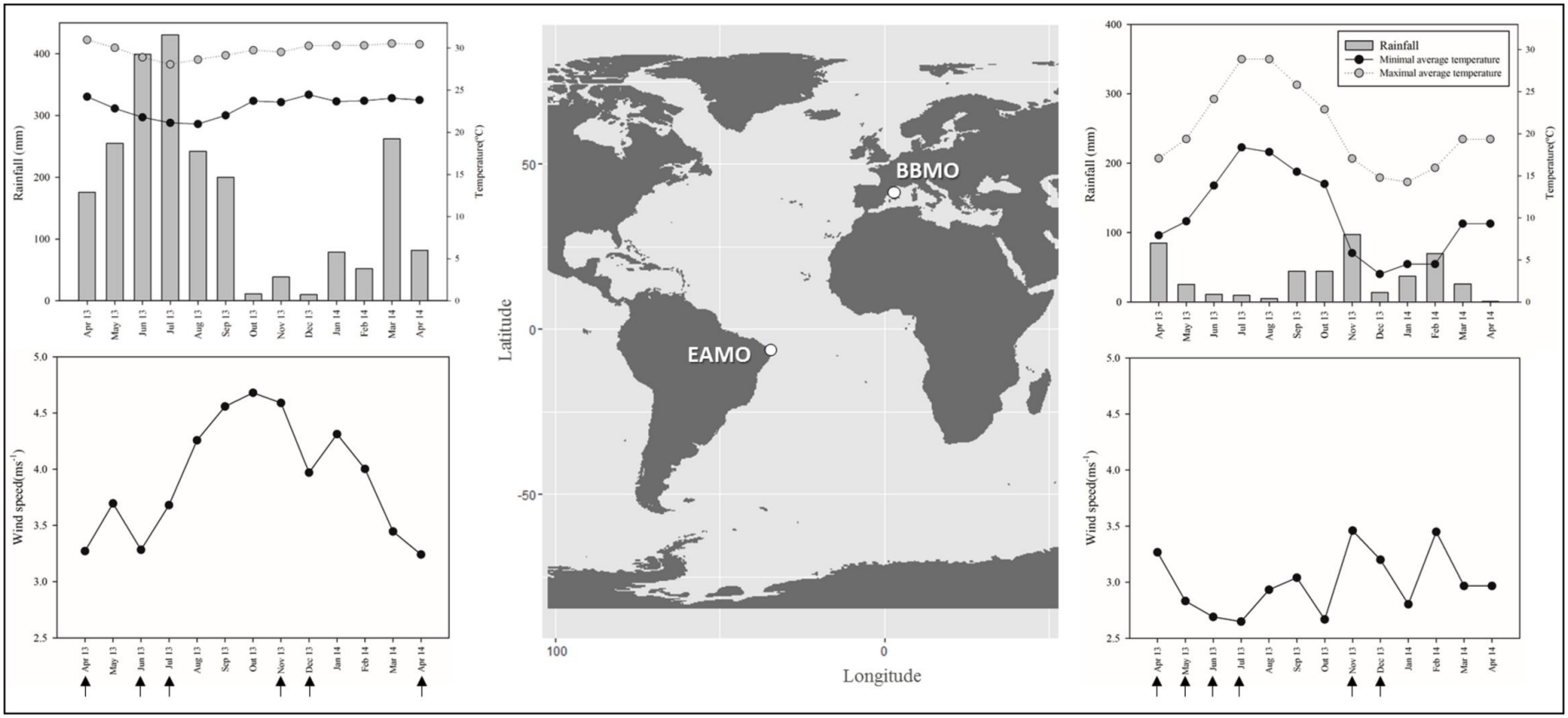

Figure 2. 1. Location of sampling stations (Equatorial Atlantic Microbial Observatory - EAMO and Mediterranean Sea in the Blanes Bay Microbial Observatory - BBMO), coordinates in the text. The plots on the sides illustrate the accumulated precipitation, air temperature (minimum and maximum) and wind speed during the period of this study in the tropical site (EAMO) on the right, and for the temperate site (BBMO) on the left. Arrows indicate the months sampled in each site.

Chemical Analyses

Water transparency was determined with a Secchi disc and water temperature, conductivity, dissolved oxygen were measured in situ with multi-parameter probes. To determine the Chlorophyll *a* concentration we filtered 2 to 4 liters of seawater, extracted pigments with acetone (90% v/v) in the dark and measured fluorescence with Turner fluorimeter.

The concentrations of dissolved organic nutrients were determined by spectrophotometry following standard procedures in 0.2 µm filtered seawater (Grasshoff *et al.*, 1999).

Bacterial abundance and biomass

The samples reserved for flow cytometry were preserved with 1% paraformaldehyde + 0.05% glutaraldehyde (final conc.). Bacterial abundance was analyzed by flow cytometry (BD FACSCalibur flow cytometer) with SybrGreen, according to Gasol and del Giorgio (2000). Picocyanobacteria were subtracted in independent counts in non-stained samples in a plot of SSC (90º side-scatter) versus FL3 (red fluorescence). Bacterial biomass was calculated using the volume-to-carbon relationship where $pgC\ cell^{-1} = 0.12\ pg\ (\mu m^3\ cell^{-1})^{0.7}$ (Norland, 1993).

Community composition

For microbial community composition analyses, 2 to 4 liters sub-surface seawater were passed through a polycarbonate filter with porosity of 3µm (called >3 µm fraction or "particle-attached organisms") and then by Sterivex "cartridge" (Millipore) of 0.22 µm porosity (called < 3 µm fraction or "free-living organisms"), using a peristaltic pump. The filters were embedded in a lysis buffer solution and maintained in the ultra-freezer (-80 °C) until extraction.

Extraction, PCR and bioinformatics

DNA extraction was carried out with a phenol-chloroform protocol, cutting the filters into small pieces (Logares, Sunagawa, *et al.*, 2014), and subsequent purification using Amicon column (Millipore® 100KDa/100.000MWCO).

PCR amplification was performed using the primers 341F (5'-CCTACGGGNGGCWGCAG-3') (Herlemann *et al.*, 2011) and 805R (5'-GACT ACHVGGGTATCTAATCC-3') (Apprill *et al.*, 2015) for the 16S rRNA gene V3-V4 region. For eukaryotes the primers used were TAReukFWD1 (5'-CCAGC ASCYGCGGTAATTCC-3') and TAReukREV3 (TTTCGTTCTTGATYRA 5'-CA-3') of the 18S rRNA gene V4 region (Stoeck *et al.*, 2010).

Samples were sequenced in an Illumina MiSeq platform and processed in a UPARSE (Edgar, 2013) based pipeline implemented internally (available in: https://github.com/ramalok/amplicon_processing). In summary, we obtained the pairing of sequences using PEAR (Zhang *et al.*, 2014). All sequences shorter than 100 nucleotides were discarded. Quality dereplication checking, OTU clustering (UPARSE algorithm, similarity ≥ 99%) and filtering of chimeras (using SILVA v.119 (Quast *et al.*, 2013) as a reference database), were made via USEARCH (Edgar, 2010). The taxonomic classification was made by comparison with the database through the BLASTn 119.1 SILVA (Zhang *et al.*, 2000) (at least 75% similarity). The final result of this pipeline is a clean OTU table that was rarefied, obtaining a relative abundance OTU table.

Statistical treatment

We used the software R version 3.2.3 (R Development CoreTeam, 2011) with the packages vegan (Oksanen *et al.*, 2015), MASS (Venables and Ripley, 2002), BiodiversityR (Kindt and Coe, 2005) for data processing and statistical treatment.

We calculated the coefficient of variation (defined as the ratio of the standard deviation to the mean) for OTUs that occurred in all samples, as a proxy for population stability in the tropical and temperate ocean coastal sites. Then, we used the Mantel test to explore environmental, temporal (Euclidean distance) and biological (eukaryotic community) standardized variables as explanatory factors of community dissimilarity matrices (Bray-Curtis distance) and in both regions.

Results

As expected, seawater temperatures were significantly lower in the temperate coastal site, and more constant (warmer) values in the tropical site (Tab. 1). Nutrient concentrations were also different between sites, excepting silica and nitrate concentrations. Phosphate and nitrite concentrations were higher in the temperate site, while ammonium was significantly higher in the tropical costal site. Water transparency was higher in the temperate Mediterranean coastal site, up to 20 m, while the maximum Secchi disk depth in the tropical coastal site was only 7 m (Tab. 1).

Average chlorophyll *a* did not differed among sites, but the smaller fraction (chlorophyll *a* < 3μm) was significantly higher in the tropical coastal site. Concerning picophytoplankton abundance, *Prochlorococcus* and picoeukaryotes given, by flow cytometry, were not significantly different between sites. In contrast, *Synechococcus* was more abundant (in average) in the tropical site. The average percentage of high nucleic acid (HNA) prokaryotes was significantly higher in the temperate costal site (Tab. 1) but no differences could be inferred in bacterial abundance nor biomass.

Table 1. Minimum, mean and maximum values of environmental and biological parameters collected in coastal waters of the Mediterranean Sea (temperate) and tropical Atlantic Ocean (asterisks indicate statistical significant differences (*p < 0.05, **p < 0.01).

	Temperate			Tropical		
	Min.	**Avg.**	Max.	Min.	**Avg.**	Max.
Temperature (°C)	14.4	**16.7**	21.6	27.3	**28.5**	29.6**
NH_4^+ (µM)	0.32	**0.81**	1.96	1.20	**1.79**	2.19*
NO_2^- (µM)	0.11	**0.19**	0.38	0.02	**0.03**	0.05*
NO_3^- (µM)	0.13	**0.72**	1.53	0.31	**1.43**	5.87
PO_4^{3-} (µM)	0.07	**0.10**	0.17	0.04	**0.05**	0.08*
SiO_4^{2-} (µM)	0.46	**1.20**	1.88	0.88	**1.70**	3.04
Secchi disk (m)	8.5	**15.1**	20.0	4.0	**5.3**	7.0*
Chlorophyll *a* (mg/m³)	0.31	**0.51**	0.75	0.17	**0.31**	0.79
Chlorophyll *a* < 3µm (%)	21.3	**41.6**	59.2	49.2	**59.7**	67.9*
Synechococcus abundance (10^4 cell/ml)	1.1	**3.1**	7.9	4.0	**9.0**	11.0*
Prochlorococcus abundance (10^2 cell/ml)	0.4	**36.9**	118.5	18.6	**34.7**	65.7
Picoeukaryots abundance (10^3 cell/ml)	0.7	**2.5**	5.1	0.4	**1.4**	2.6
Bacterial abundance (10^5 cell/ml)	7.5	**9.8**	144.0	4.5	**6.1**	7.9
% of HNA	47.0	**52.8**	63.7	24.0	**37.1**	54.9*
Bacterial biomass (µgC l^{-1})	15.5	**20.3**	30.2	9.5	**13.3**	18.5

Concerning the sequencing results, a total of 4.665.345 reads were obtained for the 16S amplicon and 6.631.839 reads for the 18S amplicon, at least 13.281 (up to 727.129) reads per sample for the 16S and at least 10.748 (up to 840.077) reads per sample for the 18S amplicon. The prokaryotic community rank-abundance curves point towards a greater dominance of fewer OTUs in the temperate site for both size fractions

(Fig. 2.2.). A similar and even more pronounced pattern was observed for eukaryotic communities throughout the year (Fig. 2.3.).

