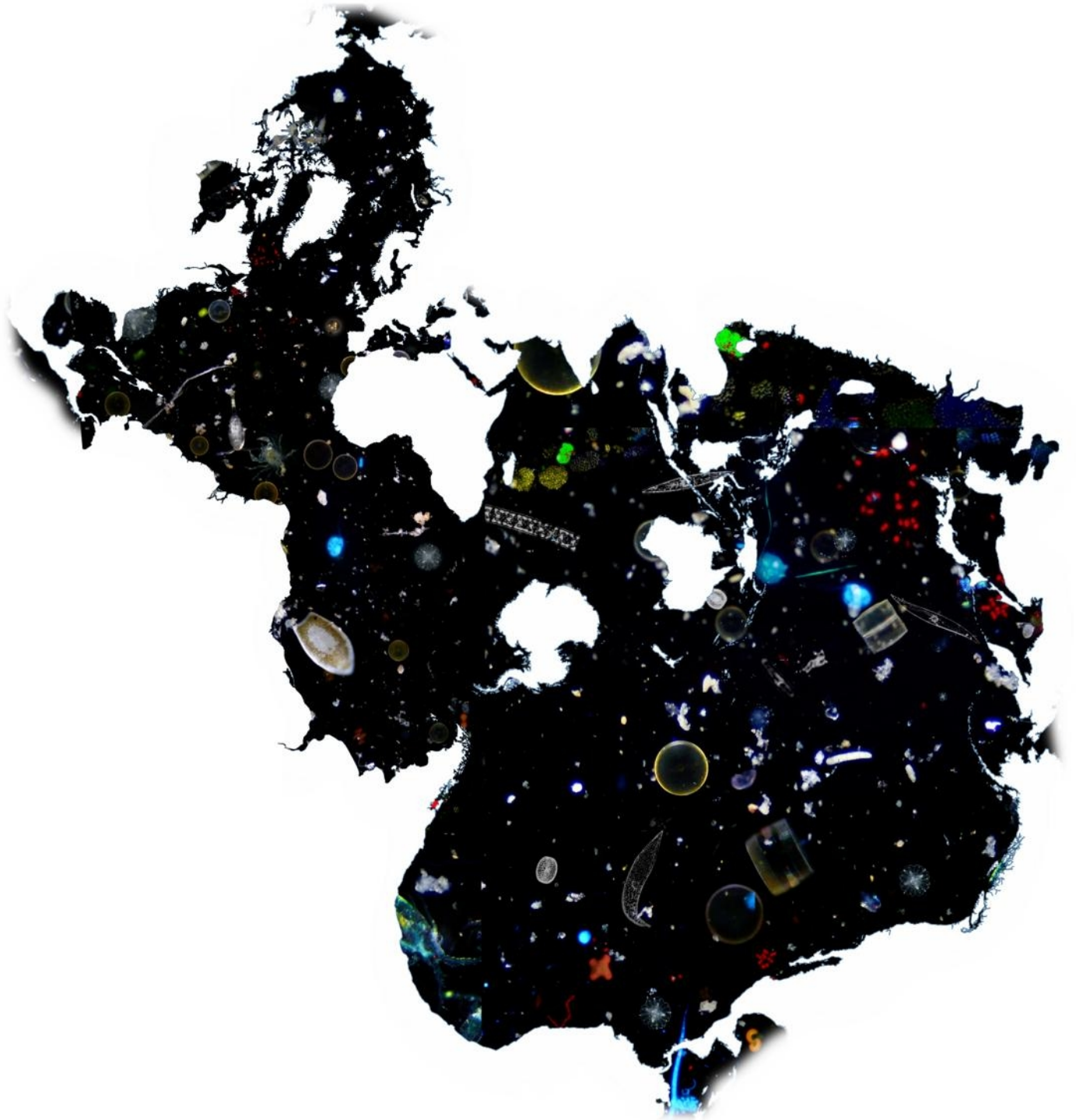


**ECOLOGICAL PROCESSES STRUCTURING  
THE OCEAN MICROBIOME  
ACROSS SPACE AND TIME**

Pedro Ciarlini Junger



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CENTRO DE CIÊNCIAS BIOLÓGICAS E DA SAÚDE  
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São Carlos

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# UNIVERSIDADE FEDERAL DE SÃO CARLOS

Centro de Ciências Biológicas e da Saúde  
Programa de Pós-Graduação em Ecologia e Recursos Naturais

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## Folha de Aprovação

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Defesa de Tese de Doutorado do candidato Pedro Ciarlini Junger Soares, realizada em 09/03/2023.

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## LISTA DE SIGLAS

AAI – average amino acid identity

ADONIS – dissimilarity analysis

ANOVA – analysis of variance

ASVs – variantes únicas de sequências de amplicones (do inglês *amplicon sequencing variants*)

BAT – bathypelagic

BBMO – Blanes Bay Microbial Observatory

$\beta$ MNTD – abundance-weighted  $\beta$ -mean nearest taxon distance

$\beta$ NTI –  $\beta$ -Nearest Taxon Index

CTD – conductivity–temperature–depth profiler

DNA – deoxyribonucleic acid

DCM – máxima de clorofila profunda (do inglês *deep chlorophyll maxima*)

EAMO – Equatorial Atlantic Microbial Observatory

eDNA – environmental DNA

HMSC – hierarchical species composition models

INMET – Instituto Nacional de Meteorologia (Brasil)

MAGs – metagenome-assembled genomes

maxEE – maximum number of expected errors

MES – mesopelagic

NMDS – non-metric multidimensional scaling

OF – orthologous fraction

PCR – polymerase chain reactions

PERMANOVA – permutational multivariate analysis of variance

RC<sub>Bray</sub> – Raup-Crick bray

rRNA – ribosomal ribonucleic acid

SAGs – single-cell amplified genomes

SCG – single-cell genomics

SRF – superfície (do inglês *surface*)



## RESUMO

O microbioma marinho, incluindo procariontes e minúsculos eucariontes unicelulares, representa uma grande parte da biodiversidade e é essencial em teias tróficas e ciclos biogeoquímicos globais. No entanto, investigar a diversidade e ecologia microbiana em escala global era um desafio por limitações metodológicas e amostrais. A combinação de ferramentas moleculares e bioinformáticas modernas à grandes expedições oceanográficas (ex.: Tara Oceans e Malaspina) permitiram a descoberta de padrões de diversidade microbiana em escala global. Estudos recentes revelaram que as comunidades microbianas apresentam padrões latitudinais, sazonais e de profundidade. No entanto, ainda há uma compreensão limitada dos mecanismos ecológicos que estão por trás destes padrões biogeográficos. Nesta tese, apliquei ecologia teórica à dados de sequenciamento de DNA para investigar os principais processos ecológicos que moldam comunidades microbianas em diferentes camadas de profundidade e regiões latitudinais do oceano. Para isso, combinei dados moleculares de duas expedições oceanográficas globais (Tara Oceans e Malaspina), de uma expedição regional (HotMix Cruise) no Mar Mediterrâneo e de observatórios microbianos localizados em diferentes latitudes. Primeiro, demonstro o alto impacto da Teoria das Comunidades Ecológicas (Vellend 2010, 2016) no campo da ecologia microbiana utilizando uma abordagem cienciométrica. Em segundo lugar, investiguei a importância relativa dos processos ecológicos (seleção, limitação de dispersão e deriva ecológica) na montagem de comunidades microbianas que habitam diferentes camadas oceânicas. Para atingir este objetivo, analisei variantes únicas de sequências de amplicons (ASVs) dos genes 16S- e 18S-rRNA de amostras (N=688) cobrindo as camadas epi- (0-200 m), meso- (200-1.000 m) e batipelágicas (1.000-4.000 m) do oceano. Enquanto a importância relativa da seleção diminuiu com a profundidade devido a um potencial diminuição da heterogeneidade do habitat, a limitação por dispersão aumentou com a profundidade devido a barreiras geográficas como massas de água segregadas e montanhas submersas. Além disso, a importância relativa do processo de seleção foi maior no observatório temperado do que no tropical. Por fim, usei ASVs do gene 16S-rRNA, além de genomas montados de metagenoma e genomas amplificados de células únicas, para explorar as bases genômicas do efeito de seleção por temperatura sobre a diferenciação de nichos de uma bactéria marinha abundante, onipresente, mas ainda pouco estudada. No geral, esta tese elucidou os processos ecológicos subjacentes aos padrões biogeográficos do microbioma oceânico no espaço, tempo e profundidade.

**Palavras-chave:** ecologia de comunidades, plâncton, microbiota, dados moleculares

## ABSTRACT

The marine microbiome, including prokaryotes and minute unicellular eukaryotes, stands for a great part of biodiversity and is essential for ocean food webs and global biogeochemical cycles. Yet, investigating their diversity and ecology was challenging due to sampling and methodological constraints. Modern molecular and bioinformatic tools, together with recent large-scale oceanographic sampling cruises (e.g., Tara Oceans and Malaspina), have helped the scientific community to assess the ocean's microbial diversity with an unprecedented depth of analysis. Recent studies have revealed that microbial communities display latitudinal, seasonal, and depth-related patterns. However, there is still a limited understanding of the mechanisms underlying these biogeographical patterns. In this thesis, I have applied theoretical ecology on DNA sequencing data to disentangle the main ecological processes shaping the ocean's microbiome across space and time. To do so, I have combined molecular data from two global oceanographic cruises (Tara Oceans and Malaspina), one regional cruise (HotMix Cruise) in the Mediterranean Sea, and from microbial observatories located in different latitudes. First, I demonstrate the high impact of the Theory of Ecological Communities (Vellend 2010, 2016) on the field of microbial ecology using a scientometric approach. Second, I investigated the relative importance of ecological processes (selection, dispersal limitation, and ecological drift) shaping picoplankton communities inhabiting different ocean layers. To accomplish this goal, I analyzed 16S- and 18S-rRNA-gene amplicon sequence variants (ASVs) from samples (N=688) covering the epi- (0-200 m), meso- (200-1,000 m) and bathypelagic (1,000-4,000 m) layers of the ocean. I found that the role of selection decreased with depth due to a potential decrease in habitat heterogeneity. Conversely, the relative importance of dispersal limitation increased with depth due to dispersal barriers such as the presence of segregated water masses and bottom topography. Furthermore, I found that the relative importance of selection was stronger in the temperate observatory than in the tropical one. Finally, I used 16S-rRNA-gene amplicons as well as metagenome-assembled genomes (MAGs) and single-amplified genomes (SAGs) to explore the genomic basis of temperature selection on niche differentiation of an abundant, ubiquitous, but previously overlooked marine bacteria. Overall, this thesis elucidates the ecological processes underlying the biogeographical patterns of microbial communities of the ocean in space, time, and depth.

**Key-words:** community ecology, plankton, microbiota, molecular data

A detailed microscopic view of a plankton sample. The background is dark, and the field is filled with numerous small, diverse organisms and particles. Visible organisms include several transparent, spherical organisms with internal structures, some with a central point and radiating lines. There are also elongated, segmented organisms, some with multiple legs or appendages, and a few larger, more complex structures. The overall appearance is that of a rich and varied community of microscopic life.

# INTRODUÇÃO GERAL

## INTRODUÇÃO GERAL

### OCEANO GLOBAL: O MAIOR ECOSISTEMA DO PLANETA TERRA

#### *A IMPORTÂNCIA DO OCEANO GLOBAL E SUA CONSERVAÇÃO*

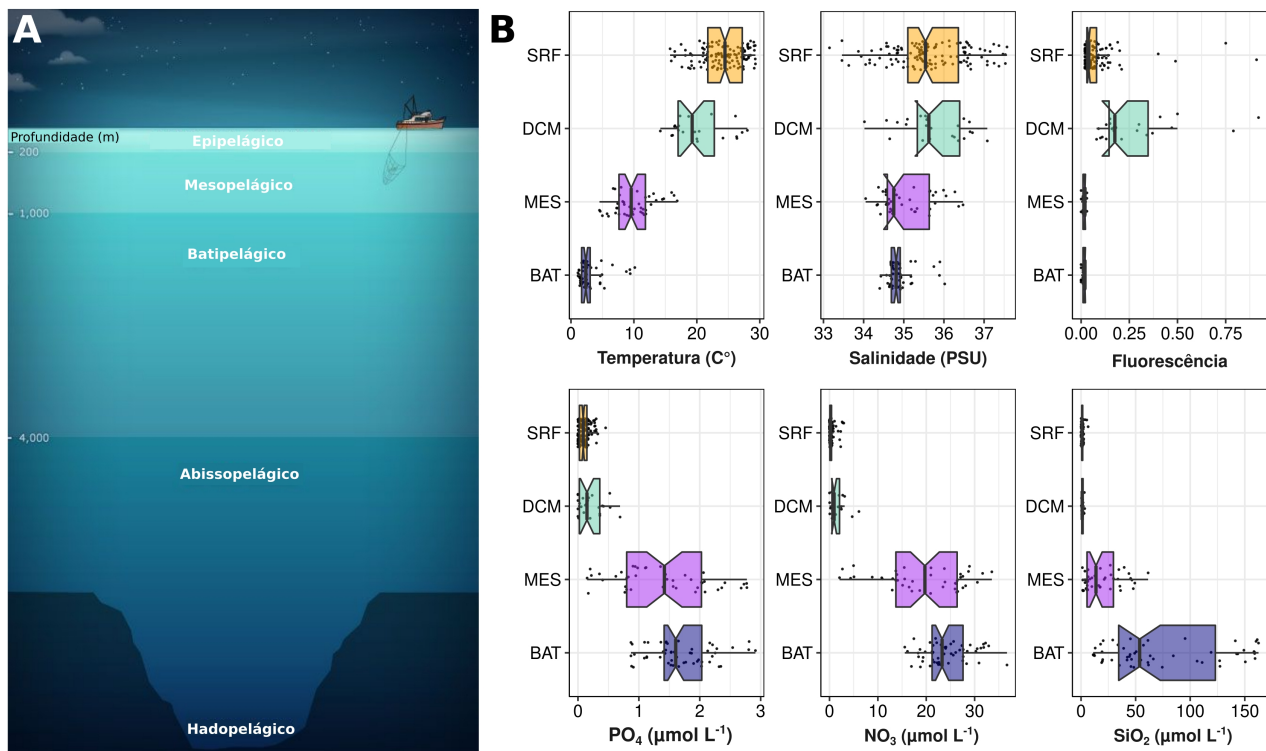
O oceano é o maior ecossistema da terra, cobrindo ~70% da superfície terrestre (~440 milhões km<sup>2</sup>), com um volume de aproximadamente 31.34 bilhões de km<sup>3</sup> (COSTELLO; CHEUNG; DE HAUWERE, 2010). O oceano foi o berço da vida na Terra e desempenha papéis fundamentais para regulação do clima, para o equilíbrio de gases na atmosfera e, conseqüentemente, para manutenção da vida e sua diversidade no nosso planeta. O oceano também provê serviços ecossistêmicos para os seres humanos como recursos pesqueiros, recreação, além de representar laços culturais e espirituais com diversas civilizações ao longo da história da humanidade (ALLISON; KURIEN; OTA, 2020). Esse ecossistema fundamental está sob forte influência das mudanças ambientais causadas por séculos de atividades antrópicas (BINDOFF et al., 2019). Nas últimas décadas, as mudanças climáticas estão causando aumento de temperatura, a acidificação e ampliação das zonas desprovidas de oxigênio (KWIATKOWSKI et al., 2020), o que tem impactado as comunidades planctônicas (CHAFFRON et al., 2021; JONKERS; HILLEBRAND; KUCERA, 2019). Além disso, há uma grande pressão econômica para o desenvolvimento de atividades de exploração (mineração, obras de infraestrutura, etc.) do oceano profundo (DANOVARO et al., 2020; LEVIN; LE BRIS, 2015), que é uma das zonas menos conhecidas dada a dificuldade logística de estudá-la em comparação com o oceano superficial. Por outro lado, há um grande potencial para o desenvolvimento da chamada ‘economia azul’ (BENNETT et al., 2019), o que inclui, por exemplo, a exploração biotecnológica do potencial genético do plâncton microbiano para o desenvolvimento de novos produtos com valor agregado como por exemplo, cosméticos, medicamentos, entre outros (ABREU et al., 2022).

Por estes motivos, a Organização das Nações Unidas (ONU) elegeu 2021-2030 como a década dos oceanos <<https://www.oceandecade.org/>> como forma de impulsionar pesquisas científicas, a educação ambiental e acordos internacionais que visem a conservação deste ecossistema fundamental (CLAUDET et al., 2020). Neste contexto, surgiram inúmeros projetos científicos que visam investigar a biologia e ecologia da vida que habita os oceanos, inclusive a diversidade planctônica, principalmente em zonas historicamente menos estudadas, como as regiões tropicais e o oceano profundo. Esta tese de doutorado está inserida neste contexto e contribui para o maior conhecimento sobre ecologia do microbioma marinho, inclusive em zonas previamente pouco exploradas como o oceano profundo e regiões tropicais.

## ZONEAMENTO VERTICAL DO OCEANO

Os oceanos podem atingir até ~11,500 m de profundidade (Fig. 1). Entre a superfície e o fundo existem distintas camadas de profundidade com diferentes características oceanográficas e ambientais que impactam a fisiologia e ecologia dos organismos que habitam esses diferentes compartimentos. O epipelágico (0-200 m) representa a camada eufótica, com disponibilidade de luz e oxigênio, além de maiores temperaturas e menores concentrações de nutrientes quando comparadas ao oceano profundo (Fig. 1). O epipelágico não é uma camada homogênea e pode ser dividido, basicamente, entre superfície e a máxima de clorofila profunda (em inglês '*deep chlorophyll maxima*', DCM). A camada superficial é uma zona exposta à alta radiação e temperatura, além de apresentar maior interação com a atmosfera, através de trocas de gases e aerossóis (FLORES et al., 2020), assim como a maior dispersão aérea de microrganismos (MAYOL et al., 2017). O DCM se caracteriza como a faixa de profundidade com condições ótimas de luz e nutrientes para realização da fotossíntese pelos organismos fotosintetizantes (CORNEC et al., 2021). Por este motivo, nessa camada encontra-se a maior produtividade primária e um pico de clorofila-a que pode ser identificada claramente traçando um perfil vertical de fluorescência (Fig. 1). O DCM é muito dinâmico e sua profundidade específica pode variar enormemente entre localidades e até mesmo ao longo do tempo numa mesma localidade (CORNEC et al., 2021). Pela maior facilidade logística, essas camadas mais superficiais do oceano (<200 m de profundidade) foram historicamente mais estudadas que as camadas mais profundas, que representam a maior porção do oceano.

De fato, o oceano profundo (>200 m de profundidade) possui ~31.03 bilhões de km<sup>3</sup>, representando 99% do volume total de água no oceano e, portanto, pode ser considerado como o maior ecossistema do planeta (COSTELLO; CHEUNG; DE HAUWERE, 2010). O mesopelágico (200-1,000 m) se caracteriza pela ausência de luz e fotossíntese e, conseqüentemente, uma baixa concentração de oxigênio. Nessa camada há um incremento na concentração de matéria orgânica e nutrientes, assim como da diversidade bacteriana (JUNGER et al., 2023; SUNAGAWA et al., 2015). O batipelágico (1,000-4,000 m) é o maior compartimento de profundidade do oceano e se caracteriza pela total ausência de luz e oxigênio, baixíssimas temperaturas e a presença de cadeias de montanhas submersas que podem funcionar como barreiras geográficas à dispersão de organismos (SALAZAR et al., 2016; SEBASTIÁN et al., 2021). Por fim, também existem as camadas abissopelágica (4,000-6,000 m) e hadopelágica (trincheiras profundas entre 6,000 e 11,000 m de profundidade) (Fig. 1), mas que não são exploradas nesta tese.



**Figura 1. (A)** Ilustração das zonas de profundidade do oceano. Nesta tese trabalhei com dados das zonas epipelágica (incluindo o DCM), mesopelágica e batipelágica. **(B)** Variabilidade dos dados dos parâmetros ambientais por camadas de profundidade. Dados da expedição Malaspina utilizados nesta tese. SRF -- superfície, DCM – máxima de clorofila profunda, MES – mesopelágico, BAT – batipelágico.

No capítulo 2 desta tese, utilizo dados do epi-(superfície e DCM), meso- e batipelágico para investigar como as diferentes características ambientais dessas camadas de profundidade afetam a ecologia dos principais componentes da comunidade microbiana marinha (procariontes e minúsculos eucariontes unicelulares). No capítulo 4 também utilizo dados dessas camadas de profundidade para descrever a distribuição de *Rhodospirillaceae*, um grupo de bactérias marinhas onipresente, mas ainda pouco estudado na literatura. Nos demais capítulos, utilizo dados obtidos em águas superficiais de séries temporais

#### *MARES SEMIFECHADOS: O MEDITERRÂNEO COMO ECOSISTEMA MODELO*

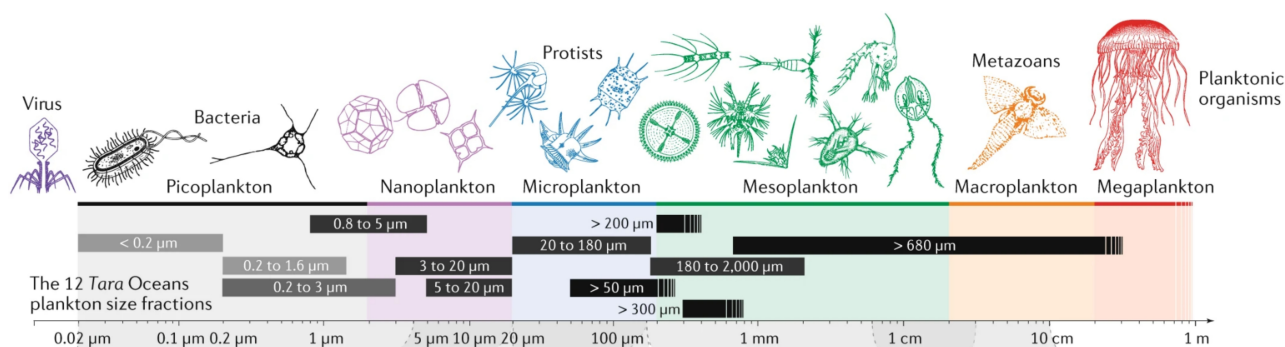
Os mares semifechados (e.g., Mar Mediterrâneo, Mar Vermelho, Mar Negro, Golfo Pérsico, etc.) estão pouco ligados ao oceano aberto (NIHOUL, 1982) e apresentam características ambientais e oceanográficas particulares, tais como temperatura e salinidade relativamente mais elevadas em águas profundas, bem como a inversão da circulação da água que afeta a dinâmica dos nutrientes (BETHOUX et al., 1999; SEBASTIÁN et al., 2021). O Mar Mediterrâneo cobre uma área de  $2,5 \times 10^6$  km<sup>2</sup>, o que o torna o maior mar semifechado da Terra. A bacia do Mediterrâneo está ligada ao



Oceano Atlântico Norte através do Estreito de Gibraltar, que tem apenas 13 km de largura e 300 m de profundidade. Essa limitada conectividade atlântico-mediterrânica leva a uma baixa mistura de águas e gera um afluxo de águas superficiais atlânticas pobres em nutrientes que excedem o fluxo de águas profundas do Mediterrâneo (SAMMARTINO et al., 2015). Assim, o Mar Mediterrâneo apresenta características oceanográficas únicas em comparação com o oceano aberto, tais como alta temperatura e salinidade em águas profundas, bem como a inversão da circulação da água que afeta a dinâmica dos nutrientes (BETHOUX et al., 1999). Como resultado, o Mar Mediterrâneo é no geral oligotrófico, mas apresenta um gradiente oeste-leste de diminuição na concentração de nutrientes (KROM et al., 1991; SEBASTIÁN et al., 2021). Além disso, as águas profundas do Mar Mediterrâneo são fisicamente divididas pelo Estreito da Sicília (500m de profundidade) entre uma sub-bacia ocidental e outra oriental. Essas características refletem na composição da comunidade planctônica (SEBASTIÁN et al., 2021) e transformam o Mar Mediterrâneo num ecossistema modelo muito interessante para testar hipóteses ecológicas numa escala espacial menor do que a do oceano global (BETHOUX et al., 1999; NIHOUL, 1982). No capítulo 2, utilizo o Mar Mediterrâneo como modelo para investigar como as diferentes características ambientais dessas camadas de profundidade afetam a ecologia dos principais componentes da comunidade microbiana marinha (procariontes e minúsculos eucariontes unicelulares).

### *O PLÂNCTON MARINHO E SUAS FRAÇÕES DE TAMANHO*

O plâncton refere-se a todas as formas de vida que não possuem a capacidade de superar a força das correntes oceânicas e que, portanto, vivem à deriva nos oceanos. Muitos dos serviços ecossistêmicos citados no início desta introdução dependem, direta ou indiretamente, das formas de vida planctônica que dominam os oceanos. A comunidade planctônica inclui organismos de diferentes domínios (vírus, procariontes e eucariontes) e tamanhos, variando desde minúsculos vírus (<0.2  $\mu\text{m}$ ) até grandes medusas (>1 m), o que representa uma variação em tamanho de mais de 12 ordens de magnitude (SUNAGAWA et al., 2020). Por esse motivo, é comum referir-se a frações de tamanho do plâncton, o que está inversamente relacionado à sua abundância (SUNAGAWA et al., 2020). Quanto maior o organismo, menor sua abundância nos oceanos, o que influencia o volume de água necessário para obter material biológico suficiente para realização dos protocolos moleculares (SUNAGAWA et al., 2020). O tamanho do plâncton também pode controlar processos biológicos relacionados ao metabolismo e influenciar os processos ecológicos que afetam sua biogeografia (LOGARES et al., 2020; SOMMERIA-KLEIN et al., 2021).



**Figura 2.** Frações de tamanho do plâncton marinho. Adaptado de SUNAGAWA et al., 2020.

Os eucariontes planctônicos apresentam uma ampla variação de tamanho e podem ser classificados em diferentes categorias de acordo com a amplitude de tamanho. Existem algumas variações nessas classificações, mas aqui utilizei as categorias de tamanho do projeto TARA Oceans (SUNAGAWA et al., 2020). As frações pico- (0,2-2 µm) e nanoplanctônica (2-20 µm) incluem eucariontes unicelulares (protistas) como, por exemplo, MASTs (do inglês *marine stramenopiles*) e Picozoas, além de diatomáceas que estão entre os principais produtores primários dos oceanos (FALKOWSKI, 2002). A fração microplanctônica (20-200 µm) inclui desde protistas maiores, como ciliados tintinídeos a organismos multicelulares pequenos (DE VARGAS et al., 2015). A fração mesoplanctônica (0,2-20 mm) inclui copépodos e cladóceros, enquanto as frações macro- (20-200 mm) e megaplanctônica (>200 mm) incluem grandes animais (Fig. 2).