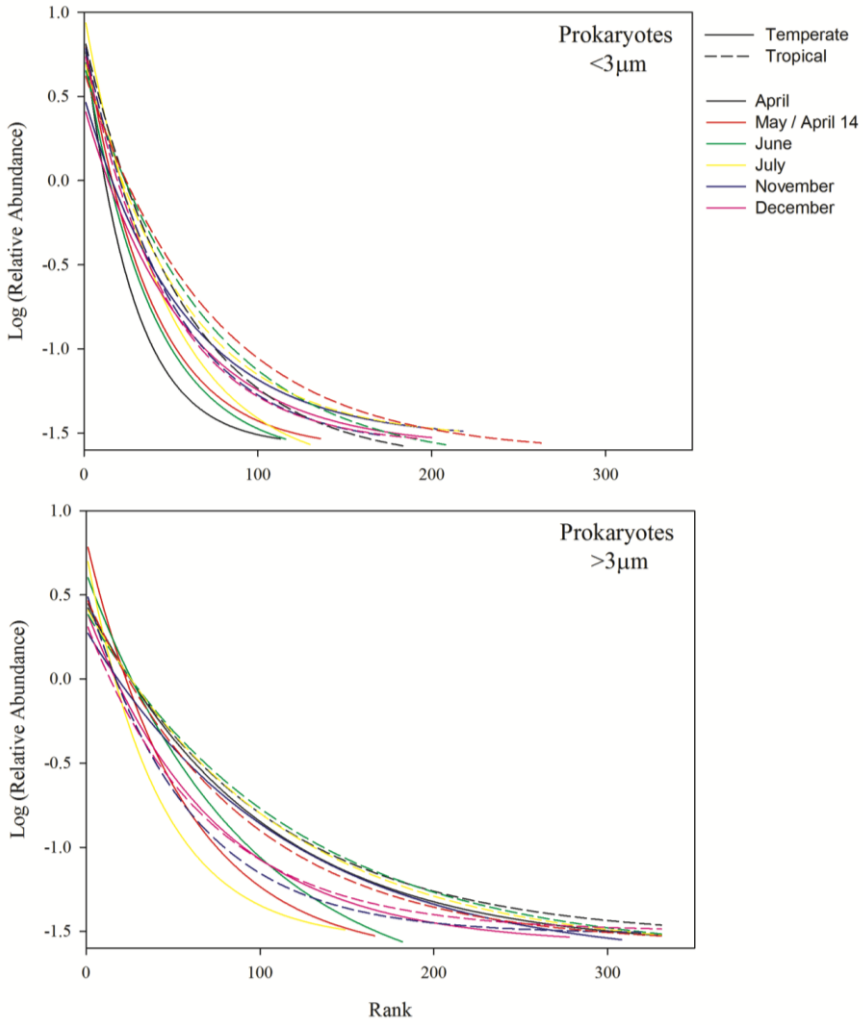

Figure 2. 2. Rank abundance of the free-living (< 3 µm) and particle-attached (> 3 µm) prokaryotes. Dashed lines represent the tropical site; solid lines represent the temperate site.

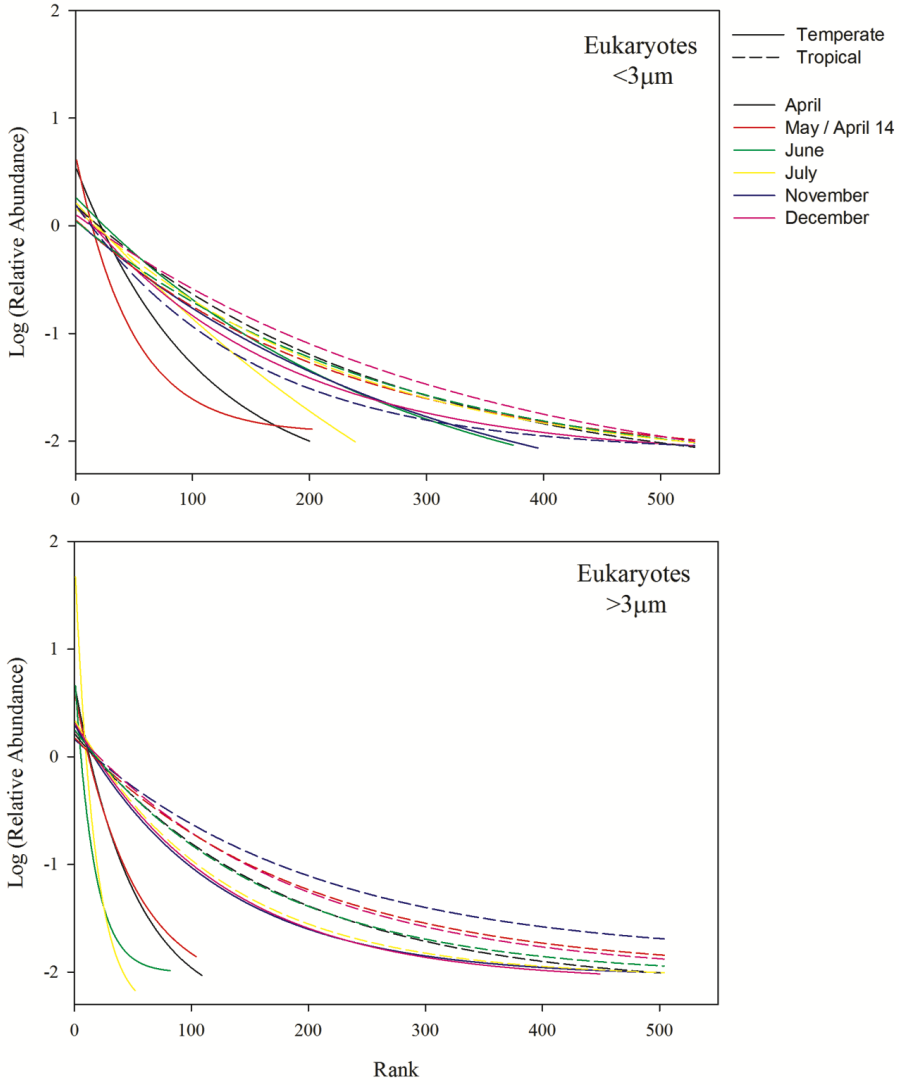

Figure 2. 3. Rank abundance of the small (< 3 µm) and large (> 3 µm) size fraction eukaryotes. Dashed lines represent the tropical site; solid lines represent the temperate site.

Prokaryotes of the larger size fractions (> 3 µm) had higher richness than smaller fractions (< 3 µm), differently from eukaryotes, where no significant differences could be pointed out in both sites (Fig. 2.4.). No statistical significant differences among sites were observed for OTU richness in the smaller size fraction of prokaryotes (< 3 µm). On the other hand, the tropical site had higher richness in the larger size fractions (> 3

μm). Significant differences could also be observed for eukaryote richness between temperate and tropical sites for both size fractions.

The patterns observed for evenness were strikingly different. There were significant differences between the two sites for prokaryotes < 3 μm and for eukaryotes > 3 μm. Furthermore, analyzing the differences within the same site, evenness was higher in eukaryotes < 3 μm in the tropical site (Fig. 2.4.). However, pairwise comparisons of the Shannon diversity index, evenness and richness indicate higher values for the tropical site (Fig. 2.5.).

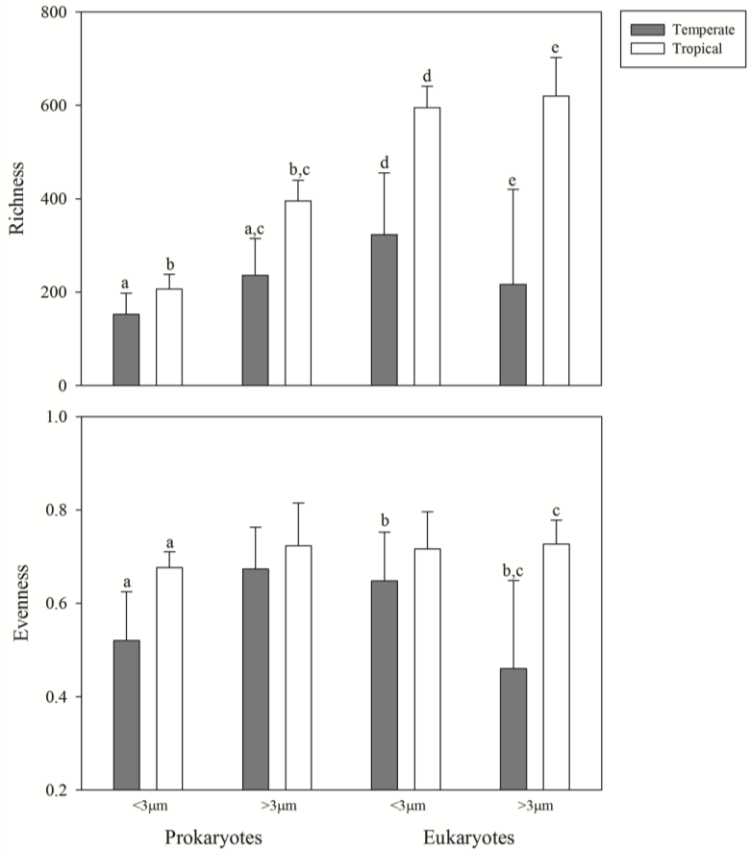

Figure 2. 4. Average and standard deviation of richness and evenness in each site. The solid bar represents the temperate costal ocean and the white bar represents the tropical costal ocean. Each set of letters indicates the significant differences between the sites and within sites.

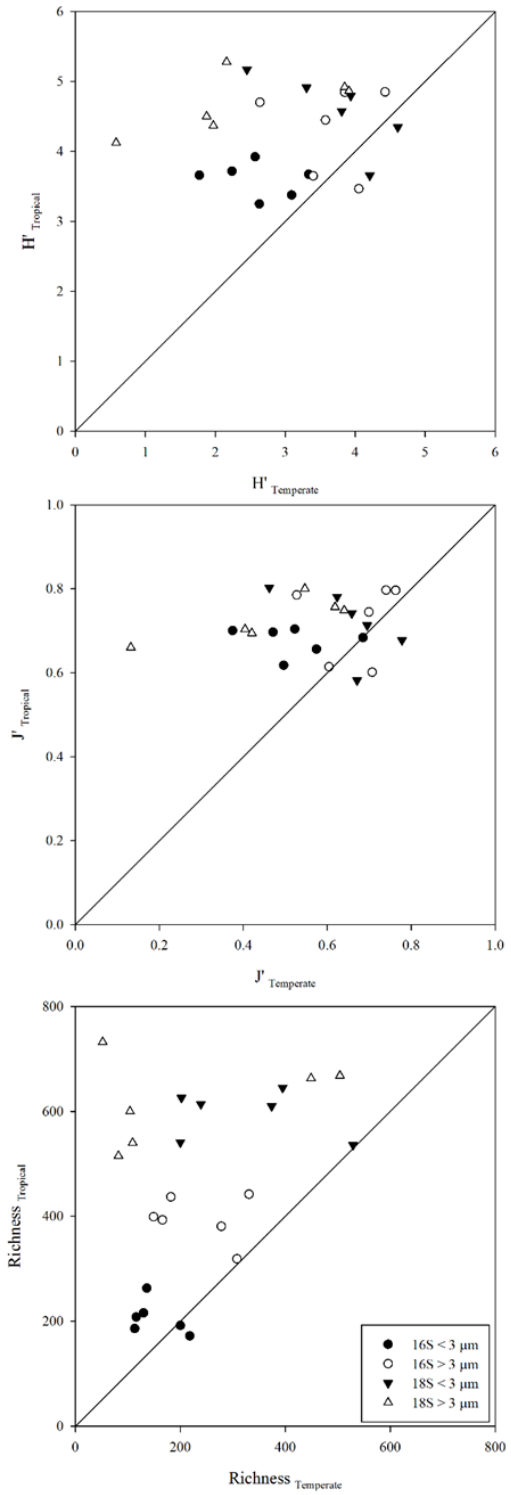

Figure 2. 5. Pairwise comparisons of diversity, evenness and richness observed in the tropical (Y-axis) and temperate (X-axis) sites. If the values would be equal the points should lay close to the 1:1 line. Higher values in the temperate site should figure in the

lower-right side of the graphs, while above-left side of the line indicate higher values in the tropical site.

In order to evaluate microbial population stability, we calculated the coefficient of variation of each OTU relative abundance in all samples. We found significant differences ($p < 0.001$) between the average coefficients of variation between sites with constantly higher values in the temperate coastal ocean (Fig. 2.5.).

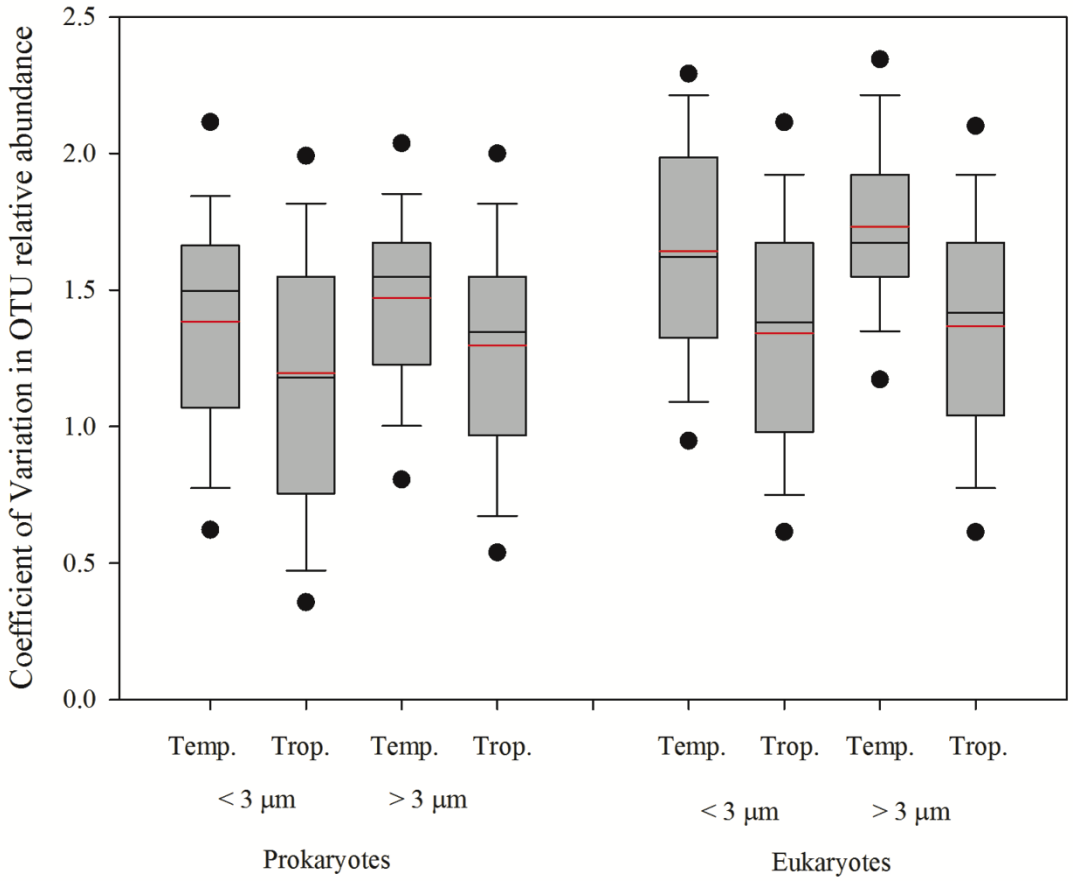

Figure 2. 6. Coefficient of variation of OTU relative abundance by site and size fraction. Temp.: temperate coastal ocean, Trop.: tropical coastal ocean. The red line represents the mean values, the central black line indicates the median value, the boxes indicate the lower and upper quartiles, the vertical lines indicate the 10th and 90th percentiles, and the dots represent the 5th and 95th percentiles. All pairwise comparisons among sites revealed statistical significant differences (see text for details).

Plotting the average coefficient of variation against average richness for both prokaryotes and eukaryotes, we observe that all points tend to go towards the lower right side of the graph, indicating more stable and richer communities in the tropical coastal ocean (Fig 2.7. upper panel). That trend was even stronger in the plot of the coefficient of variation against evenness (Fig 2.7. lower panel).

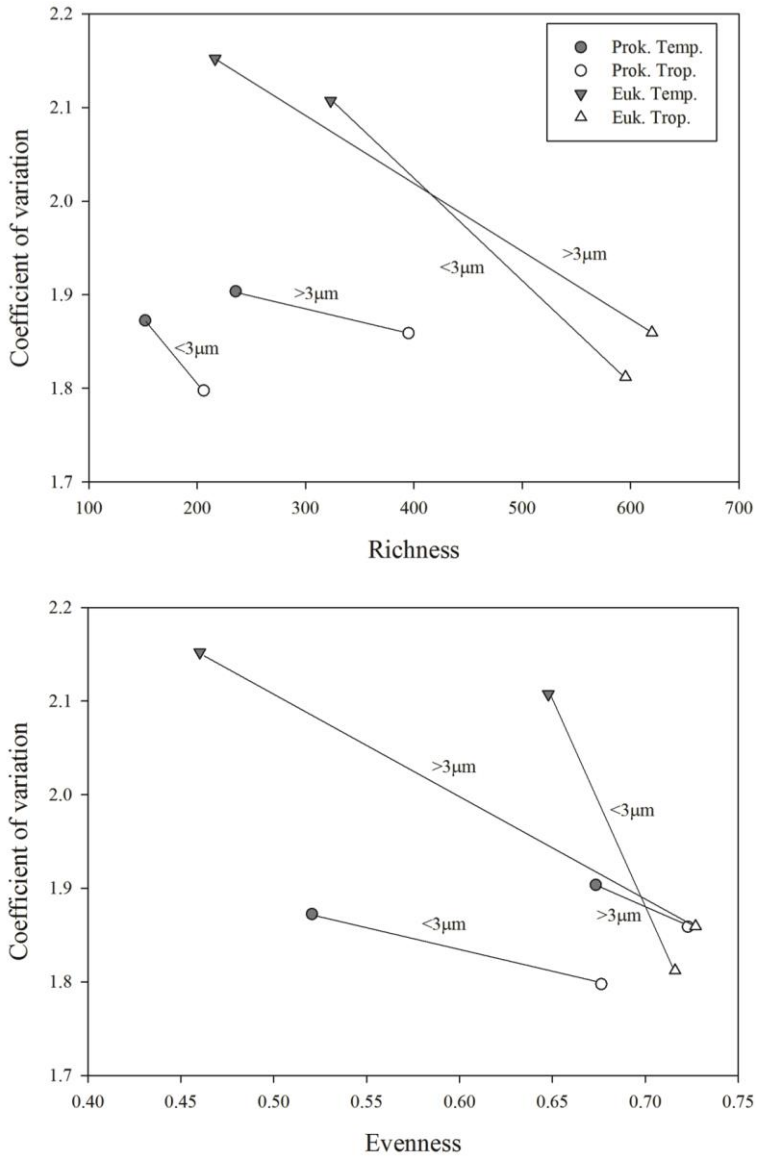

Figure 2. 7. Relationship between stability (coefficient of variation) and richness observed separated by size-fraction and site. Filled marks represent the temperate coastal ocean; empty marks represent the tropical coastal ocean. Solid lines connecting points were drawn to facilitate comparisons.

Then we tried to determine if the prokaryotic communities were responding to the same variables in contrasting latitudes, using Mantel test correlation between distance matrices. We divided the variables measured (Tab. 1) in environmental (temperature, nutrients, water transparency), potential biotic interactions (eukaryotic communities and chlorophyll *a*) or neutral factors (time). The dissimilarity matrix (Bray-Curtis distance) of the temperate free-living (< 3 μm) prokaryotic community was correlated with environmental factors such as temperature and silica. In contrast, in the tropical site, the prokaryotic community distance matrix was correlated with biological variables such as eukaryotic community (Bray-Curtis dissimilarity matrix) > 3 μm and < 3 μm and chlorophyll *a* (Tab. 2).

The attached prokaryotes (> 3 μm) in the temperate site were correlated with the larger eukaryotes (> 3 μm), while in the tropical site, the best explanatory variables were phosphate, time between samples (Euclidean distance), and percentage of chlorophyll *a* < 3 μm (Tab. 2).

Table 2. Mantel test significant relationships between dissimilarity (Bray Curtis distance) matrices of the free-living (< 3 μm) and particle-attached (> 3 μm) prokaryotes and environmental, biological and temporal (Euclidean) distance matrices.

Site	Fraction	Variable	Pearson r	p
Temperate	< 3 μm	Temperature	0.85	0.011
		SiO_4^{2-}	0.72	0.018
	> 3 μm	Eukaryotes > 3 μm	0.53	0.035
Tropical	< 3 μm	Eukaryotes > 3 μm	0.73	0.008
		Chlorophyll *a*	0.71	0.010
		Eukaryotes < 3 μm	0.70	0.011
		% Chlorophyll *a* < 3 μm	0.68	0.004
	> 3 μm	PO_4^{3-}	0.69	0.049
		Time	0.67	0.015
		% Chlorophyll *a* < 3 μm	0.52	0.040

Discussion

We found evidence of increasing stability with increasing richness and evenness in tropical marine microbial communities when compared to the temperate ones (Fig. 2.7). With the exception of the free-living bacteria (< 3 μm fraction), all fractions had higher richness in the tropical site. These observations on samples taken at different times in the same location contradicts those from snapshot-sampling approaches covering a wide latitude range (Sunagawa *et al.*, 2015; Milici *et al.*, 2016). Given that environmental conditions and microbial communities in the ocean are extremely dynamic (Fuhrman *et al.*, 2015), snapshot-sampling strategies may miss a lot of that variability, hampering the detection of consistent large-scale ecological patterns. Our observations support the view that microbial communities actually follow a similar macro-ecological pattern as animals and plants, exhibiting higher (or at least similar) richness towards the equator, at least in the coastal ocean. It is possible that these observations do not hold for the open ocean, as coastal systems have different dynamics, but our results highlight the importance of Eulerian time-series to fully understand microbial diversity patterns in the ocean.