Ainda que a grande maioria dos procariontes possuam tamanho entre 0,2 e 2 µm (fração picoplanctônica), existem bactérias e arqueias adaptadas a viver aderidas a superfícies de partículas ou mesmo sobre outros organismos maiores (MESTRE et al., 2017; SALAZAR et al., 2015). Por essa razão, a composição e diversidade do bacterioplâncton varia com diferentes frações de tamanho [e.g.: 0,2-3 µm, 3-5 µm, 5-20 µm e 20-200 µm] (MESTRE et al., 2018, 2020). No entanto, utilizar essa abordagem mais completa em todos os estudos requereria maior investimento de tempo e recursos financeiros. Portanto, é comum observar uma divisão mais clássica entre bactérias de vida-livre (0,22-3 µm) e bactérias aderidas a partículas (>3 µm). Nesta tese, foco principalmente em procariontes (bactérias e arqueias) de vida-livre (0,2-3 µm) e aderidos a partículas (>3 µm) e em eucariontes (protistas) das frações pico- (0,2-3 µm), nano- e microplanctônica (3-200 µm).

O conjunto desses microrganismos no oceano também pode ser referido na literatura como microbiota marinha (BERG et al., 2020). Quando considerada sua diversidade genética, seu potencial funcional e suas relações com o ambiente, pode-se utilizar o termo ‘microbioma marinho’.

## O MICROBIOMA MARINHO

O microbioma marinho representa uma grande proporção da biodiversidade na Terra (LOCEY; LENNON, 2016) e cerca de 57% (~3.8 Gt C) da biomassa total do oceano (BAR-ON; MILO, 2019), desempenhando papéis essenciais em interações tróficas (SHERR; SHERR, 2008) e ciclos biogeoquímicos globais (FALKOWSKI; FENCHEL; DELONG, 2008; GUIDI et al., 2016). Ter uma amostragem representativa do microbioma marinho para investigar sua distribuição e funcionalidade em escala global era muito desafiador, uma vez que o oceano cobre mais de 70% da superfície do planeta. No entanto, expedições oceanográficas de grande escala, como *Tara Oceans* (PESANT et al., 2015) e *Malaspina* (DUARTE, 2015; SALAZAR et al., 2016), foram conduzidas durante a última década, possibilitando, dessa forma, a avaliação da diversidade planctônica no oceano global. Essas campanhas globais, juntamente com a revolução tecnológica em ferramentas moleculares e bioinformáticas (SALAZAR; SUNAGAWA, 2017), permitiram, finalmente, a abordagem de questões científicas relacionadas à ecologia, diversidade e evolução de microrganismos nos oceanos (IBARBALZ et al., 2019; LOGARES et al., 2020; SALAZAR et al., 2016, 2019). Nesse contexto, o advento de tecnologias de sequenciamento de alto rendimento (*high throughput sequencing* – HTS), combinadas com ferramentas bioinformáticas (CAPORASO et al., 2012; LOGARES et al., 2014), permitiram a caracterização de populações microbianas diretamente de amostras ambientais, trazendo, assim, uma nova perspectiva para a ecologia de comunidades microbianas (AMEND et al., 2013; LOCEY; LENNON, 2016; SHOEMAKER; LOCEY; LENNON, 2017).

Os primeiros estudos que utilizaram esses métodos para investigar a relação entre latitude e diversidade microbiana no oceano revelaram padrões nem sempre convergentes (FUHRMAN et al., 2008; GHIGLIONE et al., 2012; RAES et al., 2011; SUL et al., 2013; SUNAGAWA et al., 2015). Porém, estudos mais recentes confirmam que tanto procariontes como protistas apresentam um padrão de distribuição latitudinal semelhante a macrorganismos, ou seja, com uma diminuição da riqueza de espécies em direção aos polos (IBARBALZ et al., 2019). No entanto, tanto procariontes como picoeucariontes aparentam ter um pico de riqueza não nos trópicos, mas em latitudes intermediárias (IBARBALZ et al., 2019; MILICI et al., 2016; SUNAGAWA et al., 2015). Esse gradiente latitudinal de diversidade não é observado nas camadas mais profundas, abaixo da zona fótica (>200 m de profundidade), do oceano (IBARBALZ et al., 2019). A razão principal para essa ausência de padrão é a baixa variabilidade espacial e temporal de parâmetros ambientais importantes (e.g.: temperatura e luz) no oceano profundo (IBARBALZ et al., 2019). Dessa forma, a diversidade picoplanctônica no oceano profundo aparenta ser governada por outros parâmetros como disponibilidade de recursos (HENSON; SANDERS; MADSEN, 2012; POMEROY; WIEBE,

2001) e composição de massas de água (GÓMEZ-LETONA et al., 2022; JUNGER et al., 2023; SUN et al., 2022).

O microbioma marinho também apresenta padrões de abundância, diversidade e composição com a profundidade (ARÍSTEGUI et al., 2009; BROWN et al., 2009; GINER et al., 2020; JUNGER et al., 2023; SUNAGAWA et al., 2015). No geral, a abundância e atividade microbianas decrescem drasticamente em direção ao oceano profundo (ARÍSTEGUI et al., 2009). A composição da comunidade microbiana também é fortemente estruturada entre as diferentes capas de profundidade no oceano (GINER et al., 2020; JUNGER et al., 2023; SEBASTIÁN et al., 2021; SUNAGAWA et al., 2015). Existe um padrão inverso de diversidade entre os componentes da comunidade picoplânctônica: enquanto a diversidade de procariontes aumenta com a profundidade (JUNGER et al., 2023; SEBASTIÁN et al., 2021; SUNAGAWA et al., 2015), a diversidade de picoeucariontes diminui drasticamente (GINER et al., 2020; JUNGER et al., 2023). Esses padrões relacionados à profundidade são fortemente moldados por diferenças na temperatura e na quantidade disponível de luz solar, oxigênio e nutrientes (SEBASTIÁN et al., 2021; SUNAGAWA et al., 2015), bem como por barreiras físicas entre as diferentes zonas de profundidade do oceano (GALAND et al., 2010; JUNGER et al., 2023; MORALES et al., 2018; VILLARINO et al., 2022). Apesar dos esforços recentes para investigar a diversidade e biogeografia do picoplâncton em diferentes zonas de profundidade do oceano, incluindo o oceano profundo (GINER et al., 2020; SEBASTIÁN et al., 2021; VILLARINO et al., 2022), pouco se sabe sobre os processos ecológicos que explicam a biogeografia desses microrganismos em escalas global e regional.

#### *COMUNIDADES MICROBIANAS NO CONTEXTO DE MUDANÇAS AMBIENTAIS NOS OCEANOS*

Entre muitos parâmetros ambientais, a temperatura exerce a mais forte pressão seletiva sobre as comunidades microbianas, principalmente bactérias, no oceano global (CHAFFRON et al., 2021; SUNAGAWA et al., 2015). Isso se deve ao fato da temperatura ser um forte regulador metabólico (ROSE; CARON, 2007; SARMENTO et al., 2010), o que é particularmente importante, uma vez que a taxa de aquecimento dos oceanos aumentou pelo menos duas vezes nos últimos dois séculos (BINDOFF et al., 2019; CHENG et al., 2020). Além disso, relatórios recentes de mudanças climáticas projetam um aquecimento oceânico adicional de 2 a 4 vezes, considerando o cenário de baixas emissões, ou de 5 a 7 vezes no cenário de altas emissões até 2100 (BINDOFF et al., 2019). Ademais, os padrões de circulação de correntes e massas de água estão sendo alterados pelas mudanças climáticas (HAYS, 2017; SILVY et al., 2020; ZIKA et al., 2021), o que pode afetar os processos de dispersão e filtro ambiental que impactam a composição e distribuição planctônica em escala global (AGOGUÉ et al., 2011; FRANK et al., 2016; RAES et al., 2018; WARD et al., 2021).

Portanto, investigar como as populações microbianas respondem a tais mudanças ambientais é crucial para entender os potenciais mecanismos de feedback que afetam o funcionamento do ecossistema em um oceano global em aquecimento.

## BASES TEÓRICAS EM ECOLOGIA DESTA TESE

### *A TEORIA DE NICHOS ECOLÓGICO*

O ‘nicho ecológico’ foi definido por Hutchinson (1957) como um espaço hiperdimensional, onde as dimensões são as condições/recursos ambientais necessários para que um indivíduo ou população persista. Em outras palavras, o conceito de nicho estabelece a relação entre a população de uma espécie e seu ambiente biótico e abiótico (HUTCHINSON, 1957). De acordo com o conceito de Hutchinson, duas espécies não podem coexistir com nichos idênticos (também conhecido como princípio de exclusão competitiva) e, portanto, deve haver uma diferenciação em pelo menos uma das dimensões do nicho para evitar a extinção (HUTCHINSON, 1957). A partição de nicho refere-se ao mecanismo pelo qual a seleção natural leva as espécies concorrentes a diferentes nichos (MACARTHUR, 1958). Tal mecanismo é fundamental para a promoção da coexistência entre as espécies e manutenção da diversidade biológica e funcional (LEVINE; HILLERISLAMBERS, 2009).

Existem exemplos clássicos de partição de nicho na natureza, como o caso dos lagartos *Anolis* da ilha de Bimini (SCHOENER, 1968), os tentilhões de Darwin nas ilhas Galápagos (DE LEÓN et al., 2014) e a partição de recursos vegetais entre abelhas *Bombus* com diferentes comprimentos de probóscides (PYKE, 1982). Também existem muitos exemplos de partição de nicho em procariontes (AULADELL et al., 2022), muitas vezes referidos na literatura como “diversificação de ecótipos” (do inglês, ecotype diversification). Por exemplo, a diversificação relacionada à profundidade entre as populações microbianas simpátricas foi descrita nos oceanos para o filo das arqueias Thaumarchaeota (REJI et al., 2019) e vários grupos bacterianos, como *Prochlorococcus* (JOHNSON et al., 2006), *Nitrospinae* (NGUGI et al., 2016), *Bacteroidetes* (DÍEZ-VIVES et al., 2019) e o onipresente clado SAR86 (HOARFROST et al., 2020). O importante clado SAR11 também possui ecótipos impulsionados pela partição de nicho com base na profundidade, bioma e estação do ano (SALTER et al., 2015; VERGIN et al., 2013). No entanto, há diversos grupos microbianos com alta abundância relativa que apresentam padrões de partição de nicho que ainda não foram estudados profundamente. No capítulo 4 desta tese, investigo padrões de partição de nicho da família *Rhodospirillaceae*, um grupo de bactérias abundante, mas pouco estudado nos oceanos.

O conceito de nicho ecológico está representado como processo de *seleção* no marco da Teoria de Comunidades Ecológicas (VELLEND, 2010, 2016), sobre a qual apresento uma revisão no item a seguir e uma avaliação no capítulo 1 desta tese.

### UNIFICAÇÃO DE TEORIAS DE COMUNIDADES ECOLÓGICAS

Padrões globais de distribuição geográfica da biodiversidade são objeto de curiosidade e investigação científica desde o princípio do século XIX, quando naturalistas clássicos, como Alexander von Humboldt (1769-1859) e Charles Darwin (1809-1882), realizaram suas primeiras expedições científicas de escala global. Desde então, muitos padrões macroecológicos foram revelados, como a distribuição da abundância de espécies (“abundance-distribution relationship”; (BROWN, 1984)), a relação espécie-área (“species-area relationship”; (PRESTON, 1962)), decaimento da semelhança pela distância (“distance-decay of similarity”; (MORLON et al., 2008)) e espécie-tempo (“species-time-area relationship; (ADLER et al., 2005)), padrões latitudinais da diversidade (MACARTHUR, 1965; PIANKA, 1966), dentre outros. Nesse cenário surge o campo da ecologia de comunidades, a qual tem como objetivo principal entender os padrões de diversidade, composição e distribuição de espécies no espaço e no tempo (VELLEND, 2010).

Nas últimas cinco décadas nosso conhecimento nesse campo de pesquisa avançou muito com o acúmulo de evidências que ajudaram na formulação de várias hipóteses e teorias (PALMER, 1994) que descrevem os principais mecanismos por trás dos padrões observados de distribuição e estruturação da biodiversidade. No entanto, ao contrário de outras áreas, como a genética de populações (PIGLIUCCI, 2007), a ecologia de comunidades carecia de uma teoria síntese que conciliasse todos os avanços científicos historicamente adquiridos. Nesse contexto surge a Teoria de Comunidades Ecológicas (VELLEND, 2010, 2016), a qual adapta o arcabouço conceitual da genética de populações para resumir e unificar as principais teorias desenvolvidas ao longo do tempo na ecologia de comunidades (Tabela 1). A citada teoria sugere que todos os mecanismos responsáveis pela montagem de comunidades descritos na literatura podem ser atribuídos, basicamente, a quatro tipos de processos: seleção, deriva ecológica, dispersão e diversificação (VELLEND, 2010, 2016).

A Teoria de Comunidades Ecológicas de Mark Vellend foi apresentada à comunidade científica em seu artigo “*Conceptual Synthesis in Community Ecology*”, publicado no histórico periódico científico “*The Quarterly Review of Biology*” (VELLEND, 2010) e, posteriormente, desenvolvida e explorada com mais detalhes e estudos de caso em seu livro “*The Theory of Ecological Communities*” (VELLEND, 2016). De acordo com essa teoria, a estruturação de comunidades ecológicas é direcionada pela interação de quatro classes fundamentais de processos

ecológicos, dos quais dois são responsáveis pelo ganho de espécies (diversificação e dispersão) e dois pela perda de espécies pela comunidade (seleção e deriva ecológica) (Fig. 3).

**Tabela 1.** Resumo de como os quatro processos ecológicos fundamentais da Teoria de Comunidades Ecológicas são contemplados pelas principais teorias em ecologia de comunidades (adaptado de VELLEND, 2016).

Seleção	Dispersão	Deriva	Diversificação	Teoria	Referência
X				Nicho Ecológico	(HUTCHINSON, 1957)
	X		X	Biogeografia de Ilhas	(MACARTHUR; WILSON, 1967)
	X	X	X	Teoria Neutra	(HUBBELL, 2001)
X	X		X	Metacomunidades	(LEIBOLD et al., 2004)
X	X	X	X	Síntese conceitual	(VELLEND, 2010)

*Seleção* é definida como o conjunto de forças ecológicas que alteram a estrutura da comunidade devido a diferenças na aptidão dos indivíduos de determinadas espécies (VELLEND, 2010). O processo de seleção resulta, obrigatoriamente, de fatores determinísticos, como condições abióticas (ex.: temperatura, pH e salinidade) e interações bióticas (ex.: competição, facilitação, mutualismo e predação). Esse mecanismo de seleção de espécies por fatores determinísticos não é novidade na ecologia e tem sido o processo ecológico mais amplamente estudado e testado na literatura desde o final dos anos 1950, quando Evelyn Hutchinson (1957) formulou a teoria de nicho ecológico (Tabela 1). Posteriormente, outros ecólogos também focaram seus esforços em estudar, por exemplo, a importância das diferentes escalas espaciais e temporais no efeito dos filtros ambientais (processo de seleção) (BROWN, 1997; RICKLEFS, 1987). Muitos estudos observacionais e experimentais demonstraram, também, que a influência da seleção na montagem de comunidades é dependente da densidade das populações das espécies quando os fatores determinantes são interações ecológicas, como competição (ADLER et al., 2006; DESCAMPS-JULIEN; GONZALEZ, 2005; TILMAN, 1977) ou predação (FUSSMANN et al., 2000; HUFFAKER; SHEA; HERMAN, 1963; KREBS et al., 2001).

*Deriva ecológica* é definida como mudanças aleatórias na abundância relativa das espécies (VELLEND, 2010, 2016). Apesar de ecólogos citarem de maneira mais genérica a relevância de eventos estocásticos na estruturação de comunidades há muitas décadas (VELLEND, 2010), a real importância desse processo foi incorporada pela primeira vez à ecologia de comunidades pela

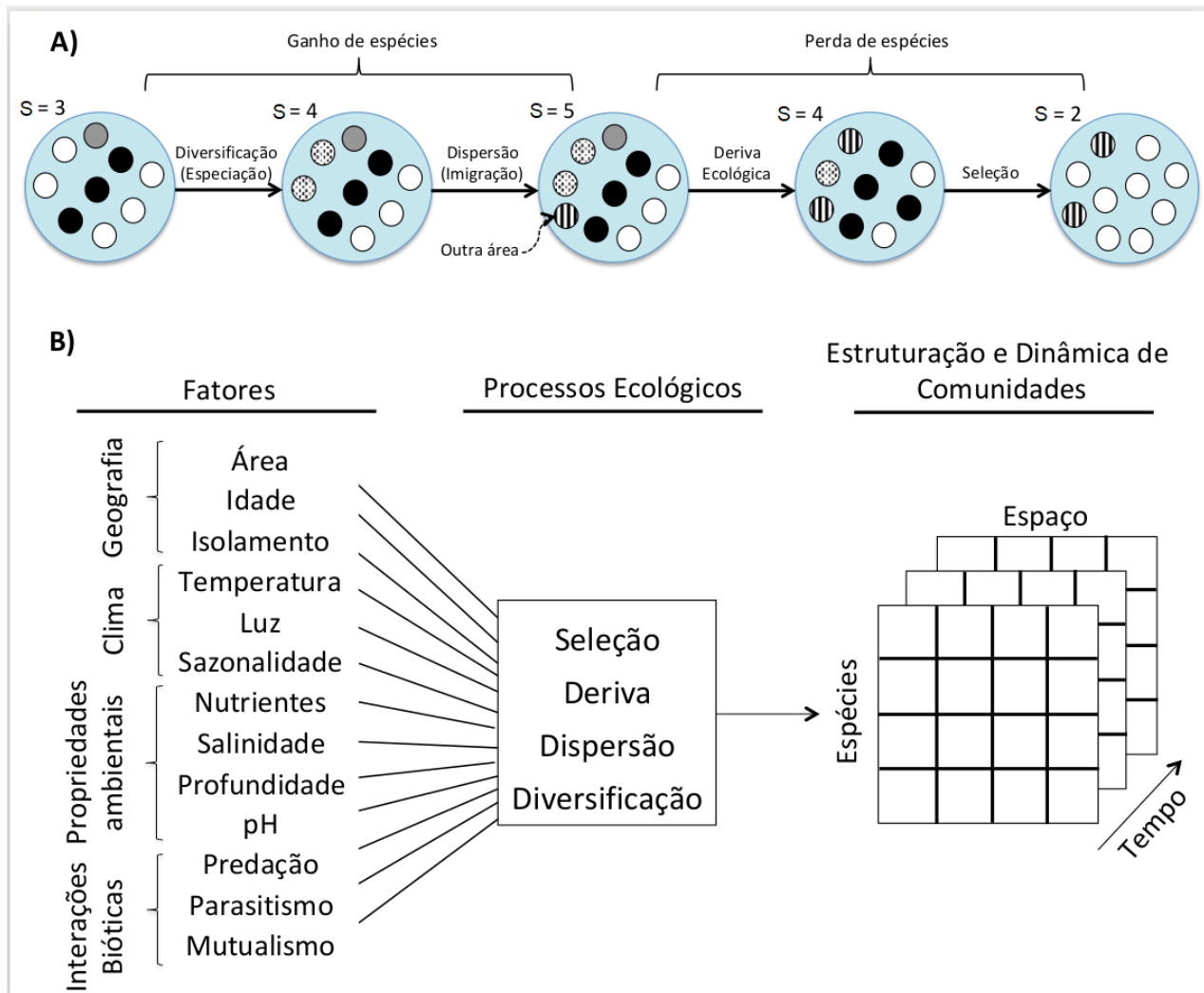
Teoria Neutra de Hubbel (2001). A deriva ecológica é um processo estocástico que pode causar a extinção de espécies com pequenas populações na comunidade (Fig. 3), o que tem bastante relevância para conservação da biodiversidade. Medir o efeito da deriva ecológica sobre comunidades é um desafio, pois, muitas vezes, pode ser confundido com fatores determinísticos não medidos no estudo (VELLEND, 2010). No entanto, alguns estudos experimentais de competição comprovam que em interações entre duas espécies ecologicamente equivalentes, pode haver efeitos aleatórios que favorecem alternadamente uma das espécies envolvidas, mesmo que sob condições iguais e controladas (MERTZ; CAWTHON; PARK, 1976; SIEPIELSKI et al., 2010).

*Diversificação* é um processo evolutivo de geração de novas variantes genéticas como resultado do balanço entre especiação e extinção (VELLEND, 2016). Este processo é particularmente importante na determinação da estrutura de comunidades quando consideradas regiões amplas, como continentes, ou áreas isoladas, como ilhas (MACARTHUR, 1969; RICKLEFS, 2008). Porém, a diversificação também pode atuar em escalas espaciais relativamente menores e, provavelmente, contribui, junto com a seleção, para alguns padrões descritos de relações entre diversidade e gradientes ambientais (RICKLEFS, 2004). Além disso, comunidades ecológicas podem se diversificar (diversificação taxonômica, filogenética, funcional e genética) em diferentes escalas temporais. De maneira geral, microrganismos se diversificam numa escala de tempo muito menor que macrorganismos devido a suas elevadas taxas de reprodução, mutação gênica e transferência horizontal de material genético (ZHOU; NING, 2017). Desse modo, pode-se esperar que este processo seja mais relevante e mensurável em comunidades microbianas. Porém, a influência da diversificação tende a ser relativamente menor em ambientes com alta taxa de dispersão (STEGEN et al., 2015), como é o caso dos oceanos.

*Dispersão* é o processo de movimentação e estabelecimento de organismos no espaço (VELLEND, 2010, 2016). Esse processo tem sido abordado por diferentes teorias ecológicas (Tabela 1) ao longo dos últimos 50 anos, como a Teoria de Biogeografia de Ilhas (MACARTHUR; WILSON, 1967) e a Teoria Neutra (HUBBELL, 2001), mas principalmente pela mais recente Teoria de Metacomunidades (HOLYOAK; LEIBOLD; HOLT, 2005; LEIBOLD et al., 2004), a qual enfatizou o papel da dispersão influenciando padrões de comunidades em múltiplas escalas espaciais. Esse processo interage com outros, como seleção ou deriva, para influenciar os padrões de comunidades locais e regionais (VELLEND, 2010). A dispersão pode ser considerada tanto um processo determinístico como estocástico, de acordo com a biologia dos organismos e as características do ecossistema em questão. Por exemplo, enquanto a dispersão de animais ou plantas pode ser considerada determinística, pois envolve decisões comportamentais e interações



ecológicas, a dispersão de microrganismos planctônicos é geralmente considerada estocástica devido ao seu caráter passivo (ZHOU; NING, 2017).



**Figura 3.** Esquema do arcabouço conceitual da Teoria de Comunidades Ecológicas (VELLEND, 2016) explicando como **(A)** os quatro processos ecológicos fundamentais influenciam o número de espécies (Grafada na imagem com S, relacionado ao termo em inglês “species number”) em uma determinada comunidade ecológica (cada círculo representa um indivíduo e cada preenchimento indica espécies diferentes); e **(B)** como estes processos mediam o efeito de fatores geográficos, ambientais e bióticos sobre a estruturação espacial e temporal de comunidades ecológicas (adaptado de VELLEND, 2016).

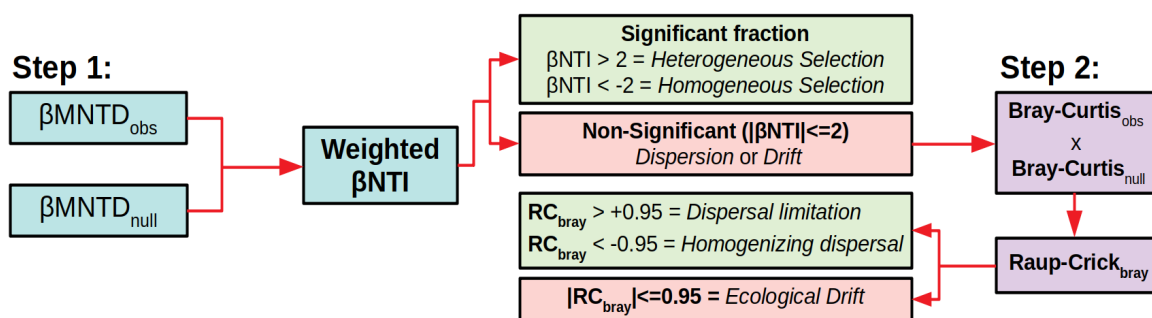
Após cerca de dez anos de sua publicação, a síntese ecológica proposta por Vellend aparenta ter grande impacto no campo da ecologia e representa, até então, a melhor tentativa de unificar teorias em comunidades ecológicas. Por essa razão, utilizo a referida teoria como principal arcabouço teórico desta tese. Com efeito, no capítulo 1, demonstro o impacto dessa teoria no campo da ecologia, utilizando uma abordagem cienciométrica para avaliar quais áreas de pesquisa foram

mais impactadas, e se essa teoria foi mais aproveitada em um contexto teórico ou aplicado. Ademais, pude avaliar nesses estudos que tipos de ambientes e grupos biológicos foram estudados. Por fim, nesta revisão apresento alguns exemplos de estudos que foram capazes de aplicar esse arcabouço teórico a dados reais de comunidades ecológicas, sobretudo de microrganismos.

## MODELOS PARA MENSURAR PROCESSOS ECOLÓGICOS

A importância relativa dos diferentes mecanismos de montagem de comunidade microbiana pode ser estimada por diferentes abordagens estatísticas, todas com suas potencialidades e limitações (VASS et al., 2020). Por exemplo, o método ‘elementos de estrutura de metacomunidades’ (em inglês *the elements of metacommunity structure* – EMS) pode ser utilizado para distinguir seleção local (em inglês *species sorting*) de processos aleatórios na estruturação de comunidades ecológicas (LEIBOLD; MIKKELSON, 2002; PRESLEY; HIGGINS; WILLIG, 2010). Além disso, o modelo de betadiversidade baseado em incidência de Raup-Crick (em inglês *Raup-Crick incidence-based beta-diversity*) tem sido muito utilizado para diferenciar processos determinísticos daqueles estocásticos na montagem de comunidades ecológicas (CHASE; MYERS, 2011; RAUP; CRICK, 1979). Finalmente, uma abordagem mais recente combina modelos nulos baseados na comparação das composições taxonômica e filogenética de comunidades microbianas para quantificar a importância relativa dos processos de seleção, dispersão e deriva ecológica (STEGEN et al., 2013). Em síntese, essa análise consiste em duas etapas principais (Fig. 4): 1) inferência do processo de seleção a partir do turnover filogenético de cada espécie; e 2) inferência dos processos dispersão e deriva ecológica a partir do turnover composicional de cada espécie (STEGEN et al., 2013). A existência de sinal filogenético (CAVENDER-BARES et al., 2009) é uma suposição da primeira etapa desse método (STEGEN et al., 2013), fazendo-se imprescindível primeiramente testar se táxons intimamente relacionados (com base na filogenia do gene rRNA 16S e 18S) são mais semelhantes em termos de preferências de habitat do que táxons distantes.

No capítulo 1, demonstro que a maior parte dos trabalhos que aplicaram a teoria e mensuraram os processos descritos por Mark Vellend foram aplicados a comunidades microbianas. Esse fato se deve, principalmente, a essa abordagem metodológica proposta por Stegen et al. (2013), já citado em 988 trabalhos no Google Scholar até 22/02/2023. Por ter sido amplamente utilizada em diferentes ecossistemas, também para efeitos comparativos, nesta tese aplico essa abordagem nos capítulos 2 e 3 (ver também ANEXO V) para mensurar os processos ecológicos descritos na seção anterior.



**Figura 4.** Fluxograma simplificado da abordagem metodológica proposta por STEGEN et al., 2013. Esses modelos foram utilizados para estimar processos ecológicos (seleção, dispersão e deriva ecológica) nos capítulos 2 e 3 desta tese.

## ANÁLISES DE REDES EM ECOLOGIA MICROBIANA

Microorganismos planctônicos formam redes complexas de interações ecológicas (FAUST; RAES, 2012), cujas combinações podem ter impacto positivo, negativo ou neutro sobre a abundância das espécies envolvidas. Essas redes de associação podem ser construídas através de métodos de inferência de rede, os quais vêm sendo aplicados para séries de dados espaciais e temporais de microbiomas de diversos ecossistemas (CHAFFRON et al., 2021; DEUTSCHMANN et al., 2021; FAUST et al., 2015; FAUST; RAES, 2012; KRABBERØD et al., 2022). Em um estudo recente, foram utilizados métodos de redes para identificar a temperatura como o mais importante estruturador de redes de coocorrência no oceano global (CHAFFRON et al., 2021). Nesse estudo, essas técnicas foram combinadas com modelos de nicho ecológico de cada espécie planctônica para simular como a perda de espécies, causada pelas mudanças climáticas, impactaria a estrutura das redes de interação no oceano (CHAFFRON et al., 2021). Estudos recentes aplicaram abordagens similares a dados temporais e encontraram resultados coerentes (DEUTSCHMANN et al., 2021). No capítulo 3 desta tese analiso redes de co-ocorrência com o objetivo de comparar diferenças entre a estrutura temporal de comunidades microbianas de dois observatórios localizados em latitudes contrastantes (tropical vs temperado).

## MÉTODOS MOLECULARES PARA ESTUDAR O MICROBIOMA MARINHO

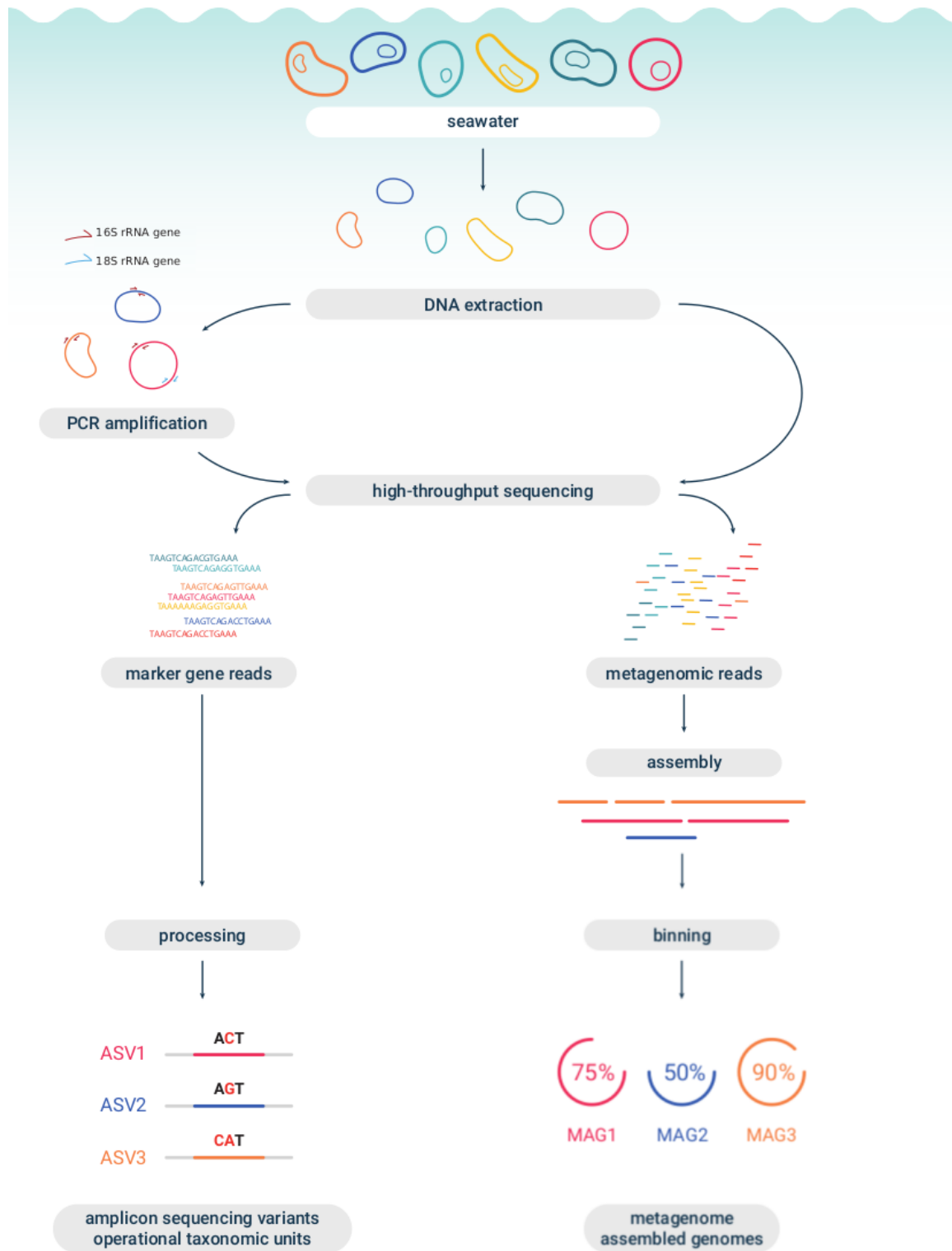
Neste estudo utilizei basicamente três tipos de métodos moleculares para investigar a ecologia de microrganismos marinhos: *a*) metabarcoding (ou sequenciamento de amplicons); *b*) genomas montados de metagenomas – MAGs (do inglês *metagenome assembled genomes*); e *c*) genomas amplificados de células únicas – SAGs (do inglês *single amplified genomes*). Nos itens

abaixo descrevo resumidamente esses métodos e suas aplicações no contexto da ecologia microbiana:

**Metabarcoding (sequenciamento de amplicons).** O método de metabarcoding consiste no sequenciamento de uma região específica do DNA de uma dada amostra ambiental (Fig. 5). Para isso, utilizam-se *primers* e realizam-se PCRs para o sequenciamento de regiões específicas do DNA (Fig. 5). Com a utilização de ferramentas bioinformáticas, pode-se utilizar essa informação para determinar unidades taxonômicas únicas para o estudo de comunidades microbianas. Atualmente, o *dada2* é o programa mais utilizado na literatura para clusterização de ‘espécies’ microbianas em variantes únicas de sequências de amplicons – ASVs (do inglês *amplicon sequencing variants*) (CALLAHAN et al., 2016). Nesta tese utilizei esse método em todos os capítulos aplicados, quais sejam: 2, 3 e 4.

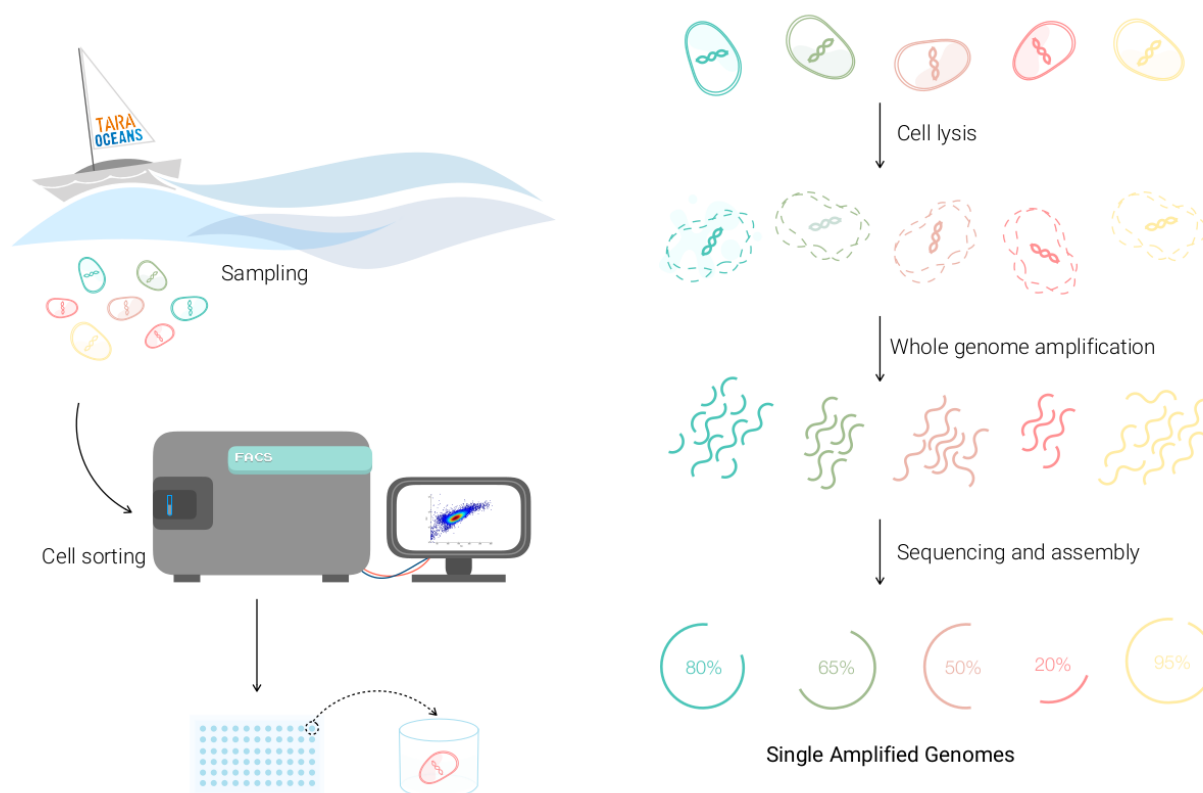
**Genomas montados de metagenomas (MAGs).** A metagenômica pode ser definida como o estudo do metagenoma, que consiste no genoma coletivo de microorganismos sequenciados do DNA extraído a partir de uma amostra ambiental (Fig. 5). Esse sequenciamento gera bilhões de sequências curtas (aproximadamente 150 pares de base) que são posteriormente processadas com métodos bioinformáticos para reconstruir fragmentos genômicos maiores (conhecidos como *contigs*). A partir desses *contigs* podem-se extrair informações sobre a abundância e diversidade funcional dos milhões de genes, além das proteínas codificadas nesses genes (Fig. 5). Além disso, esses *contigs* podem ser reunidos (*contigs binning*) para montagem de genomas de populações de organismos específicos, conhecido na literatura como MAGs (do inglês *metagenome assembled genomes*) (ALBERTSEN et al., 2013; SALAZAR; SUNAGAWA, 2017). Nesta última década, métodos bioinformáticos avançados foram desenvolvidos para a obtenção de genomas a partir de sequências de metagenomas recuperados de amostras ambientais (DELMONT; EREN, 2018; PARKS et al., 2018). Desde então, houve um grande esforço da comunidade científica para reconstrução de dezenas de milhares de novos MAGs de uma ampla variedade de ecossistemas, aumentando consideravelmente nosso conhecimento sobre a diversidade de microorganismos (NAYFACH et al., 2021). Portanto, há um crescente número de base de dados de MAGs na literatura, inclusive do oceano global (ACINAS et al., 2021; NAYFACH et al., 2021; NISHIMURA; YOSHIZAWA, 2022; PAOLI et al., 2022; ROYO-LLONCH et al., 2021; TULLY; GRAHAM; HEIDELBERG, 2018). No capítulo 4 desta tese utilizei MAGs de bactérias marinhas da família *Rhodospirillaceae* disponíveis em uma dessas base de dados (NISHIMURA; YOSHIZAWA, 2022).





**Figura 5.** Diagrama simplificado com duas das abordagens moleculares para estudar o microbioma marinho que foram utilizadas nesta tese: sequenciamento de amplicons (metabarcoding) e geração de MAGs a partir de amostras marinhas. Extraído de (AULADELL, 2021).

**Genomas amplificados de células únicas (SAGs).** SAGs são gerados a partir da amplificação do DNA diretamente de células únicas obtidas de amostras ambientais, e selecionadas por *cell-sorting* (Fig. 6). O sequenciamento de célula única (SCG, do inglês *single-cell genomics*) recupera todas as moléculas de DNA de uma célula, desta forma revelando não apenas suas funções, mas também suas interações ecológicas, como predação, infecções e simbioses (CORNEJO-CASTILLO et al., 2019; LABONTÉ et al., 2015; ROUX et al., 2014). O fato de serem células únicas representa uma vantagem em relação aos MAGs, que, por sua vez, podem mesclar genomas de diferentes cepas de um determinado grupo microbiano (MACAULAY; VOET, 2014; PACHIADAKI et al., 2019). Também há um crescente número de SAGs sequenciados e publicamente disponíveis (PACHIADAKI et al., 2019; PAOLI et al., 2022). No capítulo 4 desta tese, sequenciamos 5 novos SAGs de *Rhodospirillaceae* e os analisamos conjuntamente com SAGs publicamente disponíveis desta família bacteriana.



**Figura 6.** Diagrama simplificado do método para sequenciamento de genomas de célula única (SGC) e geração de seus genomas amplificados (SAGs) a partir de amostras marinhas. Extraído de (ROYO-LLONCH, 2020).

O uso de métodos independentes de cultura, como MAGs e SAGs, vem crescendo rapidamente no campo da ecologia microbiana (PACHIADAKI et al., 2019; ROYO-LLONCH et

al., 2021; SWAN et al., 2013). Essas técnicas são alternativas extremamente poderosas que permitem o sequenciamento de genomas – até então não cultivados em laboratório – que podem ser utilizados para avaliar diferenças genéticas, funcionais e evolutivas entre microrganismos, bem como para identificar papéis ecológicos e potencial biotecnológico de grupos microbianos não cultivados (PACHIADAKI et al., 2019; ROYO-LLONCH et al., 2021). Os resultados de MAGs e SAGs apresentam forte correlação e geram informações genômicas precisas sobre populações bacterianas ainda não cultivadas (ALNEBERG et al., 2018), provando que a combinação desses dois métodos é uma abordagem poderosa para expandir nosso conhecimento sobre a maioria da imensa diversidade microbiana que ainda não conseguimos isolar em laboratório. A maioria das análises de pangenoma baseadas em SAG em ecossistemas marinhos se concentrou em entender a conexão entre o ambiente e as chamadas ‘ilhas genômicas hipervariáveis’ (hypervariable genomic islands) de populações de *Prochlorococcus* e SAR11 (DELMONT; EREN, 2018; THOMPSON et al., 2019). Todavia, um estudo recente usou com sucesso essa tecnologia para descrever novas espécies e suas capacidades ecológicas e funcionais (ROYO-LLONCH et al., 2020). A genômica unicelular também permitiu a anotação funcional para resoluções taxonômicas mais baixas, de gênero (PACHIADAKI et al., 2019) ao nível da espécie (ROYO-LLONCH et al., 2020), o que representa um grande avanço no estudo da diversidade microbiana.

## BASES DE DADOS UTILIZADAS

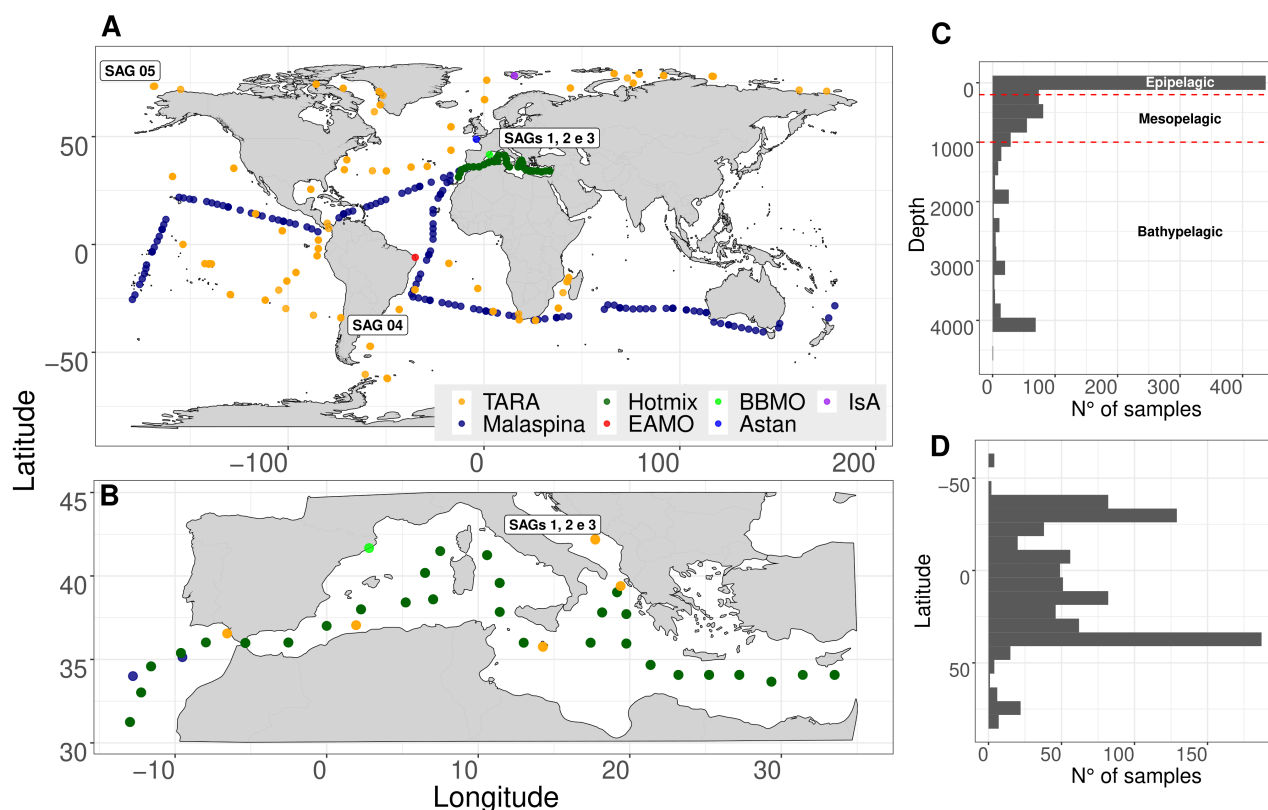
Nesta tese utilizei bases de dados moleculares e ambientais de diversas expedições científicas oceanográficas realizadas durante a última década, além de séries temporais de observatórios microbianos (Fig. 7). Conjuntamente, essas bases de dados cobrem todos os oceanos, as regiões latitudinais e diferentes zonas de profundidade, nas escalas global, regional e temporal. Também geramos uma nova base de dados de amplicons (16S-V4V5 e 18S-V4) do *Equatorial Atlantic Microbial Observatory* (EAMO), uma série temporal tropical de baixa latitude localizada uma região do Atlântico Sul ainda pouco explorada na literatura científica. Nesta seção descrevo brevemente cada uma dessas bases de dados.

## EXPEDIÇÕES GLOBAIS E REGIONAIS

**TARA** – O veleiro científico TARA (<https://fondationtaraocean.org/>) navegou 140,000 km ao longo de todos os oceanos do planeta, inclusive regiões remotas do Oceano Ártico (SUNAGAWA et al., 2020). No total, a base de dados TARA consiste de 210 estações de amostragem, cobrindo uma ampla distribuição latitudinal (62°S–79°N). Nessas estações foram coletadas mais de 35.000 amostras e sequenciados cerca de 40 milhões de genes do epipelágico (superfície e DCM) e



mesopelágico (até 1,000 m) do oceano global, o que representa o maior esforço de sequenciamento genético de microrganismos na história da ciência (PESANT et al., 2015; SUNAGAWA et al., 2020). Os primeiros trabalhos científicos produzidos a partir dessa base de dados trouxeram informações essenciais para o avanço da ecologia microbiana. No capítulo 4 desta tese, sequenciamos SAGs obtidos durante esta expedição (Fig. 7). Além disso, essa base de dados foi utilizada em dois trabalhos, com os quais colaborei, que estão diretamente relacionados ao tema desta tese (ANEXO III).



**Figura 7.** (A) Mapa com a localização geográfica dos pontos de amostragem dos projetos utilizados nesta tese. A localização das estações do TARA de onde os SAGs foram obtidos estão em destaque. (B) Localização dos pontos de amostragem no Mar Mediterrâneo. Histogramas de distribuição das amostras por (C) profundidade e (D) latitude. BBMO – Blanes Bay Microbial Observatory. EAMO – Equatorial Atlantic Microbial Observatory. Astan – SOMLIT Astan Observatory. IsA – Isfjorden Adventfjorden Time-Series.

Malaspina – A expedição Malaspina ocorreu de Dezembro 2010 a Julho 2011, tendo coletado amostras em 121 estações distribuídas nas regiões tropical e sub-tropical dos Oceanos Pacífico, Atlântico e Índico (Fig. 7). Essa expedição foi particularmente importante por ter um desenho amostral desde a superfície até o oceano profundo (4,000 m), cobrindo, assim, as zonas epi-, meso- e batipelágicas do oceano (DUARTE, 2015). Nesse sentido, essa base de dados é única ao incluir

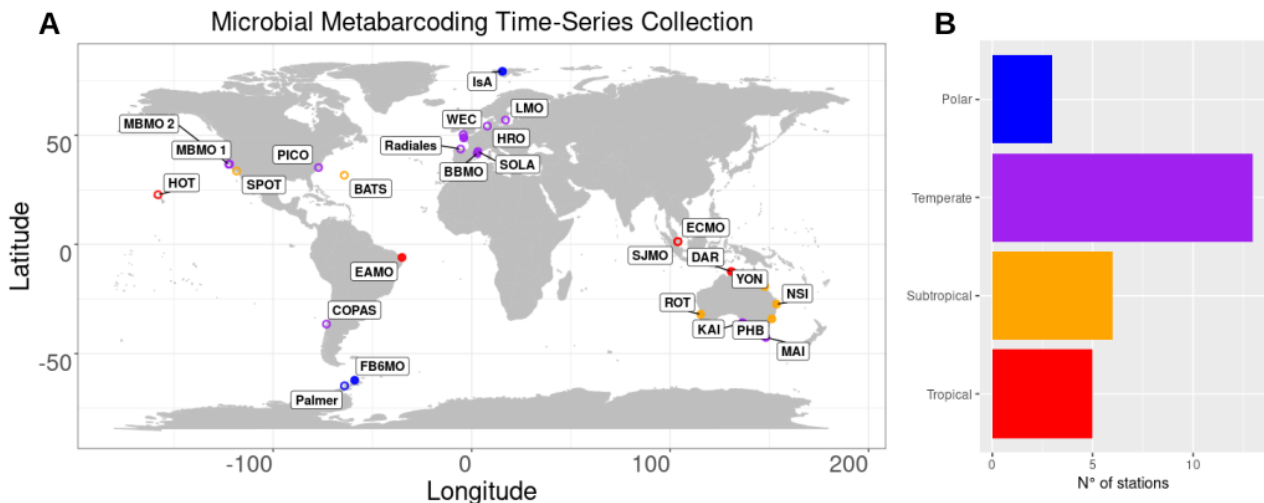
amostras do oceano profundo, o qual representa a maior parte do volume do oceano global, embora historicamente menos estudado.

*HotMix* – A expedição Hotmix foi realizada em 2014 ao longo de um transecto que cobria todas as principais regiões do Mar Mediterrâneo, assim como a região adjacente do Oceano Atlântico Norte, conectada ao Mar Mediterrâneo pelo estreito de Gibraltar (Fig. 7). Essa expedição foi realizada com uma estratégia de amostragem similar ao Malaspina ao obter amostras desde a superfície até o batipelágico ao longo desse transecto. Como descrito anteriormente, o Mar Mediterrâneo possui características únicas que o tornam um ecossistema modelo para testar hipóteses ecológicas.

### *OBSERVATÓRIOS MICROBIANOS*

Apesar das grandes expedições descritas acima representarem um grande esforço e avanço científico para compreensão do microbioma marinho em grandes escalas espaciais, estas não incluem a dimensão temporal. Os oceanos são ecossistemas muito dinâmicos e a variação sazonal das características ambientais geram modificações na composição da comunidade microbiana dificilmente capturadas com amostragens pontuais (FUHRMAN; CRAM; NEEDHAM, 2015). Considerar amostragens temporais de comunidades microbianas é, portanto, essencial para compreender integralmente a estruturação e funcionamento do plâncton marinho (BUNSE; PINHASSI, 2017). As oscilações sazonais das características ambientais (ex.: temperatura e duração do dia) e, conseqüentemente, da composição da comunidade microbiana, tendem a ser muito mais fortes em latitudes mais elevadas, particularmente em regiões temperadas e polares (BUNSE; PINHASSI, 2017). De outra banda, regiões tropicais possuem menor amplitude de variação nas condições ambientais e, por isso, apresentam menor coeficiente de variação na composição das comunidades planctônicas (ALVAIN et al., 2008).

No mundo existe uma série de observatórios microbianos marinhos onde são monitoradas a comunidade microbiana e variáveis ambientais (BUTTIGIEG et al., 2018). A maioria desses observatórios estão estabelecidos em regiões costeiras pela facilidade logística de amostrar mensalmente ou até em maior frequência. Muitos deles também coletam dados microbianos, desde abundância e produção até sequenciamento genético (BUNSE; PINHASSI, 2017; BUTTIGIEG et al., 2018). Nesta tese, realizei uma revisão da literatura, buscando os observatórios microbianos que possuíam dados de sequenciamento de amplicons (Fig. 8). Busquei também informações técnicas sobre a tecnologia de sequenciamento, a região sequenciada, os *primers* utilizados, bem como a duração e frequência de amostragem em cada observatório com dados de metabarcoding.