Nutrient concentration and Chlorophyll *a* indicated that both sites had comparable trophic state, rather oligotrophic. Still, the tropical site had a higher proportion of the smaller fraction of phytoplankton biomass (Chlorophyll *a* < 3 μm) and higher *Synechococcus* abundance (Tab. 1). This is probably due to higher amplitude of seasonal fluctuations in the temperate site, resulting in the typical phytoplankton succession in the Northwestern Mediterranean coast, with a diatom winter bloom (Margalef, 1978), which is not likely to occur in the constantly warm waters of the tropical site (Alvain *et al.*, 2008).

The higher richness of particle-attached prokaryotes already observed in the Mediterranean Sea (Ghiglione *et al.*, 2009; Acinas *et al.*, 1999) was also confirmed for tropical waters. Contrastingly, this pattern could not be observed for eukaryotes. The richness of the two size fractions were different between sites, but not between size fractions in the tropical site. Again, this could reflect the lack of true phytoplankton succession cycle in constantly warm tropical waters, usually dominated by picoplankton (Alvain *et al.*, 2008; Boyce *et al.*, 2015).

The rank-abundance plots and evenness values evidenced a higher dominance of few OTUs in the Mediterranean Sea contrasting with a more even distribution in the tropical ocean. This pattern was particularly clear for eukaryotes indicating "bloom" events. Curiously, rank-abundance curves of winter months in the Mediterranean Sea were those that resembled the most to the tropical ones, with a more even distribution for both prokaryotes and eukaryotes, corroborating the niche model predictions of higher microbial richness in winter in temperate regions (Ladau *et al.*, 2013) and also previous observations in the Western English Channel Observatory (Gilbert *et al.*, 2012). It worth noticing that taking the free-living prokaryotes alone, OTU richness was not significantly different between sites, but this fraction was less stable in the temperate site, as reflected by the lower evenness. These observations are also compatible with the niche model predictions (Ladau *et al.*, 2013) where the higher winter richness in mid and higher latitudes compensate the lower richness during summer, compared to the always moderate richness predicted for tropical regions.

Temperature was the main factor driving the free-living prokaryotic communities (< 3 µm) in the temperate site, which is a deterministic factor (Fuhrman *et al.*, 2006), followed by varying silicate concentration, which might be related to the phytoplankton succession in this region (Margalef, 1978). Interestingly, the particle-attached

prokaryote community (> 3 μm) was better explained by the concomitant "living particles" (eukaryotes > 3 μm), indicating strong associations between the prokaryotes and the particles they are sitting on (Lima-Mendez *et al.*, 2015).

Unlike the temperate site, the structure of coastal tropical ocean prokaryotic community was clearly driven by more stochastic factors such as biotic interactions (Tab. 2). We may infer that, once the deterministic factors such as day length and temperature removed, stochastic features such as the interactions between organisms emerge as drivers of free-living prokaryotic community dynamics. In one hand, being more evenly distributed and stable throughout the year, the amplitude of fluctuations in tropical plankton populations is low, with no true succession or phenology. On the other hand, biological processes occur at higher rates because they are not constrained by temperature (e.g. Sarmento *et al.*, 2010). Furthermore, high light and low nutrient conditions increase the release of photosynthates by primary producers (Zlotnik and Dubinsky, 1989; Morana *et al.*, 2014), which might increase mutualistic interactions between phytoplankton and bacterioplankton (Sarmento and Gasol, 2012) in warm oligotrophic conditions. Altogether, these factors concur to turn biological processes more coupled, with short lag times between population peaks (which are also smoother), and allow capturing co-varying populations of eukaryotes and prokaryotes in the same sample (Figure 2.8.). This probably can not be captured in higher latitudes, where higher amplitude events and varying time lags between peaks (depending on the season/temperature) can not be captured in an Eulerian sampling strategy at fixed times.

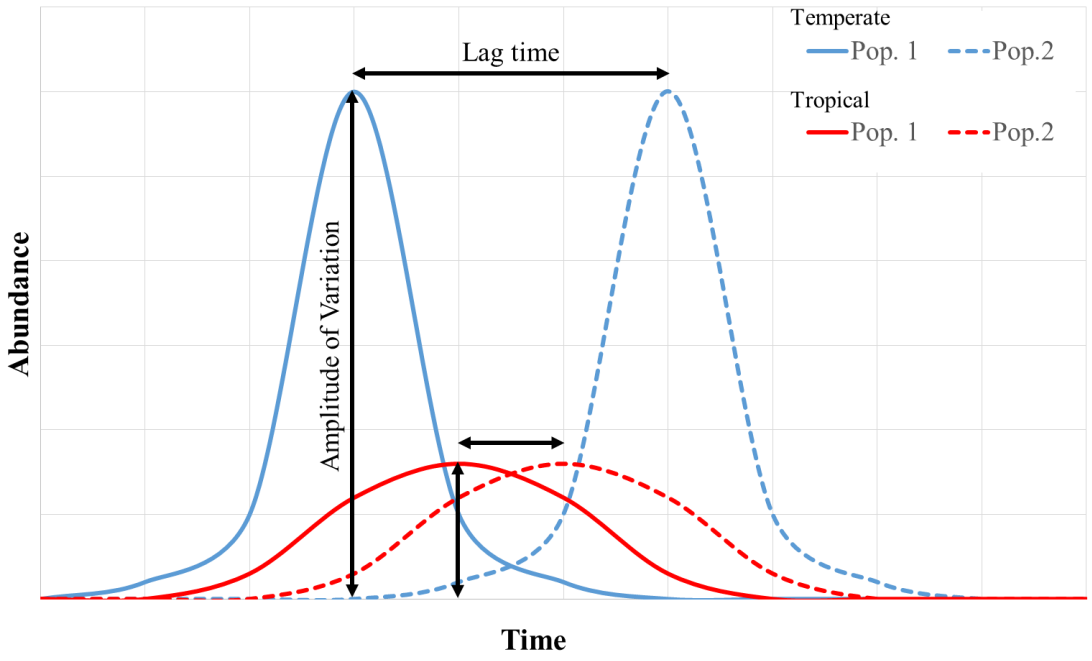

Figure 2. 8. Theoretical interpretation of the succession time lag between two random populations (horizontal arrows) and the amplitude of abundance variation (vertical arrows) in contrasting latitudes. The blue line represents a temperate site and the red line represents a tropical site.

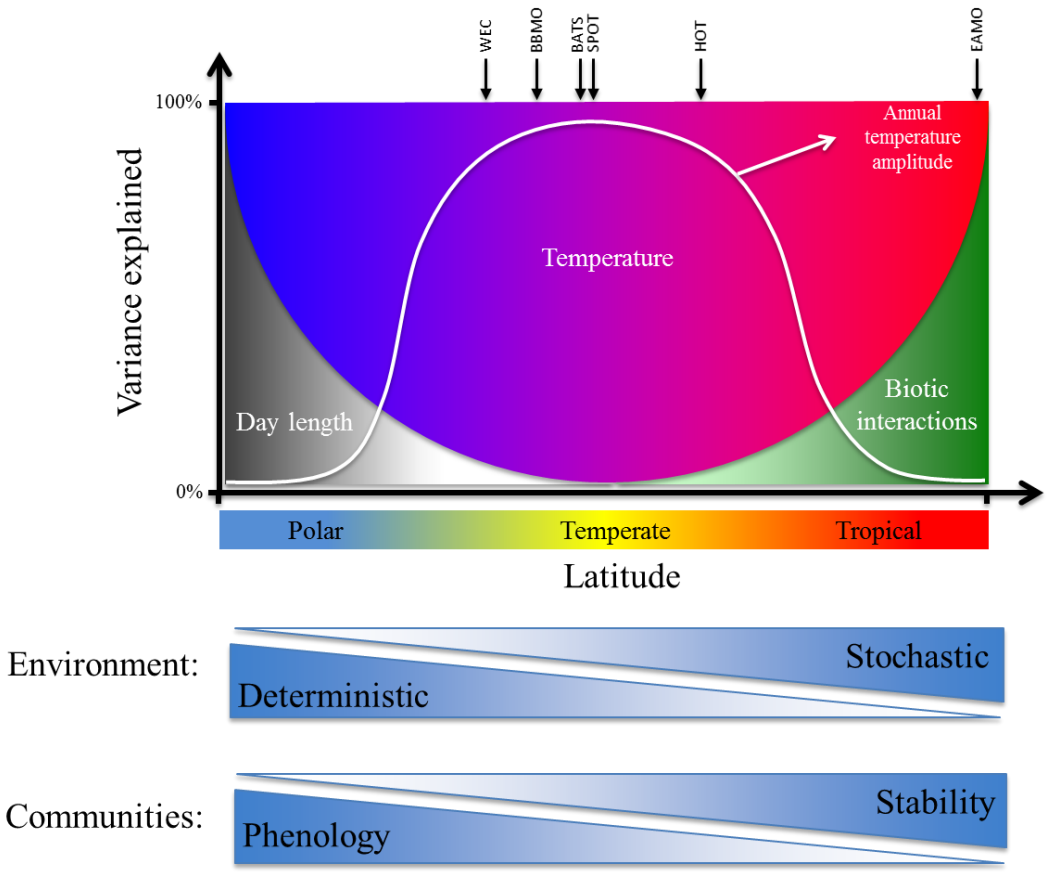

Figure 2. 9. Conceptual view of the different variables explaining variance in microbial communities across latitudes. At low latitudes (tropical regions) higher stability and more stochasticity (such as biotic interactions) are expected to drive the community structure. At high latitudes, less stability (marked succession) and more deterministic factors would affect microbial community composition. At intermediary latitudes (temperate regions) high annual temperature amplitude would be the main factor driving microbial communities. The arrows at the top indicate the location of the marine microbial observatories: WEC – Western English Channel (50ºN), BBMO - Blanes Bay Microbial Observatory (42ºN), BATS – Bermuda Atlantic Time Series (33ºN), SPOT – San Pedro Ocean Time Series (33ºN), HOT – Hawaii Ocean Time Series (22ºN), EAMO - Equatorial Atlantic Microbial Observatory (6ºS).

We propose a conceptual framework where microbial community composition would be driven by different proportions deterministic and stochastic factors along a latitudinal gradient: in higher latitudes most of the variation would be explained by deterministic factors (day length, for example, Gilbert *et al.*, 2012) dictating a true phenology throughout seasons. In intermediate latitudes (BBMO) where the annual temperature amplitude in maximal, microbial communities would mainly dictated by temperature (Figure 2.9.). Finally, in low latitudes (EAMO), where the factor temperature is removed, more stochastic biotic interactions would be the main factors shaping microbial communities.