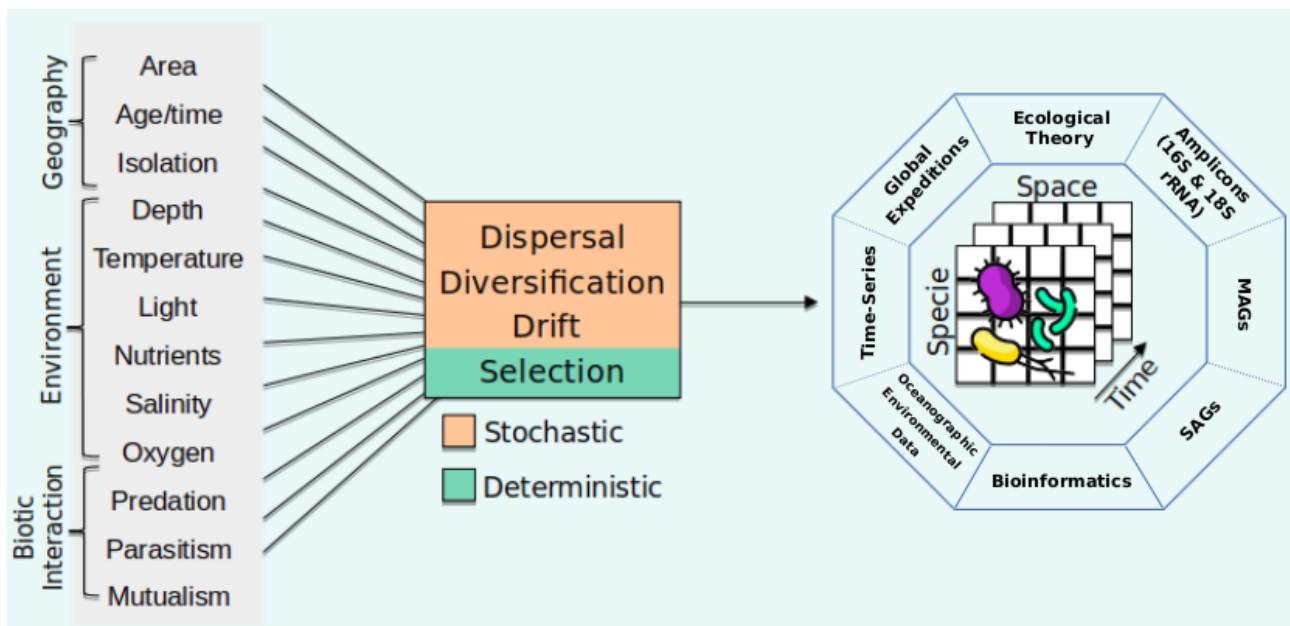
**Figure 8.** Coletânea de séries temporais com dados de metabarcoding de comunidades microbianas. **(A)** Mapa com a localização geográfica dos observatórios microbianos encontrados na literatura. **(B)** Número de observatórios de acordo com a região climática em que se encontram. As cores das estações no mapa e no gráfico de barras correspondem às regiões climáticas: Polar (azul), Temperada (roxo), Subtropical (laranja) e Tropical (vermelho).

Encontrei 27 observatórios microbianos com dados disponíveis de metabarcoding, incluindo um novo observatório microbiano (EAMO) de baixa latitude, cujos dados apresento no manuscrito do capítulo 3 desta tese. Como esperado, a maior parte dos observatórios estão concentrados em regiões temperadas e no hemisfério norte. Recentemente, o número de observatório no sul global aumentou consideravelmente com a publicação dos 7 observatórios australianos (BROWN et al., 2018). Alguns oceanos, no entanto, ainda não possuíam observatórios microbianos com dados de metabarcoding, como é o caso do Atlântico Sul. Esses números destacam a importância do EAMO em um contexto global. No tocante às regiões polares, ainda há poucos dados de séries temporais (TREFALT et al., 2021), o que se explica pela dificuldade logística de acessar essas áreas ao longo de muito tempo, principalmente durante o inverno. Também encontrei uma maior padronização das regiões e *primers* utilizados para sequenciamento do gene 18S, utilizado para estudar comunidades de eucariontes. Há uma maior variabilidade em regiões e *primers* para sequenciamento do gene 16S, utilizado para estudo de comunidades de procariontes. Nesta tese, utilizo dados de metabarcoding de quatro observatórios microbianos: um tropical – Equatorial Atlantic Microbial Observatory (EAMO), dois temperados – Blanes Bay Microbial Observatory (BBMO) e SOMLIT-Astan Observatory (SOMLIT-Astan), além de um polar – Isfjorden Adventfjorden Time-Series (IsA). (Fig. 8):

## OBJETIVOS

Os primeiros trabalhos publicados por grandes projetos – como TARA Oceans e Malaspina – foram essenciais ao colocar nosso conhecimento sobre microrganismos planctônicos marinhos em outro patamar, revelando padrões globais da biogeografia, ecologia e evolução do microbioma marinho. No entanto, esses trabalhos inicialmente responderam questões muito gerais, havendo uma grande quantidade de dados gerados ainda por explorar. Diversas questões ecológicas fundamentais continuam permanecem sem resposta, notadamente sob a luz de modelos teórico-conceituais de ecologia de comunidades.

Nesse contexto, esta tese tem como objetivo geral investigar três processos ecológicos (seleção, dispersão e deriva ecológica), assim como os fatores ambientais e geográficos – que exercem pressão de seleção ou influenciam taxas de dispersão – atuando na estruturação do microbioma marinho (procariontes e eucariontes unicelulares) no tempo e em duas dimensões espaciais (horizontal e vertical) do oceano (Fig. 9).



**Figura 9.** Síntese teórica e metodológica aplicada nesta tese para compreender a ecologia de comunidades microbianas marinhas no espaço e tempo.

## OBJETIVOS ESPECÍFICOS

A presente tese está estruturada em 4 capítulos, representando os seguintes objetivos específicos:

Capítulo 1: Revisar a literatura e avaliar a aplicação da teoria de ecologia de comunidades de VELLEND, 2010;

Capítulo 2: Determinar os processos ecológicos (seleção, dispersão e deriva ecológica) e os fatores ambientais que exercem pressão seletiva sobre comunidades picoplanctônicas habitando distintas camadas de profundidades (epi-, meso- e batipelágico) do oceano, em escala global e regional;

Capítulo 3: I) Determinar quais processos (seleção ou estocásticos) estruturam as comunidades microbianas em dois observatórios marinhos costeiros localizados em latitudes contrastantes (Tropical vs. Temperado); II) Construir redes de co-ocorrência estáticas e dinâmicas para determinar métricas topológicas indicadoras de associações biológicas em cada observatório;

Capítulo 4: Identificar padrões de partição de nicho em bactérias da família *Rhodospirillaceae* no oceano global, buscando descrever os mecanismos genômicos que conferem adaptação desse grupo bacteriano a temperaturas contrastantes.

## REFERÊNCIAS

ABREU, Andre et al. Priorities for ocean microbiome research. **Nature Microbiology**, [S. l.], v. 7, n. 7, p. 937–947, 2022. DOI: 10.1038/s41564-022-01145-5. Disponível em: <https://doi.org/10.1038/s41564-022-01145-5>.

ACINAS, Silvia G. et al. Deep ocean metagenomes provide insight into the metabolic architecture of bathypelagic microbial communities. **Communications Biology**, [S. l.], v. 4, n. 1, p. 604, 2021. DOI: 10.1038/s42003-021-02112-2. Disponível em: <https://doi.org/10.1038/s42003-021-02112-2>.

ADLER, Peter B.; HILLERISLAMBERS, Janneke; KYRIAKIDIS, Phaedon C.; GUAN, Qingfeng; LEVINE, Jonathan M. Climate variability has a stabilizing effect on the coexistence of prairie grasses. **Proceedings of the National Academy of Sciences**, [S. l.], v. 103, n. 34, p. 12793 LP-12798, 2006. Disponível em: <http://www.pnas.org/content/103/34/12793.abstract>.

ADLER, Peter B.; WHITE, Ethan P.; LAUENROTH, William K.; KAUFMAN, Dawn M.; RASSWEILER, Andrew; RUSAK, James a. Evidence for a general species time area relationship. **Ecology**, [S. l.], v. 86, n. 8, p. 2032–2039, 2005. DOI: 10.1890/05-0067. Disponível em: [Journals %5CEcology%5CAdler2005ecology.pdf](#).

AGOGUÉ, Hélène; LAMY, Dominique; NEAL, Phillip R.; SOGIN, Mitchell L.; HERNDL, Gerhard J. Water mass-specificity of bacterial communities in the North Atlantic revealed by massively parallel sequencing. **Molecular Ecology**, [S. l.], v. 20, n. 2, p. 258–274, 2011. DOI: 10.1111/j.1365-294X.2010.04932.x. Disponível em: <https://onlinelibrary.wiley.com/doi/10.1111/j.1365-294X.2010.04932.x>.

- ALBERTSEN, Mads; HUGENHOLTZ, Philip; SKARSHEWSKI, Adam; NIELSEN, Kåre L.; TYSON, Gene W.; NIELSEN, Per H. Genome sequences of rare, uncultured bacteria obtained by differential coverage binning of multiple metagenomes. **Nature Biotechnology**, [S. l.], v. 31, n. 6, p. 533–538, 2013. DOI: 10.1038/nbt.2579. Disponível em: <https://doi.org/10.1038/nbt.2579>.
- ALLISON, Edward H.; KURIEN, John; OTA, Yoshitaka. The human relationship with our ocean planet. [S. l.], 2020. Disponível em: <https://oceanpanel.org/blue-papers/%0AHumanRelationshipwithOurOceanPlanet>.
- ALNEBERG, Johannes et al. Genomes from uncultivated prokaryotes: a comparison of metagenome-assembled and single-amplified genomes. **Microbiome**, [S. l.], v. 6, n. 1, p. 173, 2018. DOI: 10.1186/s40168-018-0550-0. Disponível em: <https://doi.org/10.1186/s40168-018-0550-0>.
- ALVAIN, S.; MOULIN, C.; DANDONNEAU, Y.; LOISEL, H. Seasonal distribution and succession of dominant phytoplankton groups in the global ocean: A satellite view. **Global Biogeochemical Cycles**, [S. l.], v. 22, n. 3, p. 1–15, 2008. DOI: 10.1029/2007GB003154.
- AMEND, Anthony S. et al. Macroecological patterns of marine bacteria on a global scale. **Journal of Biogeography**, [S. l.], v. 40, n. 4, p. 800–811, 2013. DOI: 10.1111/jbi.12034.
- ARÍSTEGUI, Javier; GASOL, Josep M.; DUARTE, Carlos M.; HERNDL, Gerhard J. Microbial oceanography of the dark ocean's pelagic realm. **Limnology and Oceanography**, [S. l.], v. 54, n. 5, p. 1501–1529, 2009. DOI: 10.4319/lo.2009.54.5.1501. Disponível em: <https://doi.org/10.4319/lo.2009.54.5.1501>.
- AULADELL, Adrià. **Seasonality of marine prokaryotes using taxonomic and functional diversity approaches**. 2021. Universitat Autònoma de Barcelona, [S. l.], 2021.
- AULADELL, Adrià; BARBERÁN, Albert; LOGARES, Ramiro; GARCÉS, Esther; GASOL, Josep M.; FERRERA, Isabel. Seasonal niche differentiation among closely related marine bacteria. **The ISME Journal**, [S. l.], v. 16, n. 1, p. 178–189, 2022. DOI: 10.1038/s41396-021-01053-2. Disponível em: <https://doi.org/10.1038/s41396-021-01053-2>.
- BAR-ON, Yinon M.; MILO, Ron. The Biomass Composition of the Oceans: A Blueprint of Our Blue Planet. **Cell**, [S. l.], v. 179, n. 7, p. 1451–1454, 2019. DOI: 10.1016/j.cell.2019.11.018. Disponível em: <https://linkinghub.elsevier.com/retrieve/pii/S0092867419312747>.
- BENNETT, Nathan J. et al. Towards a sustainable and equitable blue economy. **Nature Sustainability**, [S. l.], v. 2, n. 11, p. 991–993, 2019. DOI: 10.1038/s41893-019-0404-1. Disponível em: <https://doi.org/10.1038/s41893-019-0404-1>.
- BERG, Gabriele et al. Microbiome definition re-visited: old concepts and new challenges. **Microbiome**, [S. l.], v. 8, n. 1, p. 103, 2020. DOI: 10.1186/s40168-020-00875-0. Disponível em: <https://doi.org/10.1186/s40168-020-00875-0>.
- BETHOUX, J. P.; GENTILI, B.; MORIN, P.; NICOLAS, E.; PIERRE, C.; RUIZ-PINO, D. The Mediterranean Sea: a miniature ocean for climatic and environmental studies and a key for the climatic functioning of the North Atlantic. **Progress in Oceanography**, [S. l.], v. 44, n. 1, p. 131–

146, 1999. DOI: [https://doi.org/10.1016/S0079-6611\(99\)00023-3](https://doi.org/10.1016/S0079-6611(99)00023-3). Disponível em: <https://www.sciencedirect.com/science/article/pii/S0079661199000233>.

BINDOFF, N. L. et al. Changing Ocean, Marine Ecosystems, and Dependent Communities. *In: **IPCC Special Report on the Ocean and Cryosphere in a Changing Climate***. [s.l.: s.n.]. Disponível em: <http://hdl.handle.net/20.500.11850/395234>.

BROWN, James H. On the Relationship between Abundance and Distribution of Species. **The American Naturalist**, [S. l.], v. 124, n. 2, p. 255–279, 1984. DOI: 10.1086/284267. Disponível em: <https://doi.org/10.1086/284267>.

BROWN, James H. **Macroecology**. Chicago (IL): The University of Chicago Press, 1997. v. 78 DOI: /10.2307/1382661. Disponível em: <http://dx.doi.org/10.2307/1382661>.

BROWN, Mark V et al. Systematic, continental scale temporal monitoring of marine pelagic microbiota by the Australian Marine Microbial Biodiversity Initiative. **Scientific Data**, [S. l.], v. 5, n. 1, p. 180130, 2018. DOI: 10.1038/sdata.2018.130. Disponível em: <https://doi.org/10.1038/sdata.2018.130>.

BROWN, Mark V; PHILIP, Gayle K.; BUNGE, John A.; SMITH, Matthew C.; BISSETT, Andrew; LAURO, Federico M.; FUHRMAN, Jed A.; DONACHIE, Stuart P. Microbial community structure in the North Pacific ocean. **The ISME Journal**, [S. l.], v. 3, n. 12, p. 1374–1386, 2009. DOI: 10.1038/ismej.2009.86. Disponível em: <https://doi.org/10.1038/ismej.2009.86>.

BUNSE, Carina; PINHASSI, Jarone. Marine Bacterioplankton Seasonal Succession Dynamics. **Trends in Microbiology**, [S. l.], v. 25, n. 6, p. 494–505, 2017. DOI: 10.1016/j.tim.2016.12.013. Disponível em: <http://dx.doi.org/10.1016/j.tim.2016.12.013>. Acesso em: 25 out. 2017.

BUTTIGIEG, Pier Luigi; FADEEV, Eduard; BIENHOLD, Christina; HEHEMANN, Laura; OFFRE, Pierre; BOETIUS, Antje. Marine microbes in 4D—using time series observation to assess the dynamics of the ocean microbiome and its links to ocean health. **Current Opinion in Microbiology**, [S. l.], v. 43, p. 169–185, 2018. DOI: <https://doi.org/10.1016/j.mib.2018.01.015>. Disponível em: <https://www.sciencedirect.com/science/article/pii/S1369527417302035>.

CALLAHAN, Benjamin J.; MCMURDIE, Paul J.; ROSEN, Michael J.; HAN, Andrew W.; JOHNSON, Amy Jo A.; HOLMES, Susan P. DADA2: High-resolution sample inference from Illumina amplicon data. **Nature Methods**, [S. l.], v. 13, p. 581, 2016. Disponível em: <https://doi.org/10.1038/nmeth.3869>.

CAPORASO, J. Gregory et al. Ultra-high-throughput microbial community analysis on the Illumina HiSeq and MiSeq platforms. **The ISME Journal**, [S. l.], v. 6, n. 8, p. 1621–1624, 2012. DOI: 10.1038/ismej.2012.8.

CAVENDER-BARES, Jeannine; KOZAK, Kenneth H.; FINE, Paul V. A.; KEMBEL, Steven W. The merging of community ecology and phylogenetic biology. **Ecology Letters**, [S. l.], v. 12, n. 7, p. 693–715, 2009. DOI: <https://doi.org/10.1111/j.1461-0248.2009.01314.x>. Disponível em: <https://doi.org/10.1111/j.1461-0248.2009.01314.x>.

CHAFFRON, Samuel et al. Environmental vulnerability of the global ocean epipelagic plankton community interactome. **Science Advances**, [S. l.], v. 7, n. 35, p. eabg1921, 2021. DOI: 10.1126/sciadv.abg1921. Disponível em: <https://doi.org/10.1126/sciadv.abg1921>.

CHASE, Jonathan M.; MYERS, Jonathan A. Disentangling the importance of ecological niches from stochastic processes across scales. **Philosophical Transactions of the Royal Society B: Biological Sciences**, 6-9 CARLTON HOUSE TERRACE, LONDON SW1Y 5AG, ENGLAND, v. 366, n. 1576, p. 2351–2363, 2011. DOI: 10.1098/rstb.2011.0063. Disponível em: <https://royalsocietypublishing.org/doi/10.1098/rstb.2011.0063>.

CHENG, Lijing et al. Record-Setting Ocean Warmth Continued in 2019. **Advances in Atmospheric Sciences**, [S. l.], v. 37, n. 2, p. 137–142, 2020. DOI: 10.1007/s00376-020-9283-7. Disponível em: <https://doi.org/10.1007/s00376-020-9283-7>.

CLAUDET, Joachim et al. A Roadmap for Using the UN Decade of Ocean Science for Sustainable Development in Support of Science, Policy, and Action. **One Earth**, [S. l.], v. 2, n. 1, p. 34–42, 2020. DOI: 10.1016/j.oneear.2019.10.012. Disponível em: <https://doi.org/10.1016/j.oneear.2019.10.012>.

CORNEC, M.; CLAUSTRE, H.; MIGNOT, A.; GUIDI, L.; LACOUR, L.; POTEAU, A.; D'ORTENZIO, F.; GENTILI, B.; SCHMECHTIG, C. Deep Chlorophyll Maxima in the Global Ocean: Occurrences, Drivers and Characteristics. **Global Biogeochemical Cycles**, [S. l.], v. 35, n. 4, p. e2020GB006759, 2021. DOI: <https://doi.org/10.1029/2020GB006759>. Disponível em: <https://doi.org/10.1029/2020GB006759>.

CORNEJO-CASTILLO, Francisco M.; MUÑOZ-MARÍN, Maria del Carmen; TURK-KUBO, Kendra A.; ROYO-LLONCH, Marta; FARNELID, Hanna; ACINAS, Silvia G.; ZEHR, Jonathan P. UCYN-A3, a newly characterized open ocean sublineage of the symbiotic N<sub>2</sub>-fixing cyanobacterium Candidatus Atelocyanobacterium thalassa. **Environmental Microbiology**, [S. l.], v. 21, n. 1, p. 111–124, 2019. DOI: <https://doi.org/10.1111/1462-2920.14429>. Disponível em: <https://doi.org/10.1111/1462-2920.14429>.

COSTELLO, Mark John; CHEUNG, Alan; DE HAUWERE, Nathalie. Surface Area and the Seabed Area, Volume, Depth, Slope, and Topographic Variation for the World's Seas, Oceans, and Countries. **Environmental Science & Technology**, [S. l.], v. 44, n. 23, p. 8821–8828, 2010. DOI: 10.1021/es1012752. Disponível em: <https://pubs.acs.org/doi/10.1021/es1012752>.

DANOVARO, Roberto et al. Ecological variables for developing a global deep-ocean monitoring and conservation strategy. **Nature Ecology & Evolution**, [S. l.], v. 4, n. 2, p. 181–192, 2020. DOI: 10.1038/s41559-019-1091-z. Disponível em: <https://doi.org/10.1038/s41559-019-1091-z>.

DE LEÓN, L. F.; PODOS, J.; GARDEZI, T.; HERREL, A.; HENDRY, A. P. Darwin's finches and their diet niches: the sympatric coexistence of imperfect generalists. **Journal of Evolutionary Biology**, [S. l.], v. 27, n. 6, p. 1093–1104, 2014. DOI: 10.1111/jeb.12383. Disponível em: <https://doi.org/10.1111/jeb.12383>.



DE VARGAS, Colomban et al. Eukaryotic plankton diversity in the sunlit ocean. **Science**, [S. l.], v. 348, n. 6237, p. 1261605–1261605, 2015. DOI: 10.1126/science.1261605. Disponível em: <http://www.sciencemag.org/cgi/doi/10.1126/science.1261605>.

DELMONT, Tom O.; EREN, A. Murat. Linking pangenomes and metagenomes: the Prochlorococcus metapangenome. **PeerJ**, [S. l.], v. 6, p. e4320, 2018. DOI: 10.7717/peerj.4320. Disponível em: <https://doi.org/10.7717/peerj.4320>.

DESCAMPS-JULIEN, Blandine; GONZALEZ, Andrew. Stable Coexistence in a Fluctuating Environment: An Experimental Demonstration. **Ecology**, [S. l.], v. 86, n. 10, p. 2815–2824, 2005. Disponível em: <http://www.jstor.org/stable/3450707>.

DEUTSCHMANN, Ina Maria; LIMA-MENDEZ, Gipsi; KRABBERØD, Anders K.; RAES, Jeroen; VALLINA, Sergio M.; FAUST, Karoline; LOGARES, Ramiro. Disentangling environmental effects in microbial association networks. **Microbiome**, [S. l.], v. 9, n. 1, p. 232, 2021. DOI: 10.1186/s40168-021-01141-7. Disponível em: <https://doi.org/10.1186/s40168-021-01141-7>.

DÍEZ-VIVES, Cristina; NIELSEN, Shaun; SÁNCHEZ, Pablo; PALENZUELA, Oswaldo; FERRERA, Isabel; SEBASTIÁN, Marta; PEDRÓS-ALIÓ, Carlos; GASOL, Josep M.; ACINAS, Silvia G. Delineation of ecologically distinct units of marine Bacteroidetes in the Northwestern Mediterranean Sea. **Molecular Ecology**, [S. l.], v. 28, n. 11, p. 2846–2859, 2019. DOI: 10.1111/mec.15068. Disponível em: <https://doi.org/10.1111/mec.15068>.

DUARTE, Carlos M. Seafaring in the 21st Century: The Malaspina 2010 Circumnavigation Expedition. **Limnology and Oceanography Bulletin**, [S. l.], v. 24, n. 1, p. 11–14, 2015. DOI: 10.1002/lob.10008. Disponível em: <https://doi.org/10.1002/lob.10008>.

FALKOWSKI, Paul G. The Ocean’s Invisible Forest. **Scientific American**, [S. l.], v. 287, n. 2, p. 54–61, 2002. Disponível em: <http://www.jstor.org/stable/26059928>.

FALKOWSKI, Paul G.; FENCHEL, Tom; DELONG, Edward F. The Microbial Engines That Drive Earth’s Biogeochemical Cycles. **Science**, [S. l.], v. 320, n. 5879, p. 1034–1039, 2008. DOI: 10.1126/science.1153213. Disponível em: <http://www.sciencemag.org/cgi/doi/10.1126/science.1153213>.

FAUST, Karoline; LAHTI, Leo; GONZE, Didier; DE VOS, Willem M.; RAES, Jeroen. Metagenomics meets time series analysis: Unraveling microbial community dynamics. **Current Opinion in Microbiology**, [S. l.], v. 25, n. May, p. 56–66, 2015. DOI: 10.1016/j.mib.2015.04.004. Disponível em: <http://dx.doi.org/10.1016/j.mib.2015.04.004>.

FAUST, Karoline; RAES, Jeroen. Microbial interactions: from networks to models. **Nature Reviews Microbiology**, [S. l.], v. 10, n. 8, p. 538–550, 2012. DOI: 10.1038/nrmicro2832. Disponível em: <http://www.nature.com/doi/10.1038/nrmicro2832>.

FLORES, J. M. et al. Tara Pacific Expedition’s Atmospheric Measurements of Marine Aerosols across the Atlantic and Pacific Oceans: Overview and Preliminary Results. **Bulletin of the American Meteorological Society**, [S. l.], v. 101, n. 5, p. E536–E554, 2020. DOI: 10.1175/BAMS-

D-18-0224.1. Disponível em: <https://journals.ametsoc.org/view/journals/bams/101/5/bams-d-18-0224.1.xml>.

FRANK, Alexander H.; GARCIA, Juan A. L.; HERNDL, Gerhard J.; REINTHALER, Thomas. Connectivity between surface and deep waters determines prokaryotic diversity in the North Atlantic Deep Water. **Environmental Microbiology**, [S. l.], v. 18, n. 6, p. 2052–2063, 2016. DOI: 10.1111/1462-2920.13237. Disponível em: <https://onlinelibrary.wiley.com/doi/10.1111/1462-2920.13237>.

FUHRMAN, Jed A.; CRAM, Jacob A.; NEEDHAM, David M. Marine microbial community dynamics and their ecological interpretation. **Nature Reviews Microbiology**, [S. l.], v. 13, n. 3, p. 133–146, 2015. DOI: 10.1038/nrmicro3417. Disponível em: <http://www.nature.com/doi/10.1038/nrmicro3417>.

FUHRMAN, Jed A.; STEELE, Joshua A.; HEWSON, Ian; SCHWALBACH, Michael S.; BROWN, Mark V.; GREEN, Jessica L.; BROWN, James H. A latitudinal diversity gradient in planktonic marine bacteria. **Proceedings of the National Academy of Sciences**, [S. l.], v. 105, n. 22, p. 7774–8, 2008. DOI: 10.1073/pnas.0803070105. Disponível em: <http://www.ncbi.nlm.nih.gov/pubmed/18509059>.

FUSSMANN, Gregor F.; ELLNER, Stephen P.; SHERTZER, Kyle W.; HAIRSTON JR., Nelson G. Crossing the Hopf Bifurcation in a Live Predator-Prey System. **Science**, [S. l.], v. 290, n. 5495, p. 1358 LP-1360, 2000. Disponível em: <http://science.sciencemag.org/content/290/5495/1358.abstract>.

GALAND, Pierre E.; POTVIN, Marianne; CASAMAYOR, Emilio O.; LOVEJOY, Connie. Hydrography shapes bacterial biogeography of the deep Arctic Ocean. **The ISME Journal**, [S. l.], v. 4, n. 4, p. 564–576, 2010. DOI: 10.1038/ismej.2009.134. Disponível em: <https://doi.org/10.1038/ismej.2009.134>.

GHIGLIONE, Jean-François et al. Pole-to-pole biogeography of surface and deep marine bacterial communities. **Proceedings of the National Academy of Sciences**, [S. l.], v. 109, n. 43, p. 17633–17638, 2012. DOI: 10.1073/pnas.1208160109. Disponível em: <http://www.pnas.org/content/109/43/17633.abstract>.

GINER, Caterina R.; PERNICE, Massimo C.; BALAGUÉ, Vanessa; DUARTE, Carlos M.; GASOL, Josep M.; LOGARES, Ramiro; MASSANA, Ramon. Marked changes in diversity and relative activity of picoeukaryotes with depth in the world ocean. **The ISME Journal**, [S. l.], v. 14, n. 2, p. 437–449, 2020. DOI: 10.1038/s41396-019-0506-9. Disponível em: <https://doi.org/10.1038/s41396-019-0506-9>.

GÓMEZ-LETONA, Markel et al. Deep ocean prokaryotes and fluorescent dissolved organic matter reflect the history of the water masses across the Atlantic Ocean. **Progress in Oceanography**, [S. l.], v. 205, p. 102819, 2022. DOI: 10.1016/j.pocean.2022.102819. Disponível em: <https://www.sciencedirect.com/science/article/pii/S0079661122000805>.

- GUIDI, Lionel et al. Plankton networks driving carbon export in the oligotrophic ocean. **Nature**, [S. l.], v. 532, n. 7600, p. 465–470, 2016. DOI: 10.1038/nature16942. Disponível em: <http://dx.doi.org/10.1038/nature16942>.
- HAYS, Graeme C. Ocean currents and marine life. **Current Biology**, [S. l.], v. 27, n. 11, p. R470–R473, 2017. DOI: <https://doi.org/10.1016/j.cub.2017.01.044>. Disponível em: <https://www.sciencedirect.com/science/article/pii/S0960982217300775>.
- HENSON, Stephanie A.; SANDERS, Richard; MADSEN, Esben. Global patterns in efficiency of particulate organic carbon export and transfer to the deep ocean. **Global Biogeochemical Cycles**, [S. l.], v. 26, n. 1, 2012. DOI: <https://doi.org/10.1029/2011GB004099>. Disponível em: <https://doi.org/10.1029/2011GB004099>.
- HOARFROST, Adrienne; NAYFACH, Stephen; LADAU, Joshua; YOOSEPH, Shibu; ARNOSTI, Carol; DUPONT, Chris L.; POLLARD, Katherine S. Global ecotypes in the ubiquitous marine clade SAR86. **The ISME Journal**, [S. l.], v. 14, n. 1, p. 178–188, 2020. DOI: 10.1038/s41396-019-0516-7. Disponível em: <https://doi.org/10.1038/s41396-019-0516-7>.
- HOLYOAK, Marcel; LEIBOLD, Mathew A.; HOLT, Robert D. **Metacommunities: spatial dynamics and ecological communities**. Chicago (IL): University of Chicago Press, 2005.
- HUBBELL, Stephen P. **The unified neutral theory of biodiversity and biogeography**. Princeton (NJ): Princeton University Press, 2001.
- HUFFAKER, C. B.; SHEA, K. P.; HERMAN, S. G. Experimental studies on predation: Complex dispersion and levels of food in an acarine predator-prey interaction. **Hilgardia**, [S. l.], v. 34, n. 9, p. 305–330, 1963. DOI: 10.3733/hilg.v34n09p305. Disponível em: <http://hilgardia.ucanr.edu/Abstract/?a=hilg.v34n09p305>.
- HUTCHINSON, M. Concluding remarks. *In*: 1957, **Anais [...]**. : Cold Spring Harb Symp Quant Biol, 1957. p. 415–427.
- IBARBALZ, Federico M. et al. Global Trends in Marine Plankton Diversity across Kingdoms of Life. **Cell**, [S. l.], v. 179, n. 5, p. 1084–1097.e21, 2019. DOI: 10.1016/j.cell.2019.10.008. Disponível em: <https://doi.org/10.1016/j.cell.2019.10.008>.
- JOHNSON, Zackary I.; ZINSER, Erik R.; COE, Allison; MCNULTY, Nathan P.; WOODWARD, E. Malcolm S.; CHISHOLM, Sallie W. Niche Partitioning Among *Prochlorococcus* Ecotypes Along Ocean-Scale Environmental Gradients. **Science**, [S. l.], v. 311, n. 5768, p. 1737 LP-1740, 2006. DOI: 10.1126/science.1118052. Disponível em: <http://science.sciencemag.org/content/311/5768/1737.abstract>.
- JONKERS, Lukas; HILLEBRAND, Helmut; KUCERA, Michal. Global change drives modern plankton communities away from the pre-industrial state. **Nature**, [S. l.], v. 570, n. 7761, p. 372–375, 2019. DOI: 10.1038/s41586-019-1230-3. Disponível em: <https://doi.org/10.1038/s41586-019-1230-3>.

- JUNGER, Pedro C. et al. Global biogeography of the smallest plankton across ocean depths. **bioRxiv**, [S. l.], p. 2023.01.13.523743, 2023. DOI: 10.1101/2023.01.13.523743. Disponível em: <http://biorxiv.org/content/early/2023/01/15/2023.01.13.523743.abstract>.
- KRABBERØD, Anders K. et al. Long-term patterns of an interconnected core marine microbiota. **Environmental Microbiome**, [S. l.], v. 17, n. 1, p. 22, 2022. DOI: 10.1186/s40793-022-00417-1. Disponível em: <https://doi.org/10.1186/s40793-022-00417-1>.
- KREBS, Charles J.; BOONSTRA, Rudy; BOUTIN, Stan; SINCLAIR, A. R. E. What Drives the 10-year Cycle of Snowshoe Hares? The ten-year cycle of snowshoe hares—one of the most striking features of the boreal forest—is a product of the interaction between predation and food supplies, as large-scale experiments in the Yukon have. **BioScience**, [S. l.], v. 51, n. 1, p. 25–35, 2001. Disponível em: [http://dx.doi.org/10.1641/0006-3568\(2001\)051\[0025:WDTYCO\]2.0.CO](http://dx.doi.org/10.1641/0006-3568(2001)051[0025:WDTYCO]2.0.CO).
- KROM, M. D.; KRESS, N.; BRENNER, S.; GORDON, L. I. Phosphorus limitation of primary productivity in the eastern Mediterranean Sea. **Limnology and Oceanography**, [S. l.], v. 36, n. 3, p. 424–432, 1991. DOI: <https://doi.org/10.4319/lo.1991.36.3.0424>. Disponível em: <https://doi.org/10.4319/lo.1991.36.3.0424>.
- KWIATKOWSKI, Lester et al. Twenty-first century ocean warming, acidification, deoxygenation, and upper-ocean nutrient and primary production decline from CMIP6 model projections. **Biogeosciences**, [S. l.], v. 17, n. 13, p. 3439–3470, 2020. DOI: 10.5194/bg-17-3439-2020. Disponível em: <https://bg.copernicus.org/articles/17/3439/2020/>.
- LABONTÉ, Jessica M. et al. Single-cell genomics-based analysis of virus–host interactions in marine surface bacterioplankton. **The ISME Journal**, [S. l.], v. 9, n. 11, p. 2386–2399, 2015. DOI: 10.1038/ismej.2015.48. Disponível em: <https://doi.org/10.1038/ismej.2015.48>.
- LEIBOLD, M. A. et al. The metacommunity concept: a framework for multi-scale community ecology. **Ecology Letters**, [S. l.], v. 7, n. 7, p. 601–613, 2004. DOI: 10.1111/j.1461-0248.2004.00608.x. Disponível em: <https://doi.org/10.1111/j.1461-0248.2004.00608.x>.
- LEIBOLD, Mathew A.; MIKKELSON, Gregory M. Coherence, species turnover, and boundary clumping: elements of meta-community structure. **Oikos**, [S. l.], v. 97, n. 2, p. 237–250, 2002. DOI: <https://doi.org/10.1034/j.1600-0706.2002.970210.x>. Disponível em: <https://doi.org/10.1034/j.1600-0706.2002.970210.x>.
- LEVIN, Lisa A.; LE BRIS, Nadine. The deep ocean under climate change. **Science**, [S. l.], v. 350, n. 6262, p. 766–768, 2015. DOI: 10.1126/science.aad0126. Disponível em: <https://doi.org/10.1126/science.aad0126>.
- LEVINE, Jonathan M.; HILLERISLAMBERS, Janneke. The importance of niches for the maintenance of species diversity. **Nature**, [S. l.], v. 461, n. 7261, p. 254–257, 2009. DOI: 10.1038/nature08251. Disponível em: <https://doi.org/10.1038/nature08251>.
- LOCEY, Kenneth J.; LENNON, Jay T. Scaling laws predict global microbial diversity. **Proceedings of the National Academy of Sciences**, [S. l.], v. 113, n. 21, p. 5970–5975, 2016. DOI:

10.1073/pnas.1521291113. Disponível em:

<http://www.pnas.org/lookup/doi/10.1073/pnas.1521291113>.

LOGARES, Ramiro et al. Metagenomic 16S rDNA Illumina tags are a powerful alternative to amplicon sequencing to explore diversity and structure of microbial communities. **Environmental Microbiology**, [S. l.], v. 16, n. 9, p. 2659–2671, 2014. DOI: 10.1111/1462-2920.12250.

LOGARES, Ramiro et al. Disentangling the mechanisms shaping the surface ocean microbiota. **Microbiome**, [S. l.], v. 8, n. 1, p. 55, 2020. DOI: 10.1186/s40168-020-00827-8. Disponível em: <https://microbiomejournal.biomedcentral.com/articles/10.1186/s40168-020-00827-8>.

MACARTHUR, Robert H. Population Ecology of Some Warblers of Northeastern Coniferous Forests. **Ecology**, [S. l.], v. 39, n. 4, p. 599–619, 1958. DOI: 10.2307/1931600. Disponível em: <https://doi.org/10.2307/1931600>.

MACARTHUR, Robert H. Patterns of Species Diversity. **Biological Reviews**, [S. l.], v. 40, n. 4, p. 510–533, 1965. DOI: 10.1111/j.1469-185X.1965.tb00815.x. Disponível em: <http://doi.wiley.com/10.1111/j.1469-185X.1965.tb00815.x>.

MACARTHUR, Robert H. Patterns of communities in the tropics. **Biological Journal of the Linnean Society**, [S. l.], v. 1, n. 1–2, p. 19–30, 1969. DOI: 10.1111/j.1095-8312.1969.tb01809.x. Disponível em: <https://doi.org/10.1111/j.1095-8312.1969.tb01809.x>.

MACARTHUR, Robert H.; WILSON, Edward O. **The Theory of Island Biogeography**. [s.l.] : Princeton University Press, 1967.

MACAULAY, Iain C.; VOET, Thierry. Single Cell Genomics: Advances and Future Perspectives. **PLOS Genetics**, [S. l.], v. 10, n. 1, p. e1004126, 2014. Disponível em: <https://doi.org/10.1371/journal.pgen.1004126>.

MAYOL, Eva et al. Long-range transport of airborne microbes over the global tropical and subtropical ocean. **Nature Communications**, [S. l.], v. 8, n. 1, p. 201, 2017. DOI: 10.1038/s41467-017-00110-9. Disponível em: <https://doi.org/10.1038/s41467-017-00110-9>.

MERTZ, David B.; CAWTHON, D. A.; PARK, Thomas. An Experimental Analysis of Competitive Indeterminacy in *Tribolium*. **Proceedings of the National Academy of Sciences of the United States of America**, [S. l.], v. 73, n. 4, p. 1368–1372, 1976. Disponível em: <http://www.jstor.org/stable/65902>.

MESTRE, Mireia; BORRULL, Encarna; SALA, Mmontserrat; GASOL, Josep M. Patterns of bacterial diversity in the marine planktonic particulate matter continuum. **ISME Journal**, [S. l.], v. 11, n. 4, p. 999–1010, 2017. DOI: 10.1038/ismej.2016.166.

MESTRE, Mireia; HÖFER, Juan; SALA, M. Montserrat; GASOL, Josep M. Seasonal Variation of Bacterial Diversity Along the Marine Particulate Matter Continuum. **Frontiers in Microbiology**, [S. l.], v. 11, 2020. DOI: 10.3389/fmicb.2020.01590. Disponível em: <https://www.frontiersin.org/articles/10.3389/fmicb.2020.01590>.

MESTRE, Mireia; RUIZ-GONZÁLEZ, Clara; LOGARES, Ramiro; DUARTE, Carlos M.; GASOL, Josep M.; SALA, M. Montserrat. Sinking particles promote vertical connectivity in the ocean microbiome. **Proceedings of the National Academy of Sciences**, [S. l.], v. 115, n. 29, p. E6799 LP-E6807, 2018. DOI: 10.1073/pnas.1802470115. Disponível em: <http://www.pnas.org/content/115/29/E6799.abstract>.

MILICI, Mathias et al. Bacterioplankton biogeography of the Atlantic ocean: A case study of the distance-decay relationship. **Frontiers in Microbiology**, [S. l.], v. 7, n. APR, p. 1–15, 2016. DOI: 10.3389/fmicb.2016.00590.

MORALES, Sergio E.; MEYER, Moana; CURRIE, Kim; BALTAR, Federico. Are oceanic fronts ecotones? Seasonal changes along the subtropical front show fronts as bacterioplankton transition zones but not diversity hotspots. **Environmental Microbiology Reports**, [S. l.], v. 10, n. 2, p. 184–189, 2018. DOI: 10.1111/1758-2229.12618. Disponível em: <https://onlinelibrary.wiley.com/doi/10.1111/1758-2229.12618>.

MORLON, Hélène; CHUYONG, George; CONDIT, Richard; HUBBELL, Stephen; KENFACK, David; THOMAS, Duncan; VALENCIA, Renato; GREEN, Jessica L. **A general framework for the distance–decay of similarity in ecological communities**. (Jerome chavez, Org.) **Ecology Letters**, 2008. DOI: 10.1111/j.1461-0248.2008.01202.x.

NAYFACH, Stephen et al. A genomic catalog of Earth’s microbiomes. **Nature Biotechnology**, [S. l.], v. 39, n. 4, p. 499–509, 2021. DOI: 10.1038/s41587-020-0718-6. Disponível em: <https://doi.org/10.1038/s41587-020-0718-6>.

NGUGI, David Kamanda; BLOM, Jochen; STEPANAUSKAS, Ramunas; STINGL, Ulrich. Diversification and niche adaptations of Nitrospina-like bacteria in the polyextreme interfaces of Red Sea brines. **The ISME Journal**, [S. l.], v. 10, n. 6, p. 1383–1399, 2016. DOI: 10.1038/ismej.2015.214. Disponível em: <https://doi.org/10.1038/ismej.2015.214>.

NIHOUL, Jacques C. J. Oceanography of Semi-Enclosed Seas: Medalpex : an international field experiment in the Western Mediterranean. In: NIHOUL, Jacques C. J. B. T. Elsevier Oceanography Series (org.). **Hydrodynamics of Semi-Enclosed Seas**. [s.l.] : Elsevier, 1982. v. 34p. 1–12. DOI: [https://doi.org/10.1016/S0422-9894\(08\)71236-4](https://doi.org/10.1016/S0422-9894(08)71236-4). Disponível em: <https://www.sciencedirect.com/science/article/pii/S0422989408712364>.

NISHIMURA, Yosuke; YOSHIZAWA, Susumu. The OceanDNA MAG catalog contains over 50,000 prokaryotic genomes originated from various marine environments. **Scientific Data**, [S. l.], v. 9, n. 1, p. 305, 2022. DOI: 10.1038/s41597-022-01392-5. Disponível em: <https://doi.org/10.1038/s41597-022-01392-5>.

PACHIADAKI, Maria G. et al. Charting the Complexity of the Marine Microbiome through Single-Cell Genomics. **Cell**, [S. l.], v. 179, n. 7, p. 1623–1635.e11, 2019. DOI: <https://doi.org/10.1016/j.cell.2019.11.017>. Disponível em: <http://www.sciencedirect.com/science/article/pii/S0092867419312735>.

- PALMER, Michael W. Variation in species richness: Towards a unification of hypotheses. **Folia Geobotanica et Phytotaxonomica**, [S. l.], v. 29, n. 4, p. 511, 1994. DOI: 10.1007/BF02883148. Disponível em: <https://doi.org/10.1007/BF02883148>.
- PAOLI, Lucas et al. Biosynthetic potential of the global ocean microbiome. **Nature**, [S. l.], v. 607, n. 7917, p. 111–118, 2022. DOI: 10.1038/s41586-022-04862-3. Disponível em: <https://doi.org/10.1038/s41586-022-04862-3>.
- PARKS, Donovan H.; CHUVOCHINA, Maria; WAITE, David W.; RINKE, Christian; SKARSHEWSKI, Adam; CHAUMEIL, Pierre-Alain; HUGENHOLTZ, Philip. A standardized bacterial taxonomy based on genome phylogeny substantially revises the tree of life. **Nature Biotechnology**, [S. l.], v. 36, n. 10, p. 996–1004, 2018. DOI: 10.1038/nbt.4229. Disponível em: <https://doi.org/10.1038/nbt.4229>.
- PESANT, Stéphane et al. Open science resources for the discovery and analysis of Tara Oceans data. **Scientific Data**, [S. l.], v. 2, n. Lmd, p. 150023, 2015. DOI: 10.1038/sdata.2015.23. Disponível em: <http://www.nature.com/articles/sdata201523>.
- PIANKA, Eric R. Latitudinal Gradients in Species Diversity: A Review of Concepts. **The American Naturalist**, [S. l.], v. 100, n. 910, p. 33–46, 1966. Disponível em: <http://www.jstor.org/stable/2459377>.
- PIGLIUCCI, Massimo. Do we need an extended evolutionary synthesis? **Evolution**, [S. l.], v. 61, n. 12, p. 2743–2749, 2007. DOI: 10.1111/j.1558-5646.2007.00246.x. Disponível em: <https://doi.org/10.1111/j.1558-5646.2007.00246.x>.
- POMEROY, LR; WIEBE, WJ. Temperature and substrates as interactive limiting factors for marine heterotrophic bacteria. **Aquatic Microbial Ecology**, [S. l.], v. 23, n. 2, p. 187–204, 2001. DOI: 10.3354/ame023187. Disponível em: <https://www.int-res.com/abstracts/ame/v23/n2/p187-204/>.
- PRESLEY, Steven J.; HIGGINS, Christopher L.; WILLIG, Michael R. A comprehensive framework for the evaluation of metacommunity structure. **Oikos**, [S. l.], v. 119, n. 6, p. 908–917, 2010. DOI: 10.1111/j.1600-0706.2010.18544.x. Disponível em: <https://doi.org/10.1111/j.1600-0706.2010.18544.x>.
- PRESTON, F. W. The Canonical Distribution of Commonness and Rarity: Part I. **Ecology**, [S. l.], v. 43, n. 2, p. 185–215, 1962. DOI: 10.2307/1931976. Disponível em: <http://www.jstor.org/stable/1931976>.
- PYKE, Graham H. Local Geographic Distributions of Bumblebees Near Crested Butte, Colorado: Competition and Community Structure. **Ecology**, [S. l.], v. 63, n. 2, p. 555–573, 1982. DOI: 10.2307/1938970. Disponível em: <https://doi.org/10.2307/1938970>.
- RAES, Eric J.; BODROSSY, Levente; VAN DE KAMP, Jodie; BISSETT, Andrew; OSTROWSKI, Martin; BROWN, Mark V.; SOW, Swan L. S.; SLOYAN, Bernadette; WAITE, Anya M. Oceanographic boundaries constrain microbial diversity gradients in the South Pacific Ocean. **Proceedings of the National Academy of Sciences**, [S. l.], v. 115, n. 35, p. E8266–E8275, 2018.

DOI: 10.1073/pnas.1719335115. Disponível em:

<http://www.pnas.org/lookup/doi/10.1073/pnas.1719335115>.

RAES, Jeroen; LETUNIC, Ivica; YAMADA, Takuji; JENSEN, Lars Juhl; BORK, Peer. Toward molecular trait-based ecology through integration of biogeochemical, geographical and metagenomic data. **Molecular Systems Biology**, [S. l.], v. 7, n. 1, 2011. Disponível em: <http://msb.embopress.org/content/7/1/473.abstract>.

RAUP, David M.; CRICK, Rex E. Measurement of Faunal Similarity in Paleontology. **Journal of Paleontology**, [S. l.], v. 53, n. 5, p. 1213–1227, 1979. Disponível em: <http://www.jstor.org/stable/1304099>.

REJI, Linta; TOLAR, Bradley B.; SMITH, Jason M.; CHAVEZ, Francisco P.; FRANCIS, Christopher A. Differential co-occurrence relationships shaping ecotype diversification within Thaumarchaeota populations in the coastal ocean water column. **The ISME Journal**, [S. l.], v. 13, n. 5, p. 1144–1158, 2019. DOI: 10.1038/s41396-018-0311-x. Disponível em: <https://doi.org/10.1038/s41396-018-0311-x>.

RICKLEFS, Robert E. Community Diversity: Relative Roles of Local and Regional Processes. **Science**, [S. l.], v. 235, n. 4785, p. 167–171, 1987. Disponível em: <http://science.sciencemag.org/content/235/4785/167.abstract>.

RICKLEFS, Robert E. A comprehensive framework for global patterns in biodiversity. **Ecology Letters**, [S. l.], v. 7, n. 1, p. 1–15, 2004. DOI: 10.1046/j.1461-0248.2003.00554.x.

RICKLEFS, Robert E. Disintegration of the Ecological Community. **The American Naturalist**, [S. l.], v. 172, n. 6, p. 741–750, 2008. DOI: 10.1086/593002. Disponível em: <https://www.journals.uchicago.edu/doi/10.1086/593002>.

ROSE, Julie M.; CARON, David A. Does low temperature constrain the growth rates of heterotrophic protists? Evidence and implications for algal blooms in cold waters. **Limnology and Oceanography**, [S. l.], v. 52, n. 2, p. 886–895, 2007. DOI: <https://doi.org/10.4319/lo.2007.52.2.0886>. Disponível em: <https://doi.org/10.4319/lo.2007.52.2.0886>.

ROUX, Simon; HAWLEY, Alyse K.; TORRES BELTRAN, Monica; SCOFIELD, Melanie; SCHWIENSTEK, Patrick; STEPANAUSKAS, Ramunas; WOYKE, Tanja; HALLAM, Steven J.; SULLIVAN, Matthew B. Ecology and evolution of viruses infecting uncultivated SUP05 bacteria as revealed by single-cell- and meta-genomics. **eLife**, [S. l.], v. 3, p. e03125, 2014. DOI: 10.7554/eLife.03125. Disponível em: <https://doi.org/10.7554/eLife.03125>.

ROYO-LLONCH, M.; SÁNCHEZ, P.; GONZÁLEZ, J. M.; PEDRÓS-ALIÓ, C.; ACINAS, S. G. Ecological and functional capabilities of an uncultured Kordia sp. **Systematic and Applied Microbiology**, [S. l.], v. 43, n. 1, p. 126045, 2020. DOI: <https://doi.org/10.1016/j.syapm.2019.126045>. Disponível em: <http://www.sciencedirect.com/science/article/pii/S0723202019303406>.



ROYO-LLONCH, Marta. **Ecogenomics of Uncultured Marine Prokaryotes**. 2020. Universitat Autònoma de Barcelona, [S. l.], 2020.

ROYO-LLONCH, Marta et al. Compendium of 530 metagenome-assembled bacterial and archaeal genomes from the polar Arctic Ocean. **Nature Microbiology**, [S. l.], v. 6, n. 12, p. 1561–1574, 2021. DOI: 10.1038/s41564-021-00979-9. Disponible em: <https://doi.org/10.1038/s41564-021-00979-9>.

SALAZAR, Guillem et al. Particle-association lifestyle is a phylogenetically conserved trait in bathypelagic prokaryotes. **Molecular Ecology**, [S. l.], v. 24, n. 22, 2015. DOI: 10.1111/mec.13419.

SALAZAR, Guillem et al. Gene Expression Changes and Community Turnover Differentially Shape the Global Ocean Metatranscriptome. **Cell**, [S. l.], v. 179, n. 5, p. 1068–1083.e21, 2019. DOI: <https://doi.org/10.1016/j.cell.2019.10.014>. Disponible em: <http://www.sciencedirect.com/science/article/pii/S009286741931164X>.

SALAZAR, Guillem; CORNEJO-CASTILLO, Francisco M.; BENÍTEZ-BARRIOS, Verónica; FRAILE-NUEZ, Eugenio; ÁLVAREZ-SALGADO, X. Antón; DUARTE, Carlos M.; GASOL, Josep M.; ACINAS, Silvia G. Global diversity and biogeography of deep-sea pelagic prokaryotes. **The ISME Journal**, [S. l.], v. 10, n. 3, 2016. DOI: 10.1038/ismej.2015.137.

SALAZAR, Guillem; SUNAGAWA, Shinichi. Marine microbial diversity. **Current Biology**, [S. l.], v. 27, n. 11, p. R489–R494, 2017. DOI: <https://doi.org/10.1016/j.cub.2017.01.017>. Disponible em: <http://www.sciencedirect.com/science/article/pii/S0960982217300179>.

SALTER, Ian; GALAND, Pierre E.; FAGERVOLD, Sonja K.; LEBARON, Philippe; OBERNOSTERER, Ingrid; OLIVER, Matthew J.; SUZUKI, Marcelino T.; TRICOIRE, Cyrielle. Seasonal dynamics of active SAR11 ecotypes in the oligotrophic Northwest Mediterranean Sea. **The ISME Journal**, [S. l.], v. 9, n. 2, p. 347–360, 2015. DOI: 10.1038/ismej.2014.129. Disponible em: <https://doi.org/10.1038/ismej.2014.129>.

SAMMARTINO, S.; GARCÍA LAFUENTE, J.; NARANJO, C.; SÁNCHEZ GARRIDO, J. C.; SÁNCHEZ LEAL, R.; SÁNCHEZ ROMÁN, A. Ten years of marine current measurements in Espartel Sill, Strait of Gibraltar. **Journal of Geophysical Research: Oceans**, [S. l.], v. 120, n. 9, p. 6309–6328, 2015. DOI: <https://doi.org/10.1002/2014JC010674>. Disponible em: <https://doi.org/10.1002/2014JC010674>.

SARMENTO, Hugo; MONTOYA, José M.; VÁZQUEZ-DOMÍNGUEZ, Evaristo; VAQUÉ, Dolors; GASOL, Josep M. Warming effects on marine microbial food web processes: how far can we go when it comes to predictions? **Philosophical Transactions of the Royal Society B: Biological Sciences**, [S. l.], v. 365, n. 1549, p. 2137–2149, 2010. DOI: 10.1098/rstb.2010.0045. Disponible em: <https://doi.org/10.1098/rstb.2010.0045>.

SCHOENER, Thomas W. The Anolis Lizards of Bimini: Resource Partitioning in a Complex Fauna. **Ecology**, [S. l.], v. 49, n. 4, p. 704–726, 1968. DOI: 10.2307/1935534. Disponible em: <https://doi.org/10.2307/1935534>.

SEBASTIÁN, Marta; ORTEGA-RETUERTA, Eva; GÓMEZ-CONSARNAU, Laura; ZAMANILLO, Marina; ÁLVAREZ, Marta; ARÍSTEGUI, Javier; GASOL, Josep M. Environmental gradients and physical barriers drive the basin-wide spatial structuring of Mediterranean Sea and adjacent eastern Atlantic Ocean prokaryotic communities. **Limnology and Oceanography**, [S. l.], v. 66, n. 12, p. 4077–4095, 2021. DOI: 10.1002/lno.11944. Disponível em: <https://onlinelibrary.wiley.com/doi/10.1002/lno.11944>.