This study demonstrates the importance of comparative studies on Eulerian time-series distributed at different latitudes to fully understand the macro-ecological patterns of diversity of microbial communities in the ocean.

Acknowledgements

This research was supported by FAPESP (process 2014/13139-3), CNPq (process 474759/2013-0), and the Brazilian Science Without Borders Program (BJT 013/2012; PVE 400313-2014-6). The authors are thankful to Marli Fiore and Armando Vieira for laboratory assistance, and Stefan Bertilsson for enriching discussions that helped improving this manuscript. We also thank all the Blanes Bay Microbial Observatory staff, especially Clara Cardelús, Vanessa Balagué, Irene Forn, Isabel Ferrera, Ramiro Logares and Josep M. Gasol.

Conclusões

1. Encontramos um aumento significativo da estabilidade nas comunidades marinhas microbianas tropicais costeiras, juntamente com um aumento na riqueza e equabilidade, quando comparado às comunidades marinhas microbianas temperadas costeiras.
2. Comunidades microbianas tropicais costeiras parecem seguir os mesmos padrões de riqueza e diversidade macroecológicos mundiais, demonstrando maior riqueza de espécies em baixas latitudes em comparação com regiões de maior latitude.
3. Amostras coletadas no inverno no mar mediterrâneo possuem riquezas semelhantes as amostras de verão dos trópicos, corroborando a ideia de que a riqueza é maior em regiões temperadas no inverno.
4. A temperatura (fator determinístico) foi preponderante na definição da estrutura das comunidades microbianas temperadas costeiras, enquanto fatores mais estocásticos como as interações biológicas parecem determinar a estrutura das comunidades microbianas tropicais costeiras
5. Finalmente, este trabalho demonstra a importância de estudos comparativos sobre as séries cronológicas (Eulerianas) distribuídas em diferentes latitudes para compreender plenamente os padrões macroecológicos da diversidade de comunidades microbianas no oceano.

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Supplementary Material

PCR conditions

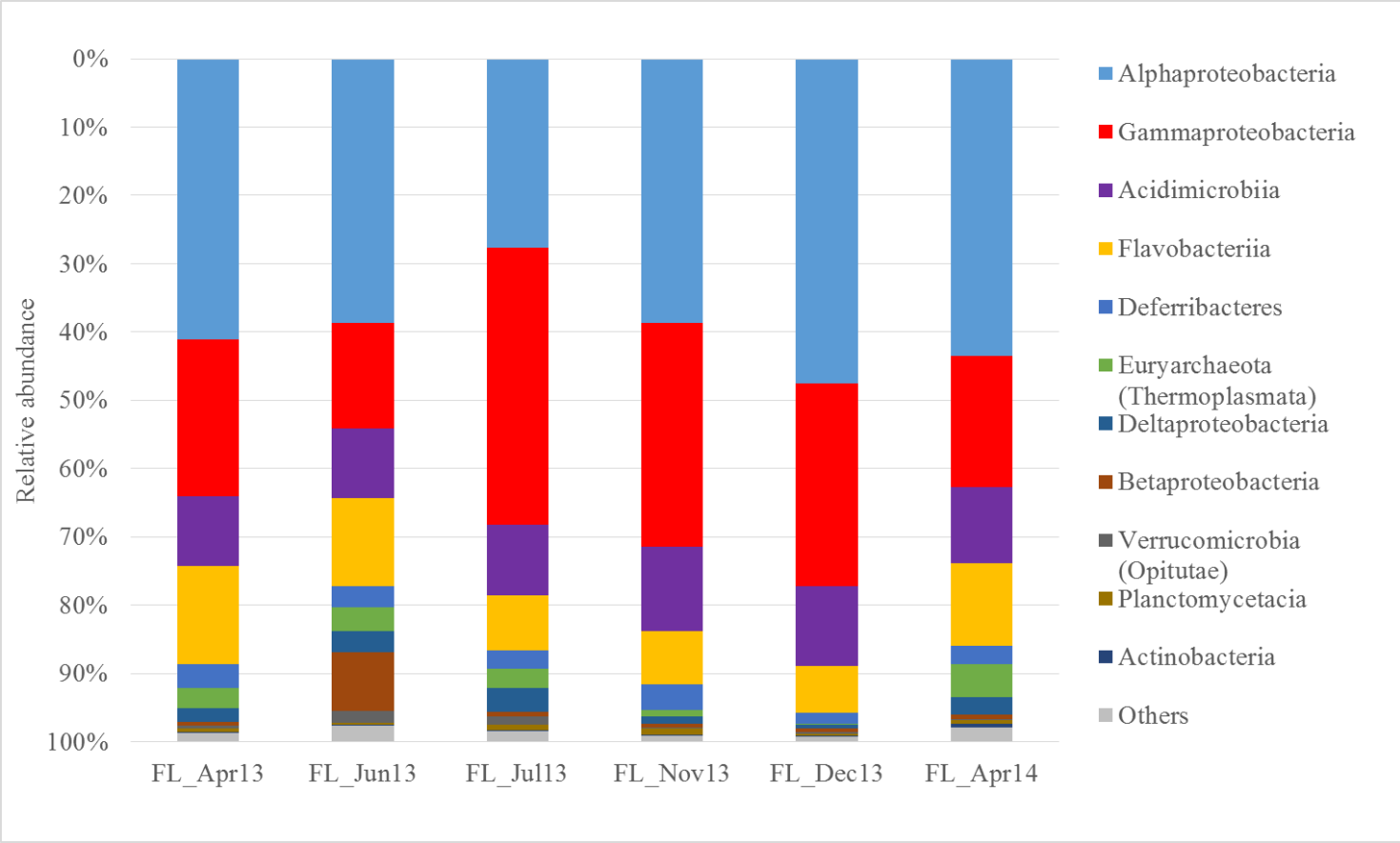
For each sample, triplicate PCR reactions were performed in 25 μl and consisted of 0.4 U of Phusion high-fidelity DNA polymerase (Finnzymes, Vantaa, Finland), 1X Phusion HF reaction buffer (Finnzymes), 200 μM of each dNTP (Invitrogen, imported by Sinapse, São Paulo, SP, Brazil), 0.5 μM of each primer (DNA Express, São Paulo, SP, Brazil), 0.4 mg ml^{-1} of BSA (Invitrogen, imported by Sinapse, São Paulo, SP, Brazil) and 5 – 10 ng of template DNA.

PCR incubations for 16S rRNA were comprised an initial denaturation step at 95 °C for 5 min, followed by 25 cycles of denaturation at 95 °C for 40 sec, annealing at 53 °C for 40 °C and extension of 72 °C for 1 min and finalized with a 10 min extension step at 72 °C. And PCR incubations for 18S rRNA were comprised 95°C during 2 min, followed by 10 cycles of 30 sec at 95°C, 60 sec at 50°C and 40s at 72°C, and afterwards by 20 cycles of 30 sec at 95°C, 30 sec at 45 °C and 40 sec at 72°C, including a final elongation step at 72°C for 10 minutes.

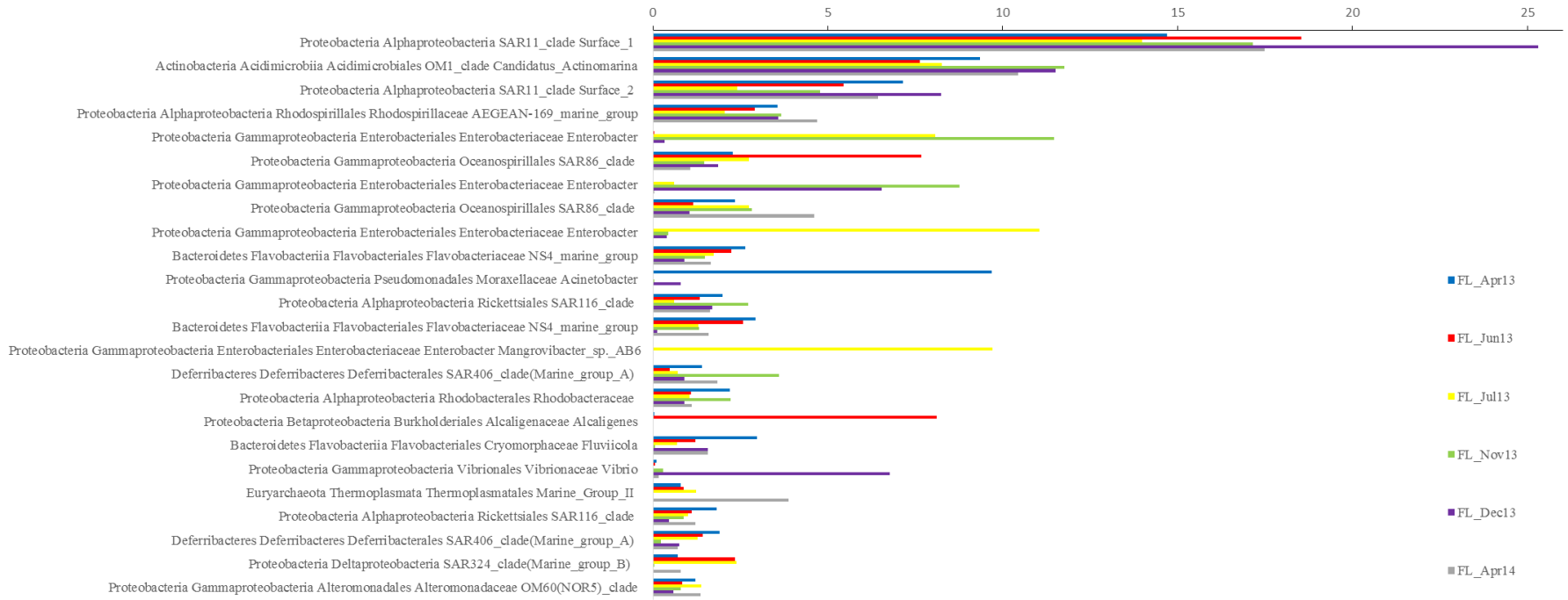
After pooling amplicons from the triplicate reactions, PCR-products were cleaned using the AMPure XP purification kit (Beckman Coulter Inc., Brea, CA, USA) following the instructions from the manufacturer.