SHERR, Evelyn B.; SHERR, Barry F. Understanding roles of microbes in marine pelagic food webs: a brief history. In: **Microbial Ecology of the Oceans**. [s.l.: s.n.]. p. 27–44.

SHOEMAKER, William R.; LOCEY, Kenneth J.; LENNON, Jay T. A macroecological theory of microbial biodiversity. **Nature Ecology & Evolution**, [S. l.], v. 1, n. 5, p. 0107, 2017. DOI: 10.1038/s41559-017-0107. Disponível em: <http://www.nature.com/articles/s41559-017-0107>.

SIEPIELSKI, Adam M.; HUNG, Keng-Lou; BEIN, Eben E. B.; MCPEEK, Mark A. Experimental evidence for neutral community dynamics governing an insect assemblage. **Ecology**, [S. l.], v. 91, n. 3, p. 847–857, 2010. DOI: 10.1890/09-0609.1. Disponível em: <https://doi.org/10.1890/09-0609.1>.

SILVY, Yona; GUILYARDI, Eric; SALLÉE, Jean-Baptiste; DURACK, Paul J. Human-induced changes to the global ocean water masses and their time of emergence. **Nature Climate Change**, [S. l.], v. 10, n. 11, p. 1030–1036, 2020. DOI: 10.1038/s41558-020-0878-x. Disponível em: <https://doi.org/10.1038/s41558-020-0878-x>.

SOMMERIA-KLEIN, Guilhem; WATTEAUX, Romain; IBARBALZ, Federico M.; KARLUSICH, Juan José Pierella; IUDICONE, Daniele; BOWLER, Chris; MORLON, Hélène. Global drivers of eukaryotic plankton biogeography in the sunlit ocean. **Science**, [S. l.], v. 374, n. 6567, p. 594–599, 2021. DOI: 10.1126/science.abb3717. Disponível em: <http://biorxiv.org/content/early/2020/12/24/2020.09.08.287524.abstract>.

STEGEN, James C.; LIN, Xueju; FREDRICKSON, Jim K.; CHEN, Xingyuan; KENNEDY, David W.; MURRAY, Christopher J.; ROCKHOLD, Mark L.; KONOPKA, Allan. Quantifying community assembly processes and identifying features that impose them. **The ISME Journal**, [S. l.], v. 7, n. 11, p. 2069–2079, 2013. DOI: 10.1038/ismej.2013.93. Disponível em: <http://dx.doi.org/10.1038/ismej.2013.93>.

STEGEN, James C.; LIN, Xueju; FREDRICKSON, Jim K.; KONOPKA, Allan E. Estimating and mapping ecological processes influencing microbial community assembly. **Frontiers in Microbiology**, AVENUE DU TRIBUNAL FEDERAL 34, LAUSANNE, CH-1015, SWITZERLAND, v. 6, n. MAY, p. 1–15, 2015. DOI: 10.3389/fmicb.2015.00370.

SUL, Woo Jun; OLIVER, Thomas A.; DUCKLOW, Hugh W.; AMARAL-ZETTLER, Linda A.; SOGIN, Mitchell L. Marine bacteria exhibit a bipolar distribution. **Proceedings of the National Academy of Sciences**, [S. l.], v. 110, n. 6, p. 2342–2347, 2013. DOI: 10.1073/pnas.1212424110. Disponível em: <http://www.pnas.org/content/110/6/2342.abstract>.

SUN, Ping; WANG, Ying; HUANG, Xin; HUANG, Bangqin; WANG, Lei. Water masses and their associated temperature and cross-domain biotic factors co-shape upwelling microbial communities.

**Water Research**, [S. l.], v. 215, p. 118274, 2022. DOI: <https://doi.org/10.1016/j.watres.2022.118274>. Disponível em: <https://www.sciencedirect.com/science/article/pii/S0043135422002378>.

SUNAGAWA, Shinichi et al. Structure and function of the global ocean microbiome. **Science**, [S. l.], v. 348, n. 6237, p. 1261359, 2015. DOI: 10.1126/science.1261359. Disponível em: <https://www.science.org/doi/10.1126/science.1261359>.

SUNAGAWA, Shinichi et al. Tara Oceans: towards global ocean ecosystems biology. **Nature Reviews Microbiology**, [S. l.], v. 18, n. 8, p. 428–445, 2020. DOI: 10.1038/s41579-020-0364-5. Disponível em: <https://doi.org/10.1038/s41579-020-0364-5>.

SWAN, Brandon K. et al. Prevalent genome streamlining and latitudinal divergence of planktonic bacteria in the surface ocean. **Proceedings of the National Academy of Sciences**, [S. l.], v. 110, n. 28, p. 11463–11468, 2013. DOI: 10.1073/pnas.1304246110. Disponível em: <http://www.pnas.org/content/110/28/11463.abstract>.

THOMPSON, Luke R. et al. Red Sea SAR11 and *Prochlorococcus*; Single-Cell Genomes Reflect Globally Distributed Pangenomes. **Applied and Environmental Microbiology**, [S. l.], v. 85, n. 13, p. e00369-19, 2019. DOI: 10.1128/AEM.00369-19. Disponível em: <http://aem.asm.org/content/85/13/e00369-19.abstract>.

TILMAN, David. Resource Competition between Plankton Algae: An Experimental and Theoretical Approach. **Ecology**, [S. l.], v. 58, n. 2, p. 338–348, 1977. DOI: 10.2307/1935608. Disponível em: <http://www.jstor.org/stable/1935608>.

TREFAULT, Nicole; DE LA IGLESIA, Rodrigo; MORENO-PINO, Mario; LOPES DOS SANTOS, Adriana; GÉRIKAS RIBEIRO, Catherine; PARADA-POZO, Génesis; CRISTI, Antonia; MARIE, Dominique; VAULOT, Daniel. Annual phytoplankton dynamics in coastal waters from Fildes Bay, Western Antarctic Peninsula. **Scientific Reports**, [S. l.], v. 11, n. 1, p. 1368, 2021. DOI: 10.1038/s41598-020-80568-8. Disponível em: <https://doi.org/10.1038/s41598-020-80568-8>.

TULLY, Benjamin J.; GRAHAM, Elaina D.; HEIDELBERG, John F. The reconstruction of 2,631 draft metagenome-assembled genomes from the global oceans. **Scientific Data**, [S. l.], v. 5, p. 170203, 2018. Disponível em: <https://doi.org/10.1038/sdata.2017.203>.

VASS, Máté; SZÉKELY, Anna J.; LINDSTRÖM, Eva S.; LANGENHEDER, Silke. Using null models to compare bacterial and microeukaryotic metacommunity assembly under shifting environmental conditions. **Scientific Reports**, [S. l.], v. 10, n. 1, p. 2455, 2020. DOI: 10.1038/s41598-020-59182-1. Disponível em: <https://doi.org/10.1038/s41598-020-59182-1>.

VELLEND, Mark. Conceptual Synthesis in Community Ecology. **The Quarterly Review of Biology**, [S. l.], v. 85, n. 2, p. 183–206, 2010. DOI: 10.1086/652373. Disponível em: <http://www.journals.uchicago.edu/doi/10.1086/652373>.

VELLEND, Mark. **The Theory of Ecological Communities. Monographs in Population Biology**, 2016. DOI: 10.1016/B978-0-7234-5558-5.00001-4.

VERGIN, Kevin L.; BESZTERI, Bánk; MONIER, Adam; CAMERON THRASH, J.; TEMPERTON, Ben; TREUSCH, Alexander H.; KILPERT, Fabian; WORDEN, Alexandra Z.; GIOVANNONI, Stephen J. High-resolution SAR11 ecotype dynamics at the Bermuda Atlantic Time-series Study site by phylogenetic placement of pyrosequences. **The ISME Journal**, [S. l.], v. 7, n. 7, p. 1322–1332, 2013. DOI: 10.1038/ismej.2013.32. Disponível em: <https://doi.org/10.1038/ismej.2013.32>.

VILLARINO, Ernesto et al. Global beta diversity patterns of microbial communities in the surface and deep ocean. **Global Ecology and Biogeography**, [S. l.], v. 31, n. 11, p. 2323–2336, 2022. DOI: 10.1111/geb.13572. Disponível em: <https://onlinelibrary.wiley.com/doi/10.1111/geb.13572>.

WARD, Ben A.; CAEL, B. B.; COLLINS, Sinead; YOUNG, C. Robert. Selective constraints on global plankton dispersal. **Proceedings of the National Academy of Sciences**, [S. l.], v. 118, n. 10, p. e2007388118, 2021. DOI: 10.1073/pnas.2007388118. Disponível em: <https://doi.org/10.1073/pnas.2007388118>.

ZHOU, Jizhong; NING, Daliang. Stochastic Community Assembly: Does It Matter in Microbial Ecology? **Microbiology and Molecular Biology Reviews**, [S. l.], v. 81, n. 4, p. e00002-17, 2017. DOI: 10.1128/MMBR.00002-17. Disponível em: <http://www.ncbi.nlm.nih.gov/pubmed/29021219>. Acesso em: 20 nov. 2017.

ZIKA, Jan D.; GREGORY, Jonathan M.; MCDONAGH, Elaine L.; MARZOCCHI, Alice; CLÉMENT, Louis. Recent Water Mass Changes Reveal Mechanisms of Ocean Warming. **Journal of Climate**, [S. l.], v. 34, n. 9, p. 3461–3479, 2021. DOI: 10.1175/JCLI-D-20-0355.1. Disponível em: <https://journals.ametsoc.org/view/journals/clim/34/9/JCLI-D-20-0355.1.xml>.

# CAPÍTULO 1

STRUCTURING PROCESSES OF  
ECOLOGICAL COMMUNITIES:  
AN EVALUATION OF MARK VELLEND'S  
THEORETICAL FRAMEWORK

## **STRUCTURING PROCESSES OF ECOLOGICAL COMMUNITIES: AN EVALUATION OF MARK VELLEND'S THEORETICAL FRAMEWORK**

### **ABSTRACT**

The field of community ecology aims to understand the patterns of species distribution across space and time. Over the last five decades, many advances have been achieved in this research field as multiple patterns of distribution were described and evidence was gathered to help formulate theories that describe the main mechanisms underpinning these patterns. However, unlike other areas such as population genetics, community ecology lacked a synthetic theory that would reconcile all scientific advances historically acquired. In this context, the Theory of Ecological Communities has been formulated by the ecologist Mark Vellend as a unified conceptual framework. Here, we present the four high-level ecological processes (Selection, Drift, Dispersion, and Diversification) structuring ecological communities that are described in this theory together with a brief contextualization of historical ideas in community ecology. We also demonstrated empirically the scientific impact of this conceptual framework on the field of community ecology through a systematic literature review. Finally, we show a few case studies that effectively estimated the relative importance of some of these processes structuring ecological communities and discussed the limitation and challenges of applying this framework to observational data.

**Keywords:** Community ecology, Theoretical ecology, Ecological processes, Scientometrics



## INTRODUCTION

Global patterns of biodiversity geographic distribution have been the subject of scientific curiosity and investigation since the early 19th century when naturalists such as Alexander von Humboldt (1769-1859) and Charles Darwin (1809-1882) made their first global scientific expeditions. Since then, many macroecological patterns have been revealed, such as the abundance-distribution relationship (BROWN, 1984), the species-area relationship (PRESTON, 1962), the distance-decay of similarity (MORLON et al., 2008), the species-time-area relationship (ADLER et al., 2005), and latitudinal patterns of diversity (MACARTHUR, 1965; PIANKA, 1966), among others. In this scenario, the field of community ecology emerges with the main aim of understanding the patterns of diversity, composition, and distribution of species across space and time (VELLEND, 2010).

In the last five decades, our knowledge in this research field has greatly advanced with the accumulation of evidence that helped formulate several ecological hypotheses and theories (PALMER, 1994), which describe the main mechanisms behind the observed biodiversity distribution patterns. However, unlike population genetics (PIGLIUCCI, 2007), community ecology lacked a theoretical synthesis to reconcile all the historically accumulated scientific advances. In this context, the Theory of Ecological Communities (VELLEND, 2010, 2016) was proposed by Mark Vellend to adapt the conceptual framework of population genetics to summarize and unify the main theories in community ecology (Table 1). This theory suggests that all the mechanisms responsible for community assembly described in the literature can be attributed to four main high-level ecological processes: selection, ecological drift, dispersal, and diversification (VELLEND, 2010, 2016).

## MARK VELLEND'S THEORETICAL IMPORTANCE

Mark Vellend's Theory of Ecological Communities was first introduced to the scientific community in his article "Conceptual Synthesis in Community Ecology", published in the landmark scientific journal "The Quarterly Review of Biology" (VELLEND, 2010). His synthesis was further developed and detailed explored with case studies in his book "The Theory of Ecological Communities" (VELLEND, 2016). According to this theory, ecological communities are structured by the interaction of four fundamental classes of ecological processes, from which two are responsible for species gain (diversification and dispersal) and two for species loss in a given community (selection and ecological drift) (Fig. 1).

*Selection* is defined as the set of ecological forces that alter community structure due to differences in the fitness of individuals of particular species (VELLEND, 2010). The *selection*

process necessarily results from deterministic factors such as abiotic conditions (e.g., temperature, pH, and salinity) and biotic interactions (e.g., competition, facilitation, mutualism, and predation). This mechanism of species selection by deterministic factors is not new in ecology. Indeed, it has been the most widely studied and tested ecological process in the literature since the late 1950s when Evelyn Hutchinson (1957) formulated the ecological niche theory (Table 1). Later, other ecologists also focused on studying, for example, the importance of different spatial and temporal scales on the effect of environmental filters (*selection* process) (BROWN, 1997; RICKLEFS, 1987). Many observational and experimental studies have also shown that the influence of selection on community assembly is dependent on the density of species populations when the determining factors are ecological interactions, such as competition (ADLER et al., 2006; DESCAMPS-JULIEN; GONZALEZ, 2005; TILMAN, 1977) or predation (FUSSMANN et al., 2000; HUFFAKER; SHEA; HERMAN, 1963; KREBS et al., 2001).

*Ecological drift* is defined as random changes in the relative abundance of species (VELLEND, 2010, 2016). Although ecologists have cited more generally the relevance of stochastic events in structuring communities for many decades (VELLEND, 2010), the real importance of this process was first incorporated into community ecology by Hubbel's neutral theory (2001). *Ecological drift* is a stochastic process that can cause the extinction of species with small populations in the community (Fig. 1), which has much relevance for biodiversity conservation. Measuring the effect of *ecological drift* on communities is challenging because it can often confound with deterministic factors not measured in the study (VELLEND, 2010). However, some experimental studies of competition prove that in interactions between two ecologically equivalent species, random effects may alternately favor one of the species involved, even under equal and controlled conditions (MERTZ; CAWTHON; PARK, 1976; SIEPIELSKI et al., 2010).

*Diversification* is an evolutionary process generating new genetic variants due to the balance between speciation and extinction (VELLEND, 2016). This process is particularly important in determining community structure when considering broad regions such as continents or isolated areas such as islands (MACARTHUR, 1969; RICKLEFS, 2008). However, *diversification* can also act at relatively smaller spatial scales and likely contributes, alongside *selection*, to described relationships between diversity and environmental gradients (RICKLEFS, 2004). Furthermore, ecological communities can diversify (taxonomic, phylogenetic, functional, and genetic diversification) over different temporal scales. In general, microorganisms diversify in a much shorter time scale than macroorganisms due to their high reproduction rates, gene mutation, and horizontal transfer of genetic material (ZHOU; NING, 2017). Therefore, this process is likely more relevant and measurable in microbial communities. However, the influence of diversification tends



to be relatively lower in environments with high dispersal rates (STEGEN et al., 2015), as is the case in oceans.

*Dispersal* is the process by which organisms move and establish across space (VELLEND, 2010, 2016). This process has been addressed by different ecological theories (Table 1) over the past 50 years, such as island biogeography theory (MACARTHUR; WILSON, 1967) and neutral theory (HUBBELL, 2001), but especially by the metacommunity theory (HOLYOAK; LEIBOLD; HOLT, 2005; LEIBOLD et al., 2004), which emphasized the role of *dispersal* influencing community patterns at multiple spatial scales. This process interacts with others, such as *selection* or *drift*, to influence local and regional community patterns (VELLEND, 2010). *Dispersal* can be considered both a deterministic and stochastic process, according to the biology of the organisms and the characteristics of the ecosystem in question. For example, while the dispersal of animals or plants is deterministic because it involves behavioral decisions and ecological interactions, the dispersal of planktonic microorganisms is generally considered stochastic due to its passive nature (ZHOU; NING, 2017).

After almost ten years of its publication, it is undeniable that the ecological synthesis proposed by Vellend had a great impact on the scientific community and, to the best of our knowledge, it is likely one of the best attempts to "tidy up the mess" of theories in community ecology. However, we have yet to understand how this synthesis has been approached by the various studies in which it has been used. Thus, this review first aims to introduce the four main ecological processes determining the structuring of ecological communities according to Mark Vellend's theoretical framework (VELLEND, 2010, 2016). Second, to demonstrate this theory's impact on the field of ecology, we also used a scientometric approach to assess which research areas were most impacted and evaluate if the citing literature is using this theory in a theoretical or application context. In addition, we could assess which types of environments and biological groups were studied in the papers citing this theory. Finally, we bring some examples of studies that applied this theoretical framework to real ecological community data.

## **METHODS**

We conducted a systematic literature search and content review of all scientific articles that cited the paper "Conceptual Synthesis in Community Ecology" (VELLEND, 2010) from its publication in June 2010 to December 2018. The literature search was conducted using the "Web of Science" system (<https://webofknowledge.com>). We used this database because it is internationally recognized and extensively used in scientometric studies (CAÑAS-GUERRERO et al., 2013; MARTINEZ et al., 2019; NETTLE; FRANKENHUIS, 2019).

The literature search resulted in a total of 500 articles. These were then individually assessed to extract information regarding the type of environment inhabited by the study's target organisms (Terrestrial, Marine, Freshwater, Host). Furthermore, we separated these studies by climate (Polar, Temperate, Subtropical, and Tropical), biological group (Plants, Vertebrates, Invertebrates, Microorganisms, Zooplankton, Phytoplankton, and General), and research type (Observational, Experimental, Theoretical, Meta-analysis, Opinion, and Review). We also selected the 150 most cited articles to assess which measured Mark Vellend's processes and which cited his work only in a generic way throughout the text. The scientometric analyses and graphs were generated with the package 'ggplot2' (WICKHAM, 2016) in the statistical program R (version 3.5.2, <[www.r-project.org](http://www.r-project.org)>).

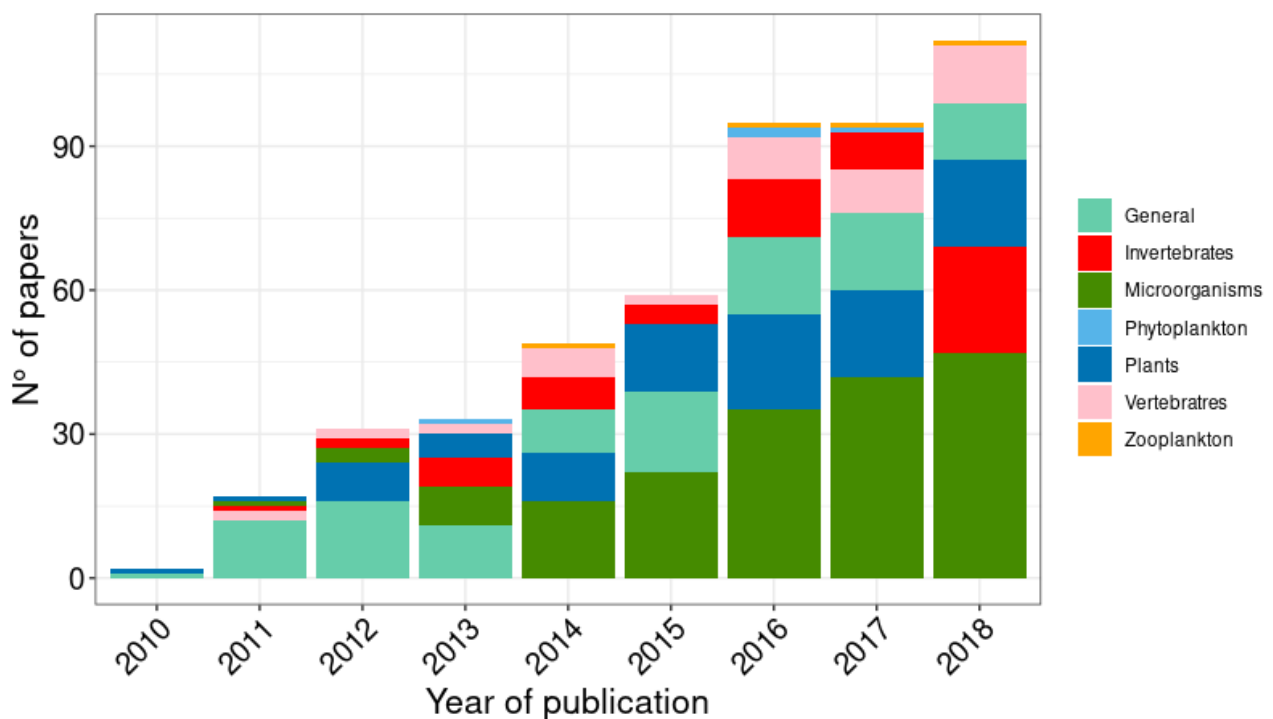
## RESULTS AND DISCUSSION

### SCIENTOMETRIC ANALYSIS

Our scientometric analysis showed that Vellend's theory has indeed been recognized by the scientific community, as evidenced by the increasing number of citations of his work (Fig. I-1) in high-impact journals in Ecology, Environmental Sciences, and other related scientific areas (Fig. I-2). Our survey pointed out that 35% of these papers were conducted with a focus on microbial communities, followed by plants (19%), invertebrates (12%), and vertebrates (9%) (Fig. I-1). There was a trend of a growing number of papers focused on microorganisms, especially from the year 2013 onwards (Fig. I-1), which is also evident by a large number of citations of this work in important journals in the field of microbial ecology, such as ISME Journal, Environmental Microbiology, FEMS Microbiology Ecology, Molecular Ecology and Frontiers in Microbiology (Fig. I-2). It was also observed that among the 500 articles analyzed, the largest number of articles focused on terrestrial and freshwater ecosystems (e.g., lakes, rivers, streams) in temperate climates (Fig. I-3). Most of these articles were categorized as observational (~34%), experimental (~29%), and theoretical (~21) (Fig. I-3).

Finally, we found that the vast majority of articles only cited Vellend's work throughout the text, mainly in the introduction (~80%) or discussion (~15%) of related concepts. Among these articles, we checked for applied approaches (n=150), and only 22 (14.6%) of them sought to test at least some of the processes described by Vellend. To achieve this goal, these papers used different methods, but the methodological approach proposed by STEGEN et al., 2013, which was applied in 7 out of 22 papers to estimate the relative importance of *selection*, *dispersal limitation*, and ecological *drift* in distinct microbial communities (see discussion in the next section). Another

paper (EVANS; MARTINY; ALLISON, 2017) proposed the use of the DEMENT (The individual-based Decomposition Model of Enzymatic Traits) model (ALLISON, 2012) to determine the influence of *dispersal* on other processes, such as *selection* and ecological *drift*. Besides, other approaches used considered null models (BERNARD-VERDIER et al., 2012; LOUDON et al., 2016; MORRISON-WHITTLE; GODDARD, 2015; PIGOT; ETIENNE, 2015), variance partitioning (SCHWALB et al., 2013) and beta-diversity partitioning to determine the importance of deterministic vs. stochastic processes (ANDERSON et al., 2011; KIMBALL et al., 2016; PUETTKER et al., 2015; SPASOJEVIC; COPELAND; SUDING, 2014). Finally, one paper proposes an experimental approach to determine the relative importance of *dispersal*, niche *selection*, and neutrals (ecological *drift*) (WEIHER et al., 2011).

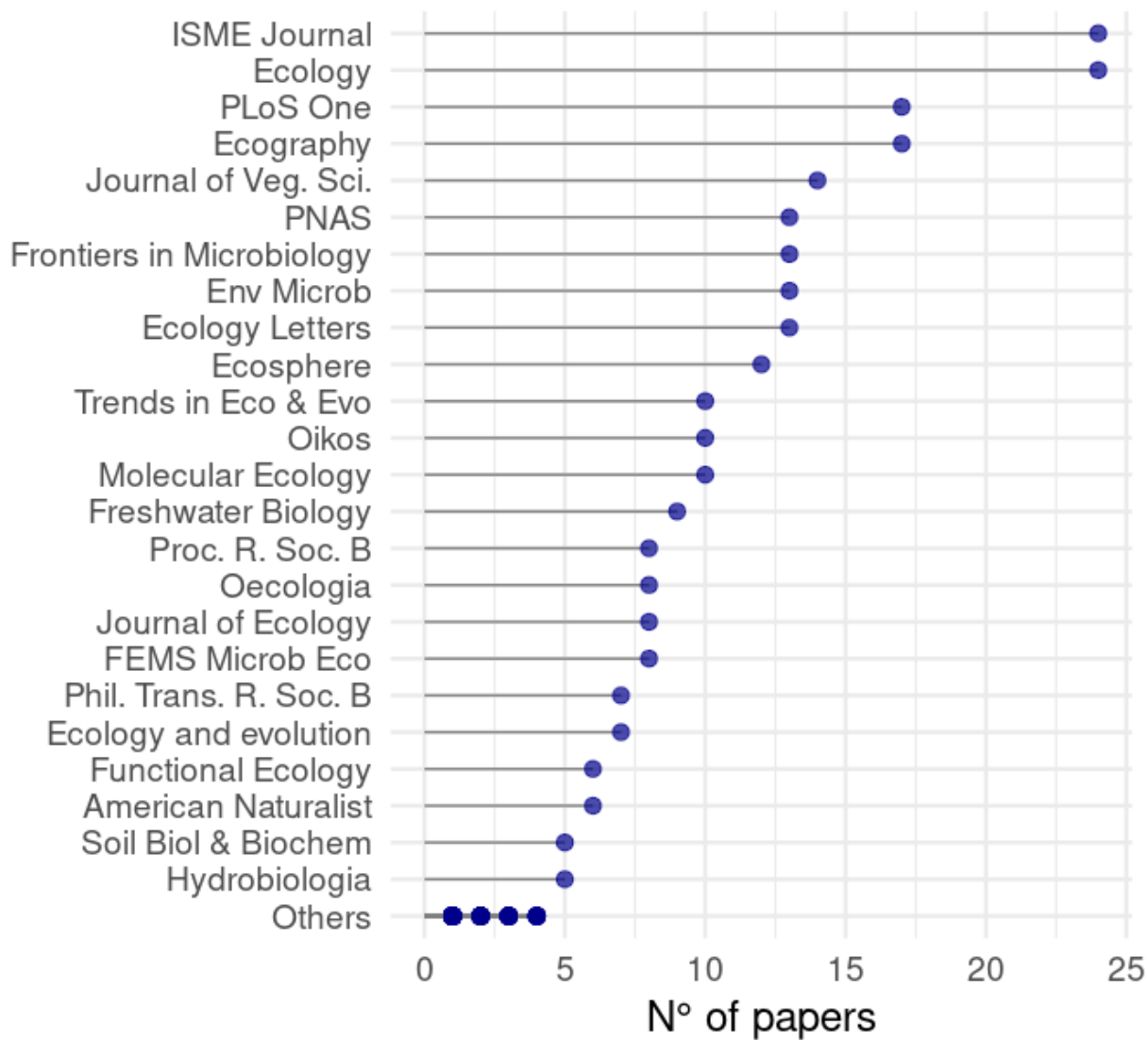


**Figure I-1.** Number of citations of (VELLEND, 2010) published per year categorized according to the target biological groups. These numbers were obtained through a systematic literature search conducted via "ISI Web of Science", as described in the Methods section.

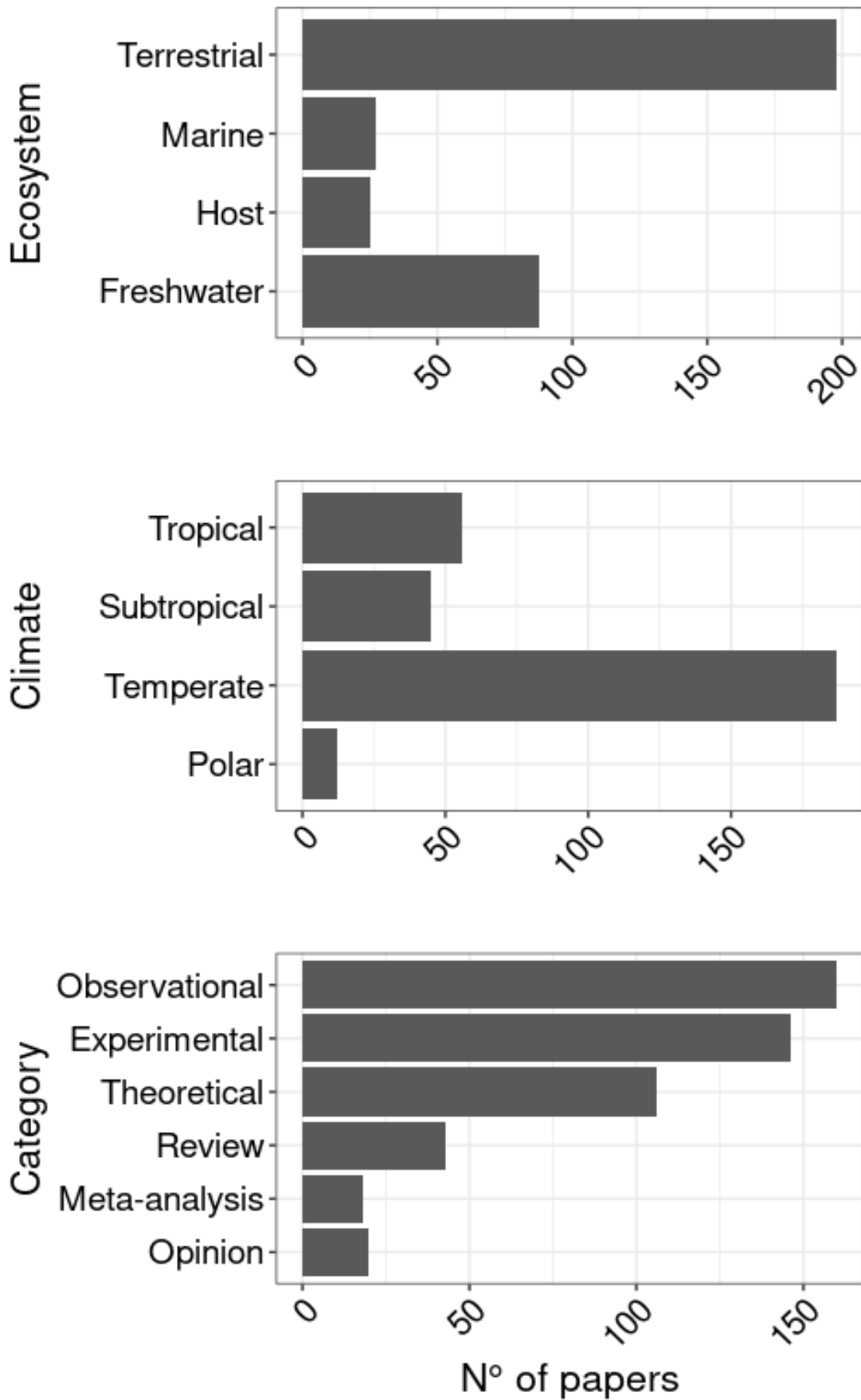
## APPLICATIONS AND LIMITATIONS

One of the great advantages of Vellend's theoretical framework is the fact that it allows the comparison of the relative importance of the processes structuring ecological communities between different organisms and ecosystems (VELLEND, 2016). However, we found that few papers simultaneously estimated at least three of these processes so that their relative importance could be

determined. Moreover, we found that a vast majority of these studies that estimated these processes were applied to microbial communities (Fig. I-1 and Fig. I-2).



**Figure I-2.** Number of articles citing (VELLEND, 2010) per scientific journal.



**Figure I-3.** Number of citations of (VELLEND, 2010) according to ecosystem, climate, and study type categories.

The reason for the greater number of papers on microorganisms and the increased permeability of Vellend’s theory in microbial ecology journals is probably associated with the publication of STEGEN et al., 2013. As previously mentioned, based on VELLEND, 2010, this work presents a methodological approach combining different ecological models to estimate the main ecological processes structuring microbial communities. In this work, bacterial communities inhabiting aquatic sediments were found to be structured primarily by selection (~42%), followed by dispersal (~35%) and ecological drift (~22%). The relative importance of these processes was also found to vary across sediment layers with different grain sizes. In shallower layers with coarser grains, dispersion showed a higher contribution (40%) compared to selection (33%). On the other hand, in deeper layers with finer and more compact grains, selection becomes the most important process (57%) compared to dispersion (15%) (STEGEN et al., 2013). Several papers were subsequently published using this method applied to microbial communities from different ecosystems, including river systems (GRAHAM et al., 2016), lakes (LOGARES et al., 2018b), soils (DINI-ANDREOTE et al., 2015), oceans (LOGARES et al., 2020) and even the gut of humans, fish and shrimp (MARTINEZ et al., 2015; XIONG et al., 2017; YAN et al., 2016) (Table I-1).

**Table I–1.** Relative contribution of selection, dispersal, and ecological drift (VELLEND, 2010) shaping different microbial communities extracted from papers that used the method of STEGEN et al., 2013.

<b>Ecosystem</b>	<b>Selection</b>	<b>Dispersion*</b>	<b>Drift</b>	<b>Reference</b>
<b>Aquatic sediments</b>	~42%	~35%	~22%	
Surface layer – thick grains	33%	40%	27%	(STEGEN et al., 2013)
Deep layer – thin grains	57%	15%	29%	
<b>Human gut</b>				
Papua New Guinea	13%	58%	28%	
United States	21%	56%	21%	(MARTINEZ et al., 2015)
Tanzania	4%	93%	3%	
Italy	11%	56%	32%	
<b>Fish gut</b>				(YAN et al., 2016)

( <i>C. idellus</i> ) Larvae	34%	63%	3%	
( <i>C. idellus</i> ) Adult	30%	2%	68%	
( <i>S. chuatsi</i> ) Larvae	38%	43%	19%	
( <i>S. chuatsi</i> ) Adult	27%	7%	66%	
( <i>S. meridionalis</i> ) Larvae	60%	15%	25%	
( <i>S. meridionalis</i> ) Adult	33%	0%	67%	
<b>Shrimp gut</b>				
Sick	~55%	~7%	~38%	(XIONG et al., 2017)
Healthy	~62%	~5%	~33%	
<b>Antarctic lakes</b>				
Prokaryotes	~72%	~23%	~2%	(LOGARES et al., 2018b)
Microeukaryotes	~5%	~22%	~72%	
<b>Global Ocean</b>				
Prokaryotes	34%	35%	31%	(LOGARES et al., 2018a)
Microeukaryotes	17%	76%	6%	

\* Dispersal Limitation + Homogenizing dispersal

Altogether, these papers exemplify how this method captures differences in the relative contribution of the main structuring processes in different ecosystems, communities, and populations (Table I-1). It is clear, for example, the high relative importance of *selection* shaping bacterial communities in diverse environments. Two studies have applied Stegen's model to determine the relative importance of these ecological processes in planktonic microbial communities, comparing prokaryotes and eukaryotes. These papers demonstrate differences in the relative importance of these processes between prokaryotic and eukaryotic communities in marine and lacustrine ecosystems (LOGARES et al., 2018a, 2018b). In the first study, conducted in Antarctic lakes, it was observed that the bacterial community is structured primarily by *selection*

(72.1% of turnover), while microeukaryote communities were structured predominantly by ecological *drift* (72.1% of turnover) (LOGARES et al., 2018b). In the oceans, *selection*, *dispersal*, and *drift* processes were found to play a balanced role (~33%) in structuring the prokaryotic community, while dispersal limitation (~76%) was the dominant process structuring the microeukaryotic community (LOGARES et al., 2018a). These studies suggest that the relative importance of the ecological processes structuring natural communities may depend on the taxonomic group analyzed. STEGEN et al., 2013 demonstrated the importance of developing a method capable of generating comparative analyses.

Applying the approach proposed by STEGEN et al., 2013 requires a phylogenetic distance matrix between species from a given community to confirm the existence of a phylogenetic signal in the community structure. For microorganisms, this task becomes relatively more straightforward since microbial diversity databases are built exclusively with genetic information by molecular methods, based mainly on the metabarcoding of the 16S-rRNA (for prokaryotes), 18S-rRNA (for microeukaryotes) and ITS (fungi and protists) regions of the DNA (CREER et al., 2016). To determine the phylogenetic diversity of macroorganisms, it would be necessary to generate a database with sequences based on reference (barcoding) genes/regions such as COx1, 12S, 16S, and 18S for fauna and ITS, matK + RbcL for plants (CREER et al., 2016). However, sampling and sequencing the DNA of individuals from a macro-organism community is a task that requires more work and financial resources compared to microbial communities. Moreover, it is practically impossible to collect all macro-organism from a community as it is feasible for microbial communities. Another possible approach to circumvent this problem is the use of, for example, environmental DNA (eDNA) sequencing, which is DNA extracted directly from environmental samples (i.e., air, feces, sediment, soil, and water) without the need to collect or isolate individuals (CREER et al., 2016). Such databases are publicly available for some groups, but still in a minimal way compared to those for microorganisms. With the expansion of eDNA databases, it would be possible to conduct more studies to determine the relative importance of ecological processes structuring different ecological communities.

A more straightforward alternative would be adapting this approach to be used on morphological diversity, especially in communities of higher organisms with well-resolved taxonomy. Large databases of species occurrence and their abundances and attributes are increasingly common for vertebrate, invertebrate, and plant communities (e.g., ATLANTIC: Data Papers from a biodiversity hotspot). The combination of this type of data with publicly available phylogenetic data (e.g., <http://vertlife.org/>) has contributed to a better understanding of community structuring and, together with the methodological approach proposed by STEGEN et al., 2013,



could be used to estimate the relative importance of ecological processes (VELLEND, 2010) in macroorganism communities. However, according to our literature survey, no studies applied a similar approach, although there are works that determined the relative importance of selection relative to stochastic processes (VELLEND et al., 2014) or dispersal (VAN DER PLAS et al., 2015). It is also evident that there is a need to expand knowledge about environments other than temperate ones, as different contexts may influence species composition at different latitudes (MARTINEZ et al., 2015; MYERS et al., 2013).

Overall, studies applied to macro-organisms are based on metacommunity theory to test the effect of dispersal on the community structure (HOLYOAK; LEIBOLD; HOLT, 2005; LEIBOLD et al., 2004). In this context, some studies use functional approaches to determine how the mechanisms of metacommunity dynamics operate in structuring the functional diversity of plant and animal communities using their traces (PAVOINE et al., 2014; SPASOJEVIC; COPELAND; SUDING, 2014). The effect of environmental filter (*selection*) on the convergence of morphological and functional traits in communities is quantified by comparing the observed pattern with null models (BERNARD-VERDIER et al., 2012). A new approach called the "dynamic null model," which has some advantages, such as more transparent assumptions compared to traditional randomization techniques, has also been presented and well-cited in the literature in recent years (PIGOT; ETIENNE, 2015). Variance partitioning (SCHWALB et al., 2013) and beta-diversity partitioning methodologies have also been commonly used to determine the importance of environmental and spatial factors, as well as deterministic vs. stochastic processes (ANDERSON et al., 2011; PUETTKER et al., 2015) on community structuring. Finally, other approaches include phylogenetic gradient analyses that merge metacommunity concepts with community phylogeny (PERES-NETO; LEIBOLD; DRAY, 2012), hierarchical species composition models (HMSC) (OVASKAINEN et al., 2017), and an experimental approach to determine the relative importance of multiple processes (i.e., dispersal, niche, and neutral) on communities (WEIHER et al., 2011).

Despite significant contributions, these previous works still need to estimate the relative contribution of three ecological processes, such as Stegen's approach. This fact undermines the systematic comparison of ecological processes across distinct ecosystems and organisms, which is the main advantage of Vellend's conceptual framework. Adopting a standard methodology capable of estimating such processes for both micro- and macro-organisms would allow unrevealing patterns of the ecological processes structuring communities across ecosystems.

## CONCLUSION

Vellend's theoretical framework has great historical value since it presents for the first time an integrated view of the critical concepts developed in Community Ecology over the last half century, overcoming the endless debate of neutral vs. niche theory. The scientific community has indeed recognized the contribution of this theoretical framework. However, most articles have cited this work only to introduce or discuss the subject more generally but have yet to measure the relative importance of each of these ecological processes. Furthermore, there is still a bias for studies with microbial communities, most likely thanks to the work of STEGEN et al., 2013 that presented a mathematical model that measures the processes of selection, dispersal, and drift as structuring these communities. More studies are needed to quantify the relative importance of these fundamental processes in structuring ecological communities of different biological groups and ecosystems. This step forward requires 1) integrating publicly available morphological to phylogenetic databases of animal and plant communities, 2) expanding environmental DNA databases, and 3) adapting the only method available so far to estimate the relative importance of these processes (STEGEN et al., 2013) or developing alternative methods that would achieve this goal.

## REFERENCES

- ADLER, Peter B.; HILLERISLAMBERS, Janneke; KYRIAKIDIS, Phaedon C.; GUAN, Qingfeng; LEVINE, Jonathan M. Climate variability has a stabilizing effect on the coexistence of prairie grasses. **Proceedings of the National Academy of Sciences**, [S. l.], v. 103, n. 34, p. 12793 LP-12798, 2006. Disponível em: <http://www.pnas.org/content/103/34/12793.abstract>.
- ADLER, Peter B.; WHITE, Ethan P.; LAUENROTH, William K.; KAUFMAN, Dawn M.; RASSWEILER, Andrew; RUSAK, James a. Evidence for a general species time area relationship. **Ecology**, [S. l.], v. 86, n. 8, p. 2032–2039, 2005. DOI: 10.1890/05-0067. Disponível em: [Journals%5CEcology%5CAdler2005ecology.pdf](#).
- ALLISON, S. D. A trait-based approach for modelling microbial litter decomposition. **Ecology Letters**, [S. l.], v. 15, n. 9, p. 1058–1070, 2012. DOI: 10.1111/j.1461-0248.2012.01807.x. Disponível em: <https://doi.org/10.1111/j.1461-0248.2012.01807.x>.
- ANDERSON, Marti J. et al. Navigating the multiple meanings of beta diversity: a roadmap for the practicing ecologist. **ECOLOGY LETTERS**, 111 RIVER ST, HOBOKEN 07030-5774, NJ USA, v. 14, n. 1, p. 19–28, 2011. DOI: 10.1111/j.1461-0248.2010.01552.x.
- BERNARD-VERDIER, Maud; NAVAS, Marie-Laure; VELLEND, Mark; VIOLLE, Cyrille; FAYOLLE, Adeline; GARNIER, Eric. Community assembly along a soil depth gradient: contrasting patterns of plant trait convergence and divergence in a Mediterranean rangeland.

**JOURNAL OF ECOLOGY**, 111 RIVER ST, HOBOKEN 07030-5774, NJ USA, v. 100, n. 6, p. 1422–1433, 2012. DOI: 10.1111/1365-2745.12003.

BROWN, James H. On the Relationship between Abundance and Distribution of Species. **The American Naturalist**, [S. l.], v. 124, n. 2, p. 255–279, 1984. DOI: 10.1086/284267. Disponível em: <https://doi.org/10.1086/284267>.

BROWN, James H. **Macroecology**. Chicago (IL): The University of Chicago Press, 1997. v. 78 DOI: /10.2307/1382661. Disponível em: <http://dx.doi.org/10.2307/1382661>.

CAÑAS-GUERRERO, Ignacio; MAZARRÓN, Fernando R.; POU-MERINA, Ana; CALLEJA-PERUCHO, Cruz; DÍAZ-RUBIO, Gonzalo. Bibliometric analysis of research activity in the “Agronomy” category from the Web of Science, 1997–2011. **European Journal of Agronomy**, [S. l.], v. 50, p. 19–28, 2013. DOI: <https://doi.org/10.1016/j.eja.2013.05.002>. Disponível em: <https://www.sciencedirect.com/science/article/pii/S116103011300066X>.

CREER, Simon; DEINER, Kristy; FREY, Serita; PORAZINSKA, Dorota; TABERLET, Pierre; THOMAS, W. Kelley; POTTER, Caitlin; BIK, Holly M. The ecologist’s field guide to sequence-based identification of biodiversity. **METHODS IN ECOLOGY AND EVOLUTION**, 111 RIVER ST, HOBOKEN 07030-5774, NJ USA, v. 7, n. 9, p. 1008–1018, 2016. DOI: 10.1111/2041-210X.12574.

DESCAMPS-JULIEN, Blandine; GONZALEZ, Andrew. Stable Coexistence in a Fluctuating Environment: An Experimental Demonstration. **Ecology**, [S. l.], v. 86, n. 10, p. 2815–2824, 2005. Disponível em: <http://www.jstor.org/stable/3450707>.

DINI-ANDREOTE, Francisco; STEGEN, James C.; VAN ELSAS, Jan Dirk; SALLES, Joana Falcao. Disentangling mechanisms that mediate the balance between stochastic and deterministic processes in microbial succession. **PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA**, 2101 CONSTITUTION AVE NW, WASHINGTON, DC 20418 USA, v. 112, n. 11, p. E1326–E1332, 2015. DOI: 10.1073/pnas.1414261112.

EVANS, Sarah; MARTINY, Jennifer B. H.; ALLISON, Steven D. Effects of dispersal and selection on stochastic assembly in microbial communities. **ISME JOURNAL**, MACMILLAN BUILDING, 4 CRINAN ST, LONDON N1 9XW, ENGLAND, v. 11, n. 1, p. 176–185, 2017. DOI: 10.1038/ismej.2016.96.

FUSSMANN, Gregor F.; ELLNER, Stephen P.; SHERTZER, Kyle W.; HAIRSTON JR., Nelson G. Crossing the Hopf Bifurcation in a Live Predator-Prey System. **Science**, [S. l.], v. 290, n. 5495, p. 1358 LP-1360, 2000. Disponível em: <http://science.sciencemag.org/content/290/5495/1358.abstract>.

GRAHAM, Emily B.; CRUMP, Alex R.; RESCH, Charles T.; FANSLER, Sarah; ARNTZEN, Evan; KENNEDY, David W.; FREDRICKSON, Jim K.; STEGEN, James C. Coupling Spatiotemporal Community Assembly Processes to Changes in Microbial Metabolism. **FRONTIERS IN MICROBIOLOGY**, AVENUE DU TRIBUNAL FEDERAL 34, LAUSANNE, CH-1015, SWITZERLAND, v. 7, 2016. DOI: 10.3389/fmicb.2016.01949.

- HOLYOAK, Marcel; LEIBOLD, Mathew A.; HOLT, Robert D. **Metacommunities: spatial dynamics and ecological communities**. Chicago (IL): University of Chicago Press, 2005.
- HUBBELL, Stephen P. **The unified neutral theory of biodiversity and biogeography**. Princeton (NJ): Princeton University Press, 2001.
- HUFFAKER, C. B.; SHEA, K. P.; HERMAN, S. G. Experimental studies on predation: Complex dispersion and levels of food in an acarine predator-prey interaction. **Hilgardia**, [S. l.], v. 34, n. 9, p. 305–330, 1963. DOI: 10.3733/hilg.v34n09p305. Disponível em: <http://hilgardia.ucanr.edu/Abstract/?a=hilg.v34n09p305>.
- KIMBALL, Sarah; FUNK, Jennifer L.; SPASOJEVIC, Marko J.; SUDING, Katharine N.; PARKER, Scot; GOULDEN, Michael L. Can functional traits predict plant community response to global change? **ECOSPHERE**, 111 RIVER ST, HOBOKEN 07030-5774, NJ USA, v. 7, n. 12, 2016. DOI: 10.1002/ecs2.1602.
- KREBS, Charles J.; BOONSTRA, Rudy; BOUTIN, Stan; SINCLAIR, A. R. E. What Drives the 10-year Cycle of Snowshoe Hares? The ten-year cycle of snowshoe hares—one of the most striking features of the boreal forest—is a product of the interaction between predation and food supplies, as large-scale experiments in the Yukon have . **BioScience**, [S. l.], v. 51, n. 1, p. 25–35, 2001. Disponível em: [http://dx.doi.org/10.1641/0006-3568\(2001\)051\[0025:WDTYCO\]2.0.CO](http://dx.doi.org/10.1641/0006-3568(2001)051[0025:WDTYCO]2.0.CO).
- LEIBOLD, M. A. et al. The metacommunity concept: a framework for multi-scale community ecology. **Ecology Letters**, [S. l.], v. 7, n. 7, p. 601–613, 2004. DOI: 10.1111/j.1461-0248.2004.00608.x. Disponível em: <https://doi.org/10.1111/j.1461-0248.2004.00608.x>.
- LOGARES, Ramiro et al. Different processes shape prokaryotic and picoeukaryotic assemblages in the sunlit ocean microbiome. **bioRxiv**, [S. l.], n. Icm, p. 37–49, 2018. a. Disponível em: <http://biorxiv.org/content/early/2018/07/23/374298.abstract>.
- LOGARES, Ramiro et al. Disentangling the mechanisms shaping the surface ocean microbiota. **Microbiome**, [S. l.], v. 8, n. 1, p. 55, 2020. DOI: 10.1186/s40168-020-00827-8. Disponível em: <https://microbiomejournal.biomedcentral.com/articles/10.1186/s40168-020-00827-8>.
- LOGARES, Ramiro; TESSON, Sylvie V. M.; CANBÄCK, Björn; PONTARP, Mikael; HEDLUND, Katarina; RENGEFORS, Karin. Contrasting prevalence of selection and drift in the community structuring of bacteria and microbial eukaryotes. **Environmental Microbiology**, [S. l.], v. 20, n. 6, p. 2231–2240, 2018. b. DOI: 10.1111/1462-2920.14265. Disponível em: <https://onlinelibrary.wiley.com/doi/10.1111/1462-2920.14265>.
- LOUDON, Andrew H.; VENKATARAMAN, Arvind; VAN TREUREN, William; WOODHAMS, Douglas C.; PARFREY, Laura Wegener; MCKENZIE, Valerie J.; KNIGHT, Rob; SCHMIDT, Thomas M.; HARRIS, Reid N. Vertebrate Hosts as Islands: Dynamics of Selection, Immigration, Loss, Persistence, and Potential Function of Bacteria on Salamander Skin. **FRONTIERS IN MICROBIOLOGY**, AVENUE DU TRIBUNAL FEDERAL 34, LAUSANNE, CH-1015, SWITZERLAND, v. 7, 2016. DOI: 10.3389/fmicb.2016.00333.

MACARTHUR, Robert H. Patterns of Species Diversity. **Biological Reviews**, [S. l.], v. 40, n. 4, p. 510–533, 1965. DOI: 10.1111/j.1469-185X.1965.tb00815.x. Disponível em: <http://doi.wiley.com/10.1111/j.1469-185X.1965.tb00815.x>.

MACARTHUR, Robert H. Patterns of communities in the tropics. **Biological Journal of the Linnean Society**, [S. l.], v. 1, n. 1–2, p. 19–30, 1969. DOI: 10.1111/j.1095-8312.1969.tb01809.x. Disponível em: <https://doi.org/10.1111/j.1095-8312.1969.tb01809.x>.

MACARTHUR, Robert H.; WILSON, Edward O. **The Theory of Island Biogeography**. [s.l.] : Princeton University Press, 1967.

MARTINEZ, Ines; STEGEN, James C.; MALDONADO-GOMEZ, Maria X.; EREN, A. Murat; SIBA, Peter M.; GREENHILL, Andrew R.; WALTER, Jens. The Gut Microbiota of Rural Papua New Guineans: Composition, Diversity Patterns, and Ecological Processes. **CELL REPORTS**, 50 HAMPSHIRE ST, FLOOR 5, CAMBRIDGE, MA 02139 USA, v. 11, n. 4, p. 527–538, 2015. DOI: 10.1016/j.celrep.2015.03.049.

MARTINEZ, Sara; DELGADO, Maria del Mar; MARTINEZ MARIN, Ruben; ALVAREZ, Sergio. Science mapping on the Environmental Footprint: A scientometric analysis-based review. **Ecological Indicators**, [S. l.], v. 106, p. 105543, 2019. DOI: <https://doi.org/10.1016/j.ecolind.2019.105543>. Disponível em: <https://www.sciencedirect.com/science/article/pii/S1470160X1930528X>.

MERTZ, David B.; CAWTHON, D. A.; PARK, Thomas. An Experimental Analysis of Competitive Indeterminacy in *Tribolium*. **Proceedings of the National Academy of Sciences of the United States of America**, [S. l.], v. 73, n. 4, p. 1368–1372, 1976. Disponível em: <http://www.jstor.org/stable/65902>.

MORLON, Hélène; CHUYONG, George; CONDIT, Richard; HUBBELL, Stephen; KENFACK, David; THOMAS, Duncan; VALENCIA, Renato; GREEN, Jessica L. **A general framework for the distance–decay of similarity in ecological communities**. (Jerome chave, Org.) **Ecology Letters**, 2008. DOI: 10.1111/j.1461-0248.2008.01202.x.