Microbial diversity at the Equatorial Atlantic Microbial Observatory (EAMO)

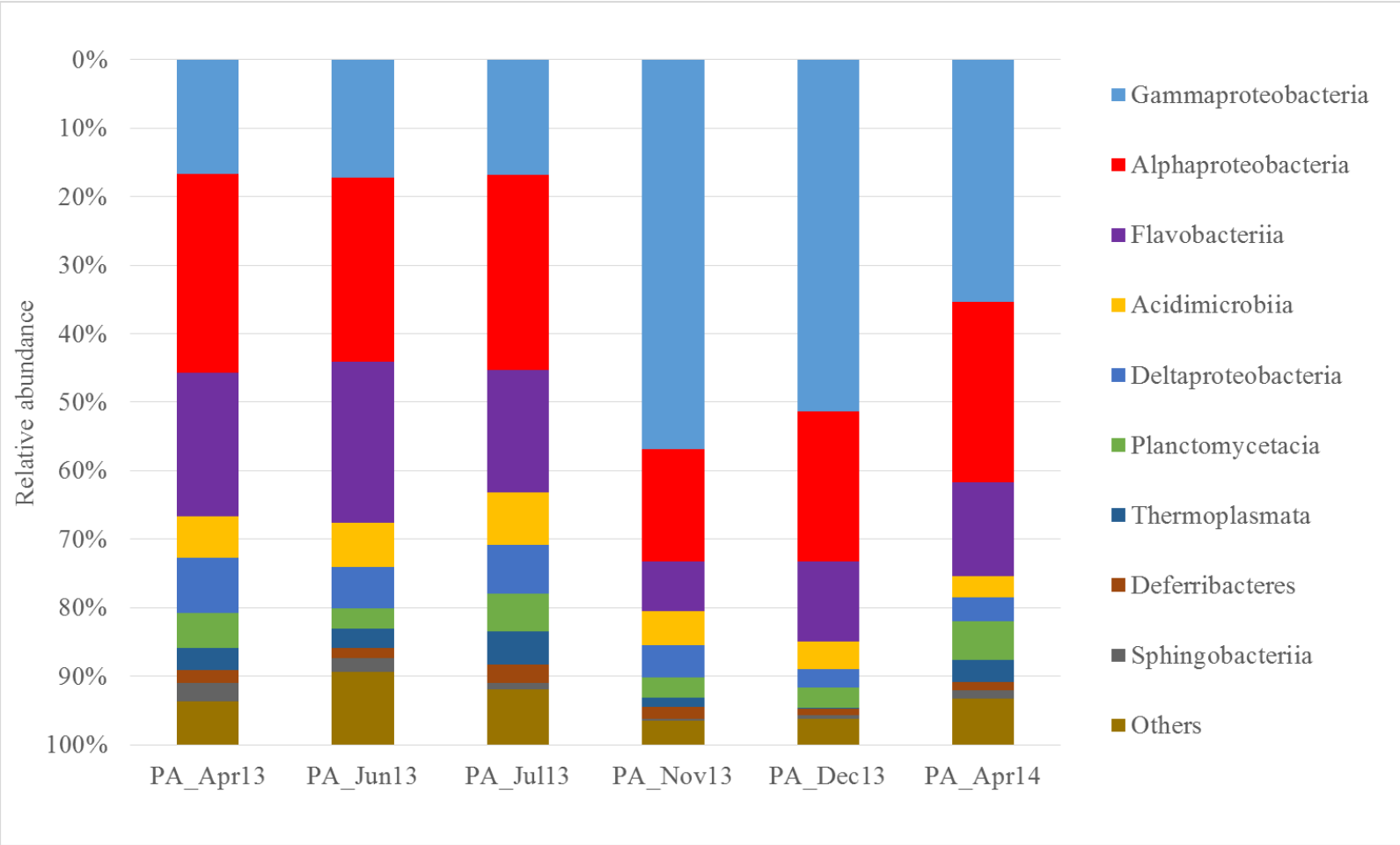
The following figures describe the prokaryotic and eukaryotic community composition from the two sizes fractions (> 3 μm and <3 μm) at the sampling dates at the EAMO, which were relatively less studied in tropical regions.



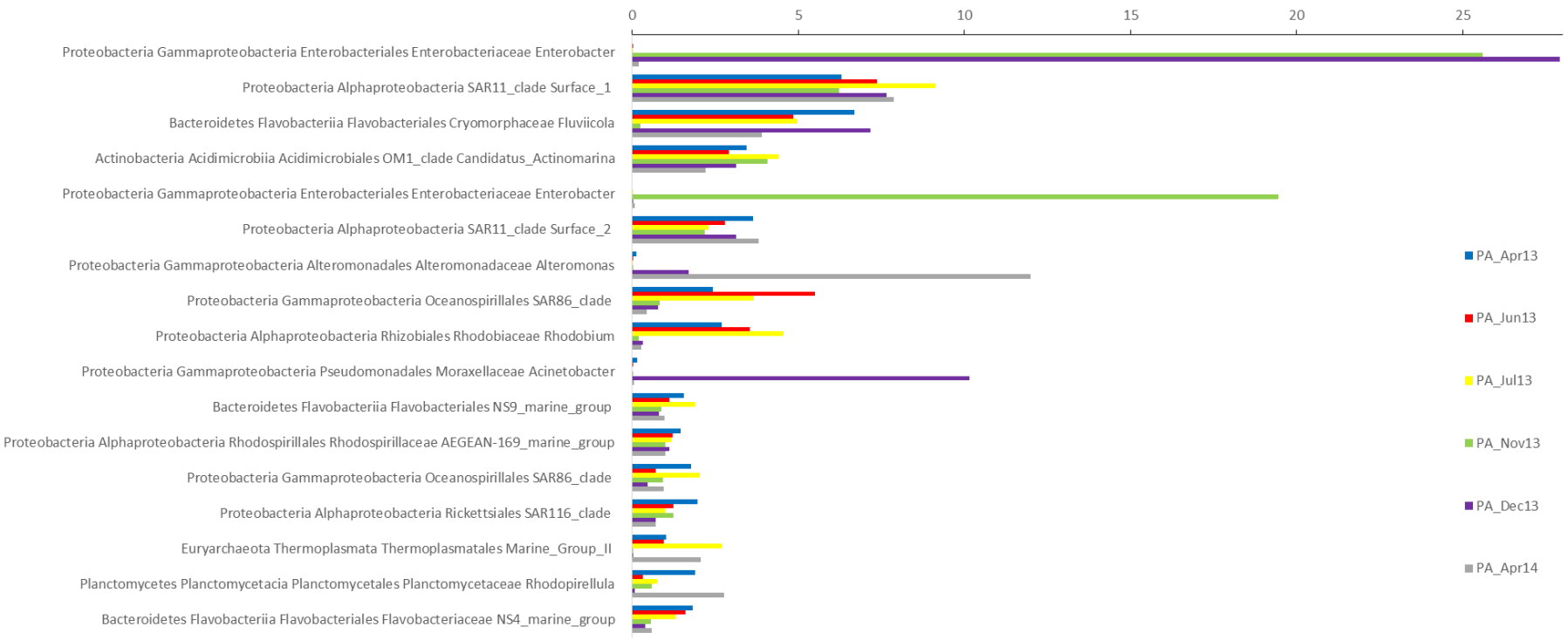
Supplementary Material 1. Free-living (FL) prokaryotic groups that contribute with at least 1% of the community at the different sampling dates at EAMO.



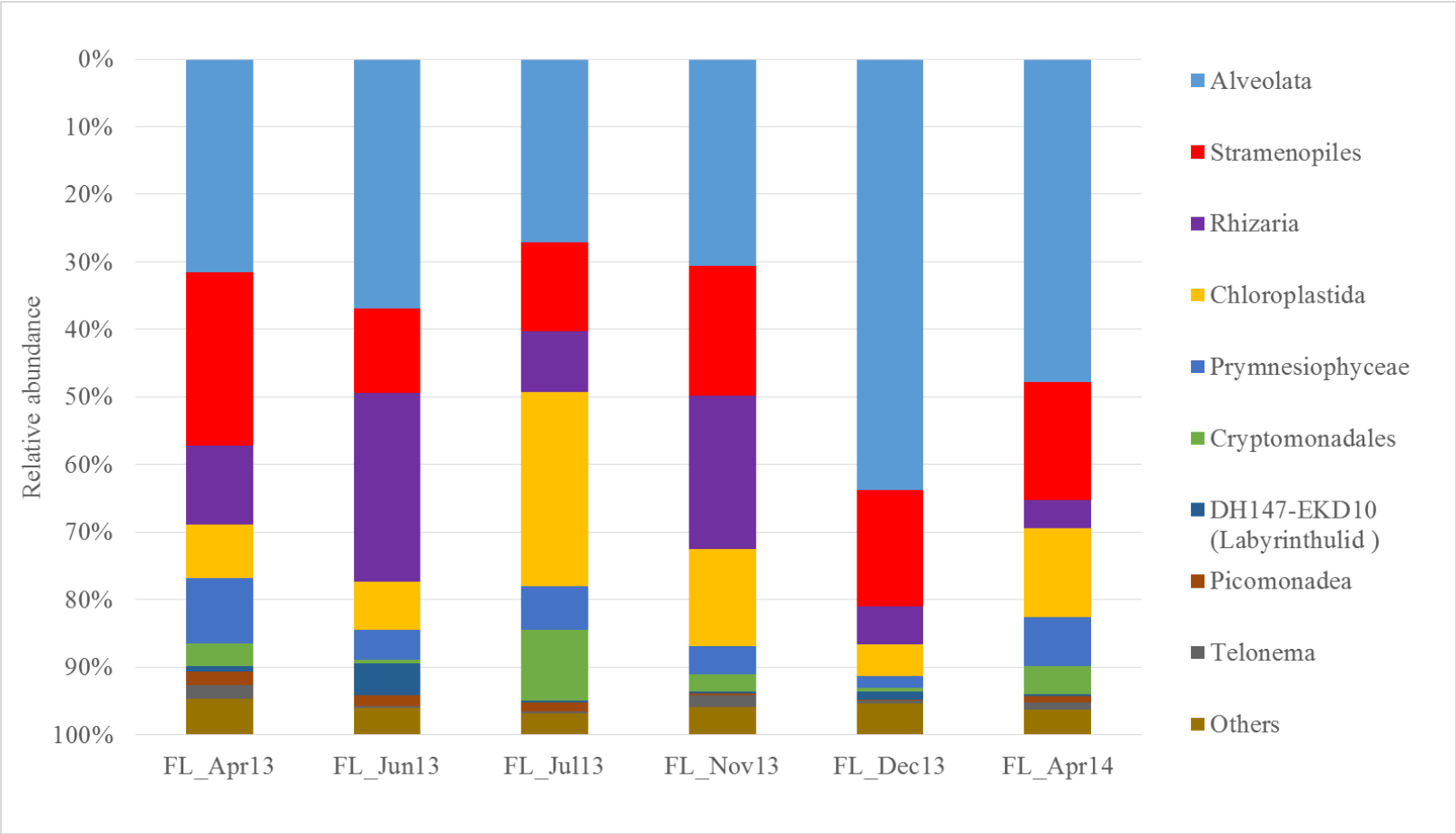
Supplementary Material 2. Free-living (FL) prokaryotic OTUs that contribute with at least 1% (sum of all dates) of the community at the different sampling dates at EAMO.



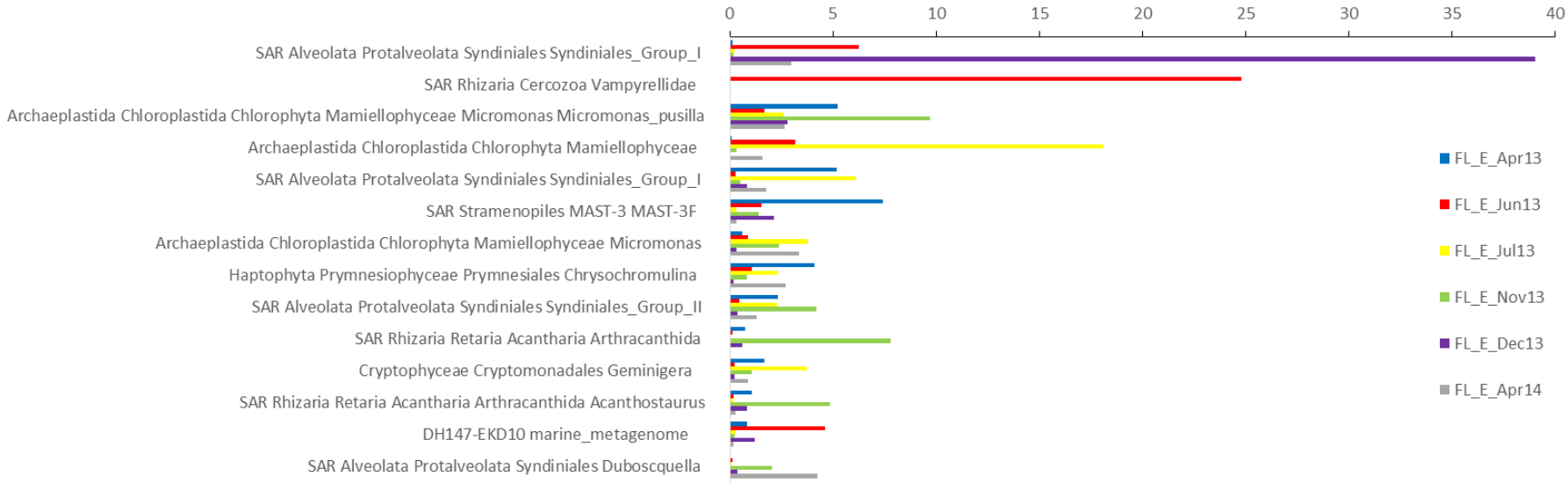
Supplementary Material 3. Particle-attached (PA) prokaryotic groups that contribute with at least 1% of the community at the different sampling dates at EAMO.



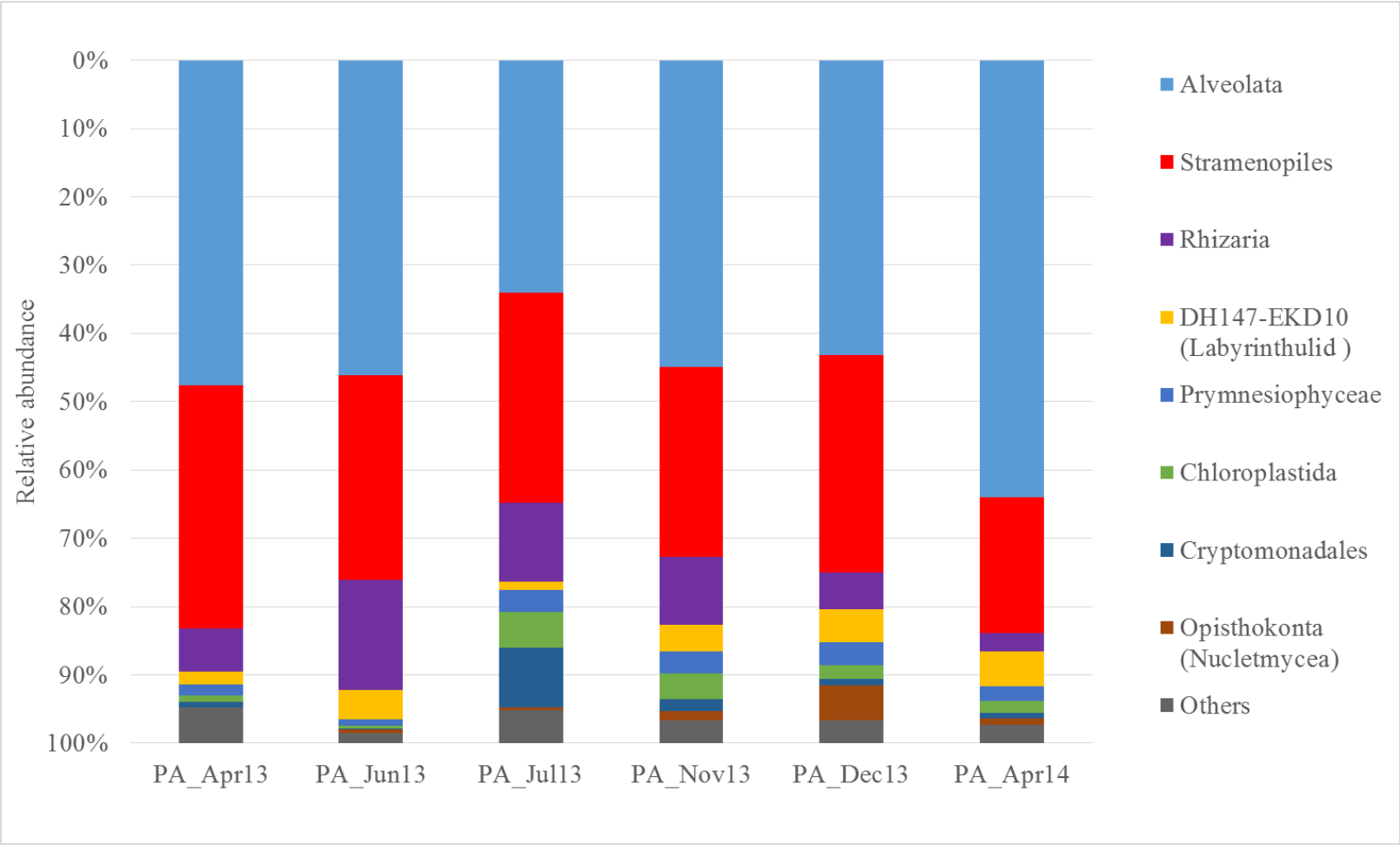
Supplementary Material 4. Particle-attached (PA) prokaryotic OTUs that contribute with at least 1% (sum of all dates) of the community at the different sampling dates at EAMO.



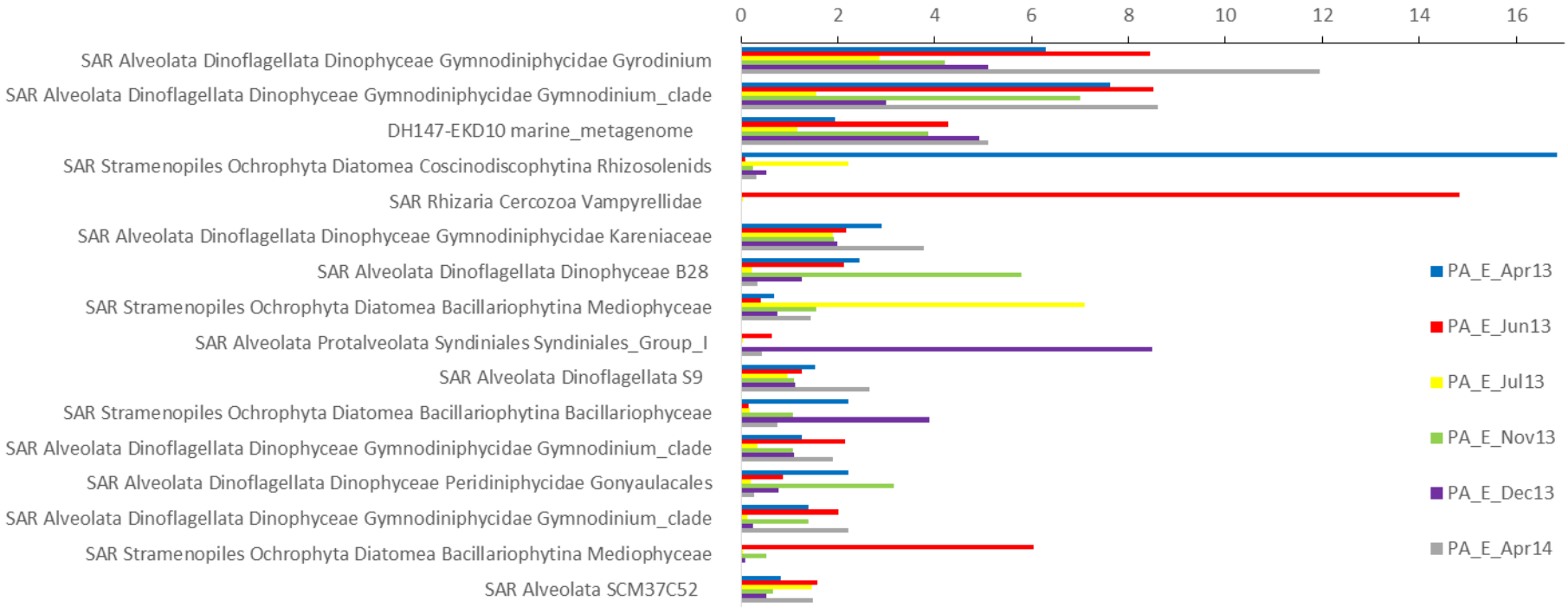
Supplementary Material 5. Free-living (FL) eukaryotic groups that contribute with at least 1% of the community at the different sampling dates at EAMO.



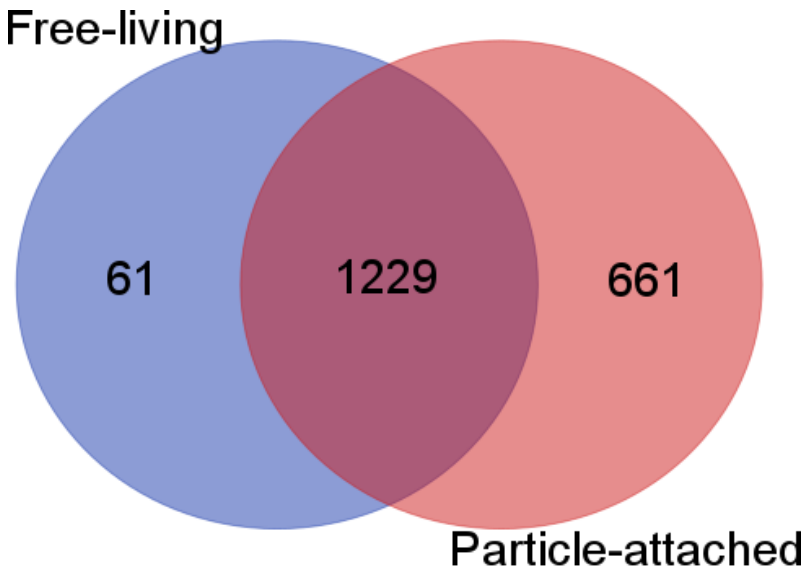
Supplementary Material 6. Free-living (FL) eukaryotic OTUs that contribute with at least 1% (sum of all dates) of the community at the different sampling dates at EAMO.



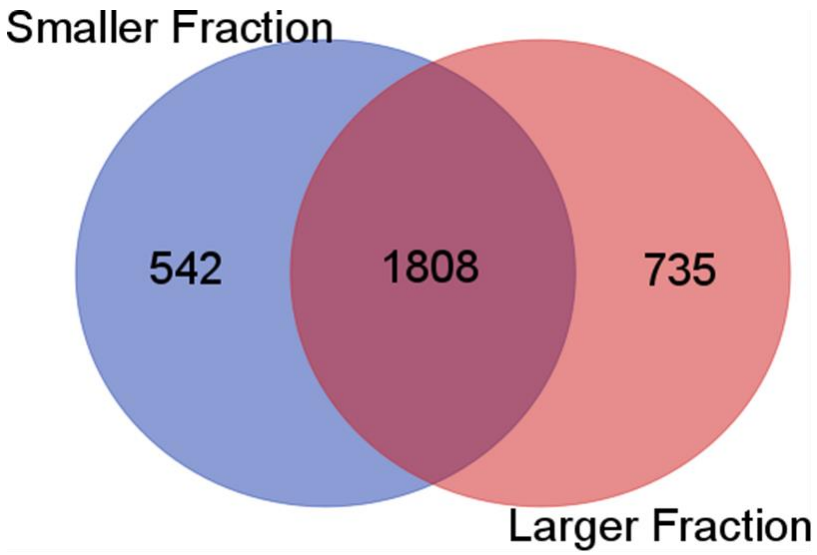
Supplementary Material 7. Larger fraction (PA) eukaryotic groups that contribute with at least 1% of the community at the different sampling dates at EAMO.



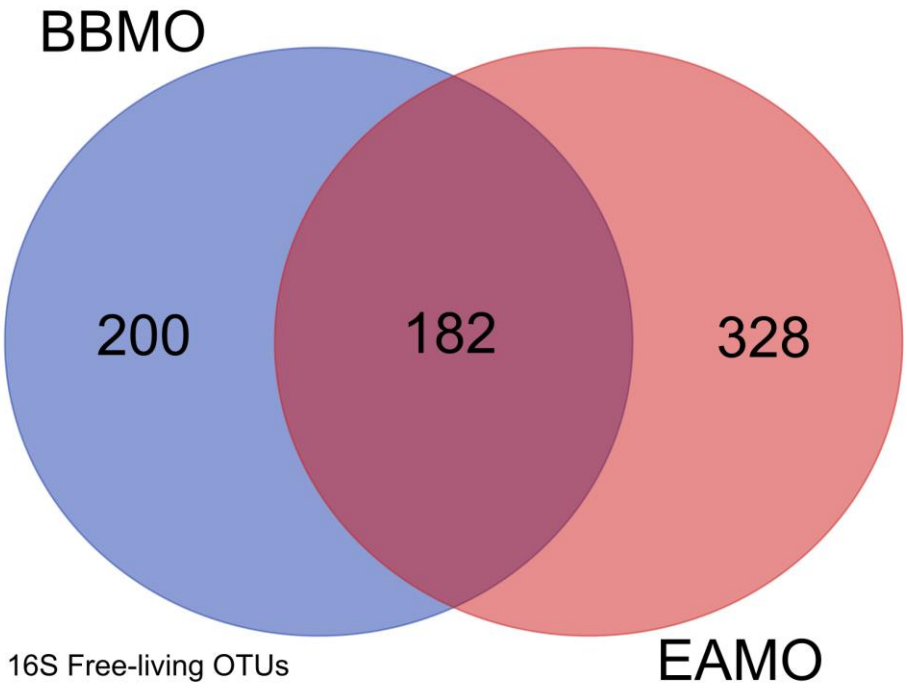
Supplementary Material 8. Larger fraction (PA) eukaryotic OTUs that contribute with at least 1% (sum of all dates) of the community at the different sampling dates at EAMO.



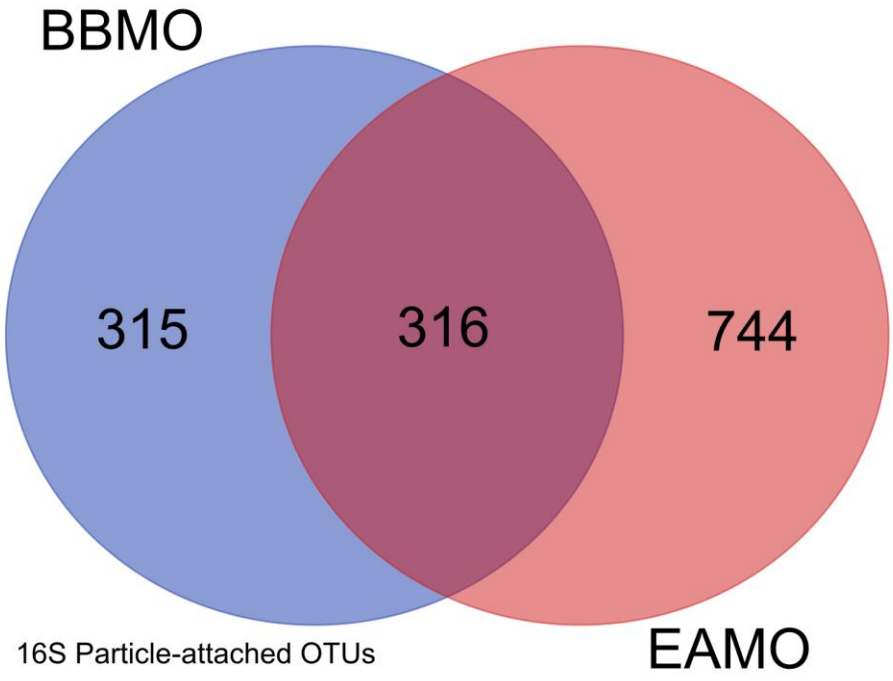
Supplementary Material 9. Prokaryote's Venn diagram of tropical site by size fraction. Free-living = < 3 µm and Particle-attached = > 3 µm.



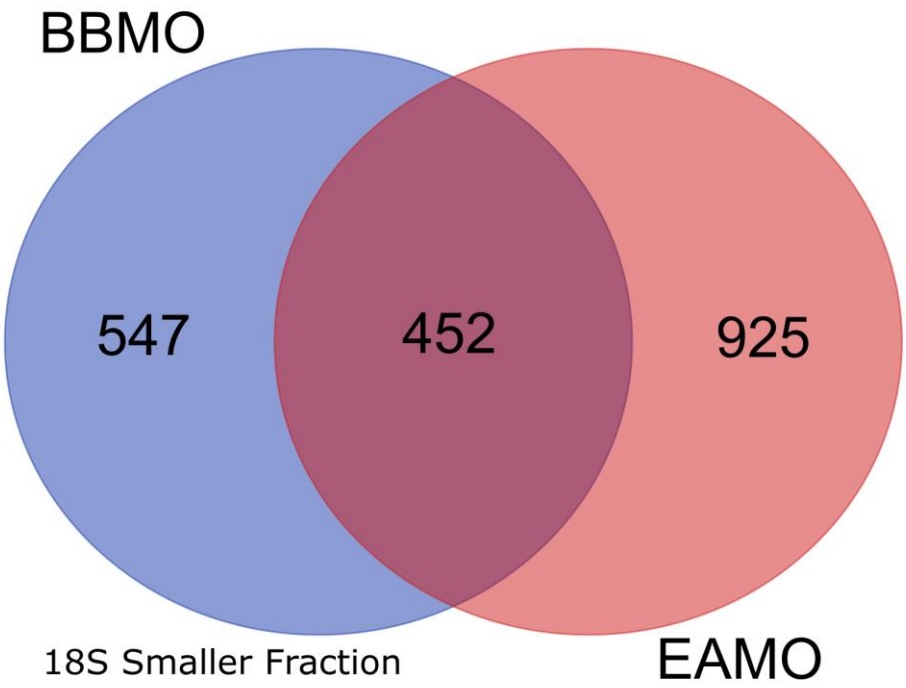
Supplementary Material 10. Eukaryote's Venn diagram of tropical site by size fraction. Smaller fraction = < 3 µm and larger fraction = > 3 µm.



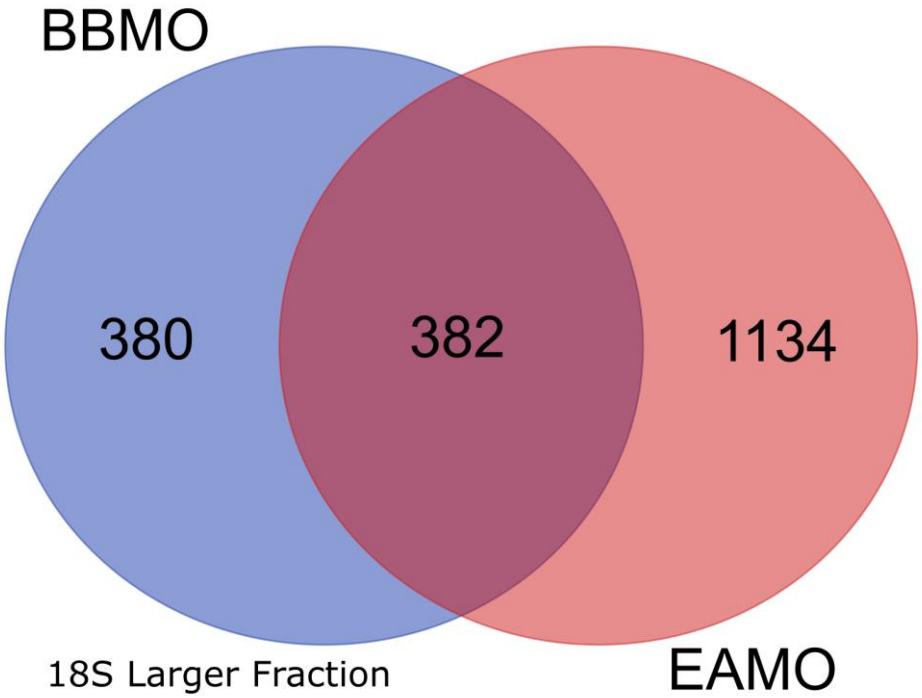
Supplementary Material 11. Prokaryote's Venn diagram of free-living OTUs between sites, BBMO = Blanes Bay Microbial Observatory and EAMO = Equatorial Atlantic Microbial Observatory. Free-living = < 3 µm.



Supplementary Material 12. Prokaryote's Venn diagram of particle-attached OTUs between sites, BBMO = Blanes Bay Microbial Observatory and EAMO = Equatorial Atlantic Microbial Observatory. Particle-attached = > 3 µm.



Supplementary Material 13. Eukaryote's Venn diagram of smaller fraction OTUs between sites, BBMO = Blanes Bay Microbial Observatory and EAMO = Equatorial Atlantic Microbial Observatory. Smaller fraction = < 3 µm.



Supplementary Material 14. Eukaryote's Venn diagram of larger fraction OTUs between sites, BBMO = Blanes Bay Microbial Observatory and EAMO = Equatorial Atlantic Microbial Observatory. Larger fraction = > 3 µm.

Fim.