MORRISON-WHITTLE, Peter; GODDARD, Matthew R. Quantifying the relative roles of selective and neutral processes in defining eukaryotic microbial communities. **ISME JOURNAL**, MACMILLAN BUILDING, 4 CRINAN ST, LONDON N1 9XW, ENGLAND, v. 9, n. 9, p. 2003–2011, 2015. DOI: 10.1038/ismej.2015.18.

MYERS, Jonathan A.; CHASE, Jonathan M.; JIMÉNEZ, Iván; JØRGENSEN, Peter M.; ARAUJO-MURAKAMI, Alejandro; PANIAGUA-ZAMBRANA, Narel; SEIDEL, Renate. Beta-diversity in temperate and tropical forests reflects dissimilar mechanisms of community assembly. **Ecology Letters**, [S. l.], v. 16, n. 2, p. 151–157, 2013. DOI: <https://doi.org/10.1111/ele.12021>. Disponível em: <https://doi.org/10.1111/ele.12021>.

NETTLE, Daniel; FRANKENHUIS, Willem E. The evolution of life-history theory: a bibliometric analysis of an interdisciplinary research area. **Proceedings of the Royal Society B: Biological**

**Sciences**, [S. l.], v. 286, n. 1899, p. 20190040, 2019. DOI: 10.1098/rspb.2019.0040. Disponível em: <https://doi.org/10.1098/rspb.2019.0040>.

OVASKAINEN, Otso; TIKHONOV, Gleb; NORBERG, Anna; BLANCHET, F. Guillaume; DUAN, Leo; DUNSON, David; ROSLIN, Tomas; ABREGO, Nerea. How to make more out of community data? A conceptual framework and its implementation as models and software. **ECOLOGY LETTERS**, 111 RIVER ST, HOBOKEN 07030-5774, NJ USA, v. 20, n. 5, p. 561–576, 2017. DOI: 10.1111/ele.12757.

PALMER, Michael W. Variation in species richness: Towards a unification of hypotheses. **Folia Geobotanica et Phytotaxonomica**, [S. l.], v. 29, n. 4, p. 511, 1994. DOI: 10.1007/BF02883148. Disponível em: <https://doi.org/10.1007/BF02883148>.

PAVOINE, Sandrine; BAGUETTE, Michel; STEVENS, Virginie M.; LEIBOLD, Mathew A.; TURLURE, Camille; BONSALL, Michael B. Life history traits, but not phylogeny, drive compositional patterns in a butterfly metacommunity. **Ecology**, [S. l.], v. 95, n. 12, p. 3304–3313, 2014. DOI: <https://doi.org/10.1890/13-2036.1>. Disponível em: <https://doi.org/10.1890/13-2036.1>.

PERES-NETO, Pedro R.; LEIBOLD, Mathew A.; DRAY, Stephane. Assessing the effects of spatial contingency and environmental filtering on metacommunity phylogenetics. **ECOLOGY**, 1990 M STREET NW, STE 700, WASHINGTON, DC 20036 USA, v. 93, n. 8, S, p. S14–S30, 2012. DOI: 10.1890/11-0494.1.

PIANKA, Eric R. Latitudinal Gradients in Species Diversity: A Review of Concepts. **The American Naturalist**, [S. l.], v. 100, n. 910, p. 33–46, 1966. Disponível em: <http://www.jstor.org/stable/2459377>.

PIGLIUCCI, Massimo. Do we need an extended evolutionary synthesis? **Evolution**, [S. l.], v. 61, n. 12, p. 2743–2749, 2007. DOI: 10.1111/j.1558-5646.2007.00246.x. Disponível em: <https://doi.org/10.1111/j.1558-5646.2007.00246.x>.

PIGOT, Alex L.; ETIENNE, Rampal S. A new dynamic null model for phylogenetic community structure. **ECOLOGY LETTERS**, 111 RIVER ST, HOBOKEN 07030-5774, NJ USA, v. 18, n. 2, p. 153–163, 2015. DOI: 10.1111/ele.12395.

PRESTON, F. W. The Canonical Distribution of Commonness and Rarity: Part I. **Ecology**, [S. l.], v. 43, n. 2, p. 185–215, 1962. DOI: 10.2307/1931976. Disponível em: <http://www.jstor.org/stable/1931976>.

PUETTKER, Thomas; BUENO, Adriana de Arruda; PRADO, Paulo I.; PARDINI, Renata. Ecological filtering or random extinction? Beta-diversity patterns and the importance of niche-based and neutral processes following habitat loss. **OIKOS**, 111 RIVER ST, HOBOKEN 07030-5774, NJ USA, v. 124, n. 2, p. 206–215, 2015. DOI: 10.1111/oik.01018.

RICKLEFS, Robert E. Community Diversity: Relative Roles of Local and Regional Processes. **Science**, [S. l.], v. 235, n. 4785, p. 167–171, 1987. Disponível em: <http://science.sciencemag.org/content/235/4785/167.abstract>.

- RICKLEFS, Robert E. A comprehensive framework for global patterns in biodiversity. **Ecology Letters**, [S. l.], v. 7, n. 1, p. 1–15, 2004. DOI: 10.1046/j.1461-0248.2003.00554.x.
- RICKLEFS, Robert E. Disintegration of the Ecological Community. **The American Naturalist**, [S. l.], v. 172, n. 6, p. 741–750, 2008. DOI: 10.1086/593002. Disponível em: <https://www.journals.uchicago.edu/doi/10.1086/593002>.
- SCHWALB, Astrid N.; MORRIS, Todd J.; MANDRAK, Nicholas E.; COTTENIE, Karl. Distribution of unionid freshwater mussels depends on the distribution of host fishes on a regional scale. **DIVERSITY AND DISTRIBUTIONS**, 111 RIVER ST, HOBOKEN 07030-5774, NJ USA, v. 19, n. 4, p. 446–454, 2013. DOI: 10.1111/j.1472-4642.2012.00940.x.
- SIEPIELSKI, Adam M.; HUNG, Keng-Lou; BEIN, Eben E. B.; MCPEEK, Mark A. Experimental evidence for neutral community dynamics governing an insect assemblage. **Ecology**, [S. l.], v. 91, n. 3, p. 847–857, 2010. DOI: 10.1890/09-0609.1. Disponível em: <https://doi.org/10.1890/09-0609.1>.
- SPASOJEVIC, Marko J.; COPELAND, Stella; SUDING, Katharine N. Using functional diversity patterns to explore metacommunity dynamics: a framework for understanding local and regional influences on community structure. **ECOGRAPHY**, 111 RIVER ST, HOBOKEN 07030-5774, NJ USA, v. 37, n. 10, p. 939–949, 2014. DOI: 10.1111/ecog.00711.
- STEGEN, James C.; LIN, Xueju; FREDRICKSON, Jim K.; CHEN, Xingyuan; KENNEDY, David W.; MURRAY, Christopher J.; ROCKHOLD, Mark L.; KONOPKA, Allan. Quantifying community assembly processes and identifying features that impose them. **The ISME Journal**, [S. l.], v. 7, n. 11, p. 2069–2079, 2013. DOI: 10.1038/ismej.2013.93. Disponível em: <http://dx.doi.org/10.1038/ismej.2013.93>.
- STEGEN, James C.; LIN, Xueju; FREDRICKSON, Jim K.; KONOPKA, Allan E. Estimating and mapping ecological processes influencing microbial community assembly. **Frontiers in Microbiology**, AVENUE DU TRIBUNAL FEDERAL 34, LAUSANNE, CH-1015, SWITZERLAND, v. 6, n. MAY, p. 1–15, 2015. DOI: 10.3389/fmicb.2015.00370.
- TILMAN, David. Resource Competition between Plankton Algae: An Experimental and Theoretical Approach. **Ecology**, [S. l.], v. 58, n. 2, p. 338–348, 1977. DOI: 10.2307/1935608. Disponível em: <http://www.jstor.org/stable/1935608>.
- VAN DER PLAS, Fons; JANZEN, Thijs; ORDONEZ, Alejandro; FOKKEMA, Wimke; REINDERS, Josephine; ETIENNE, Rampal S.; OLFF, Han. A new modeling approach estimates the relative importance of different community assembly processes. **Ecology**, [S. l.], v. 96, n. 6, p. 1502–1515, 2015. DOI: 10.1890/14-0454.1. Disponível em: <https://doi.org/10.1890/14-0454.1>.
- VELLEND, Mark. Conceptual Synthesis in Community Ecology. **The Quarterly Review of Biology**, [S. l.], v. 85, n. 2, p. 183–206, 2010. DOI: 10.1086/652373. Disponível em: <http://www.journals.uchicago.edu/doi/10.1086/652373>.
- VELLEND, Mark et al. Assessing the relative importance of neutral stochasticity in ecological communities. **Oikos**, 111 RIVER ST, HOBOKEN 07030-5774, NJ USA, v. 123, n. 12, p. 1420–1430, 2014. DOI: 10.1111/oik.01493. Disponível em: <http://doi.wiley.com/10.1111/oik.01493>.

VELLEND, Mark. **The Theory of Ecological Communities. Monographs in Population Biology**, 2016. DOI: 10.1016/B978-0-7234-5558-5.00001-4.

WEIHER, Evan; FREUND, Deborah; BUNTON, Tyler; STEFANSKI, Artur; LEE, Tali; BENTIVENGA, Stephen. Advances, challenges and a developing synthesis of ecological community assembly theory. **PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B-BIOLOGICAL SCIENCES**, 6-9 CARLTON HOUSE TERRACE, LONDON SW1Y 5AG, ENGLAND, v. 366, n. 1576, p. 2403–2413, 2011. DOI: 10.1098/rstb.2011.0056.

WICKHAM, Hadley. **Ggplot2: Elegant graphics for data analysis**. 2. ed. Cham, Switzerland: Springer International Publishing, 2016. Disponível em: <https://ggplot2.tidyverse.org>.

XIONG, Jinbo; ZHU, Jinyong; DAI, Wenfang; DONG, Chunming; QIU, Qiongfeng; LI, Chenghua. Integrating gut microbiota immaturity and disease-discriminatory taxa to diagnose the initiation and severity of shrimp disease. **ENVIRONMENTAL MICROBIOLOGY**, 111 RIVER ST, HOBOKEN 07030-5774, NJ USA, v. 19, n. 4, p. 1490–1501, 2017. DOI: 10.1111/1462-2920.13701.

YAN, Qingyun et al. Environmental filtering decreases with fish development for the assembly of gut microbiota. **ENVIRONMENTAL MICROBIOLOGY**, 111 RIVER ST, HOBOKEN 07030-5774, NJ USA, v. 18, n. 12, p. 4739–4754, 2016. DOI: 10.1111/1462-2920.13365.

ZHOU, Jizhong; NING, Daliang. Stochastic Community Assembly: Does It Matter in Microbial Ecology? **Microbiology and Molecular Biology Reviews**, [S. l.], v. 81, n. 4, p. e00002-17, 2017. DOI: 10.1128/MMBR.00002-17. Disponível em: <http://www.ncbi.nlm.nih.gov/pubmed/29021219>. Acesso em: 20 nov. 2017.



A composite image showing the surface of the ocean at the top and a microscopic view of various plankton species at the bottom. The plankton are illuminated with different colors (blue, green, red, yellow) against a dark background.

## CAPÍTULO 2

### GLOBAL BIOGEOGRAPHY OF THE SMALLEST PLANKTON ACROSS OCEAN DEPTHS

## GLOBAL BIOGEOGRAPHY OF THE SMALLEST PLANKTON ACROSS OCEAN DEPTHS

### ABSTRACT

Tiny ocean plankton (picoplankton) are fundamental for the functioning of the biosphere, but the ecological mechanisms shaping their biogeography are partially understood. Comprehending whether these microorganisms are structured by niche vs. neutral processes is highly relevant in the context of global change. The ecological drivers structuring picoplankton communities differ between prokaryotes and minute eukaryotes (picoeukaryotes) in the global surface ocean: while prokaryotic communities are shaped by a balanced combination of *dispersal*, *selection*, and *drift*, picoeukaryotic communities are mainly shaped by *dispersal limitation*. Yet, whether or not the relative importance of these processes in structuring picoplankton varies as we dive into the deep ocean was unknown. Here we investigate the mechanisms structuring picoplanktonic communities inhabiting different ocean depths. We analyzed 451 samples from the tropical and subtropical global ocean and the Mediterranean Sea covering the epi- (0-200m), meso- (200-1,000m), and bathypelagic (1,000-4,000m) depth zones. We found that selection decreased with depth possibly due to lower habitat heterogeneity. In turn, dispersal limitation increased with depth, possibly due to dispersal barriers such as water masses and bottom topography. Picoplankton  $\beta$ -diversity positively correlated with environmental heterogeneity and water mass variability in both the open-ocean and the Mediterranean Sea. However, this relationship tended to be weaker for picoeukaryotes than for prokaryotes. Community patterns were generally more pronounced in the Mediterranean Sea, probably because of its substantial cross-basin environmental heterogeneity and deep-water isolation. Altogether, we found that different combinations of ecological mechanisms shape the biogeography of the smallest members of the ocean microbiome across ocean depths.

**Keywords:** Marine microbiota, Prokaryotes, Picoeukaryotes, Metabarcoding, Metacommunity

## INTRODUCTION

The smallest eukaryotes and prokaryotes (picoplankton, 0.2 - 3  $\mu\text{m}$ ) play essential roles in the global ocean: from trophic interactions (SHERR; SHERR, 2008) to biogeochemical cycles (FALKOWSKI; FENCHEL; DELONG, 2008; GUIDI et al., 2016). They account for 57% (~3.8 Gt C) of the ocean's biomass (BAR-ON; MILO, 2019) and are the main contributors to the taxonomic and functional diversity of the ocean (DE VARGAS et al., 2015; MASSANA, 2011; SUNAGAWA et al., 2015; WORDEN et al., 2015). Therefore, understanding the mechanisms determining their global biogeography is fundamental to predict how they will respond to environmental changes. Picoplankton abundance, diversity, and composition are relatively well described across ocean depths (ARÍSTEGUI et al., 2009; BROWN et al., 2009): prokaryotes' diversity increases with depth (SEBASTIÁN et al., 2021; SUNAGAWA et al., 2015), while picoeukaryotes' diversity sharply decreases (GINER et al., 2020). These depth-related patterns are strongly shaped by gradients in sunlight, temperature, oxygen, and nutrients (SEBASTIÁN et al., 2021; SUNAGAWA et al., 2015) as well as by physical barriers such as water masses, currents, and fronts (BALTAR; ARÍSTEGUI, 2017; GALAND et al., 2010; MORALES et al., 2018; RAES et al., 2018). However, the ecological processes underpinning picoplankton biogeography are only partially understood (LOGARES et al., 2020; VILLARINO et al., 2022), specially considering different ocean depth zones and geographic scales. Given that the deep ocean is the largest ecosystem on our planet and harbors a massive microbial genetic diversity (ACINAS et al., 2021) – responsible for essential global ecosystem services – understanding how these processes shape the microbiota in the understudied and vast deep ocean is particularly important.

The biogeography of organisms is the result of four high-level ecological processes that act in different proportions: selection, dispersal, ecological drift, and diversification (VELLEND, 2016). *Selection* is a deterministic force emerging from combinations of biotic and abiotic variables that lead to differences in the fitness of individuals of a species and, as a consequence, to changes in community structure. *Selection* can either restrict (homogeneous selection) or promote (heterogeneous selection) the divergence of communities (ZHOU; NING, 2017). *Dispersal* is the movement of organisms across space and their establishment in new locations, affecting local community assembly by adding individuals from the regional species pool. Dispersal is considered a stochastic process for small plankton as they passively drift with currents (ZHOU; NING, 2017). Microbial dispersal rates may be high (homogenizing dispersal), moderate, or low (dispersal limitation) (ZHOU; NING, 2017), depending on organism and population sizes, geographic scale, and the presence of physical barriers (FODELIANAKIS et al., 2021; LOUCA, 2022; VILLARINO et al., 2022). *Dispersal limitation* takes place when species are not present in suitable habitats

because colonizers cannot reach them (HEINO et al., 2015). Thus, the relative importance of *dispersal limitation* usually increases with geographic scales (HUBBELL, 2001) or barriers (LOUCA, 2022). Ecological drift (hereafter *drift*) refers to random changes in community structure due to stochastic demographic events (i.e., birth, death, immigration, and emigration) in a local community (VELLEND, 2016). *Drift* is a stochastic process that tends to be most important for the local extinction of low-abundant microbial taxa with small populations (NEMERGUT et al., 2013), especially under a low dispersal scenario (FODELIANAKIS et al., 2021). Finally, diversification (also referred to as ‘speciation’) is the emergence of new species by evolution (VELLEND, 2016), which occurs more frequently for microbes than for larger organisms due to their short generation times, high mutation rates as well as horizontal gene transfer (NEMERGUT et al., 2013; ZHOU; NING, 2017). Yet, diversification is expected to have a relatively small impact on the turnover of communities that are highly connected via dispersal (STEGEN et al., 2013), as is the case for ocean picoplankton (LOUCA, 2022). *Diversification*, as measured by the evolution of the rRNA gene sequence, will not be further considered here, given that its impact on measured ecological processes is likely minor considering the low evolutionary rates of this marker (WOESE, 1987).

A recent study – using *Malaspina* and TARA data – found that the relative importance of these processes differs between the components of the surface ocean picoplankton community: while prokaryotes are shaped by a balanced combination of dispersal, selection, and drift, picoeukaryotes are mainly driven by dispersal limitation (LOGARES et al., 2020). However, we do not fully understand whether these processes change across ocean depth zones. These zones display striking differences in environmental and geographic features that may influence selection, dispersal, and drift. First, environmental heterogeneity – potentially exerting heterogeneous selection on microbial communities (HUBER et al., 2020; LOGARES et al., 2020) – is higher in the upper ocean due to stronger horizontal environmental gradients (RUIZ-GONZÁLEZ et al., 2019) than in the deep ocean (REID, 1981). Second, the presence of aerial dispersal (MAYOL et al., 2017) and faster oceanic currents likely increases dispersal at the surface (RICHTER et al., 2022; VILLARINO et al., 2018), while the presence of sharper geographical barriers (e.g. water masses and bottom topography) may limit microbial dispersal in the low-turbulent deep ocean (PERNICE et al., 2016; SALAZAR et al., 2016; VILLARINO et al., 2022). Third, smaller population sizes in the deep ocean (ARÍSTEGUI et al., 2009) may lead to reduced dispersal and increased drift (FODELIANAKIS et al., 2021), as compared to the surface ocean (LOGARES et al., 2020; VILLARINO et al., 2018). Recently, using a subset of the *Malaspina* dataset, it has been shown that picoplankton community assembly differed between a water layer in the surface ocean

(3 m) and a counterpart in the deep ocean (~4,000 m), with dispersal limitation being relatively more important in the deep layer than in the surface counterpart (VILLARINO et al., 2022).

In addition, we do not know whether these processes would be different in an ocean basin presenting strong environmental gradients and obvious geographic barriers. In this regard, the Mediterranean Sea – the largest semi-enclosed sea on Earth – is an ideal ocean model to test ecological hypotheses at a smaller scale (BETHOUX et al., 1999; NIHOUL, 1982). Although the Mediterranean Sea is connected to the adjacent Atlantic Ocean through the Strait of Gibraltar, it is so in a rather restricted way (SAMMARTINO et al., 2015). As a consequence, the Mediterranean Sea has developed unique oceanographic features in comparison to the open ocean, such as higher temperature and salinity in deep waters as well as a west-to-east gradient of decreasing nutrient concentration and increasing salinity in surface waters (KROM et al., 1991; SEBASTIÁN et al., 2021). Additionally, the Mediterranean Sea deep (> 1,000 m) waters are physically divided by the Sicily Strait (500 m deep) into Western and Eastern basins. These features are expected to influence the processes shaping picoplankton biogeography and, ultimately, be reflected in its community composition (SEBASTIÁN et al., 2021).

In the last few years, it has been found that different processes shape prokaryotes and picoeukaryotes in the surface ocean (LOGARES et al., 2020). In addition, a recent report points to differences in the picoplankton biogeography between specific waters layers in the surface (3 m) and deep ocean (4,000 m) (VILLARINO et al., 2022). However, we still lacked a broad examination of the ecological processes driving picoplankton community assembly and biogeography across all depth zones of the global ocean that takes into account environmental heterogeneity, potential dispersal barriers, and geography. Here, we addressed the previous challenge. We determined the relative importance of the ecological processes structuring picoplanktonic communities inhabiting three ocean depth zones at the global and basin scales: epi- (0-200 m), meso- (200-1,000 m) and bathypelagic (1,000-4,000 m). We also aimed at understanding to what extent water masses, deep-sea topography as well as environmental heterogeneity are potentially limiting dispersal or exerting selection on the picoplanktonic communities. To do so, we used 16S and 18S rRNA gene amplicon sequence variants (ASV) from both prokaryotes and picoeukaryotes collected during global and regional expeditions covering the tropical and subtropical global ocean as well as the Mediterranean Sea. Overall, we hypothesize that the role of heterogeneous selection will decrease with depth due to a potential decrease in habitat heterogeneity, while homogeneous selection is expected to be higher in the bathypelagic compared to the meso- and epipelagic. In turn, the relative importance of dispersal limitation is expected to increase with depth, given the decrease in current speed in deep waters, the existence of

geographical barriers (e.g. fronts, deep sea topography), and the absence of aerial dispersal. We also hypothesize that these patterns should be more pronounced in the Mediterranean Sea due to its strong environmental gradients and constrained communities exchange in deep waters.

## RESULTS

### *Different ecological processes shape picoplankton communities in depth zones of the ocean*

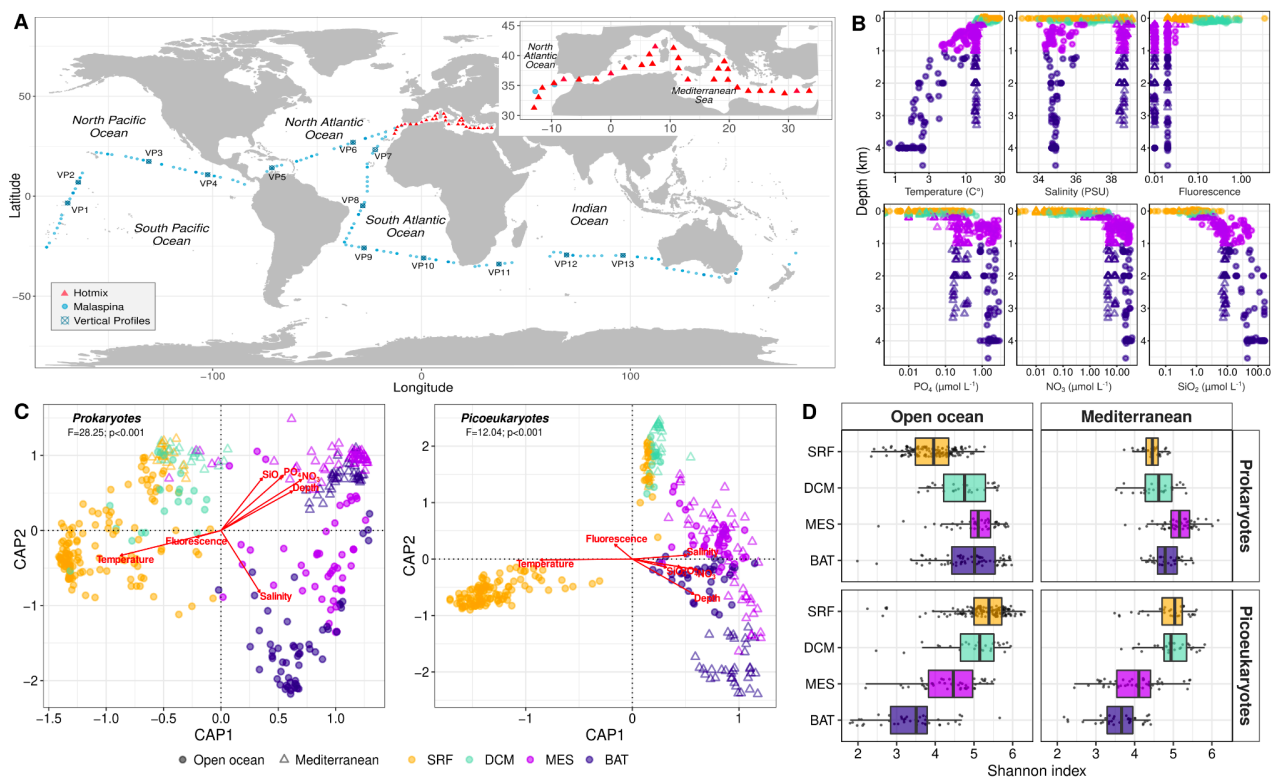
We analyzed picoplankton community composition in 451 samples across three ocean depth zones: epi- (0-200 m – including the deep chlorophyll maxima, DCM), meso- (200-1,000 m), and bathypelagic (1,000-4,000 m) using metabarcoding of the 16S and 18S rRNA genes (Fig. II-1A and Fig. II-S1A; see *Methods* for details on standard protocols). These zones display contrasting environmental features across the water column (Fig. II-1B and Fig. II-S1B), reflected in a depth-structured picoplankton community composition (Fig. II-1C). Our data also makes evident an inverted diversity pattern between the two main components of the picoplankton community: while prokaryotic diversity (richness, Shannon index, and phylogenetic diversity) increased with depth, picoeukaryotic diversity decreased towards the deep ocean (Fig. II-1D and Fig. II-S2). While the Mediterranean Sea displayed higher temperature and salinity as well as lower nutrients than the oceanic basins, particularly in the meso- and bathypelagic (Fig. II-1B), the diversity patterns were similar in both ocean sets. The environmental features, however, were reflected in differences in picoplankton community composition (Bray-Curtis Dissimilarity) between the Mediterranean Sea and the rest of the oceanic basins (Fig. II-1C). The Mediterranean Sea was evaluated separately from the open ocean in downstream analyses to test whether the large scale patterns are reflected at the regional scale of a smaller basin with strong environmental gradients and sharp geographic barriers.

We found differences in the biodiversity metrics ( $\beta$ NTI,  $RC_{\text{Bray}}$  and  $\beta$ -diversity partitioning; Fig. II-S3 and Fig. II-S4) and, ultimately, in the balance between ecological processes shaping picoplankton communities across depth zones of the ocean (Fig. II-2A). *Selection* explained a similar percentage of the turnover of picoeukaryotes as compared to prokaryotes in the epi- (~37% vs. ~36%), meso- (~32% vs. ~31%) and bathypelagic (~32% vs. ~26%) of the open ocean (Fig. II-2A). *Heterogeneous selection* tended to increase with depth for both domains: while for prokaryotes it increased from ~10% to ~19% and ~13% in the meso- and bathypelagic, it increased from ~13% in the epi- to ~27% and ~31% in the meso- and bathypelagic for picoeukaryotes, respectively (Fig. II-2A). Accordingly, the relative importance of *homogeneous selection* for prokaryotes decreased from ~26% in the epi- to ~13% in the bathypelagic. Similarly, the relative importance of *homogeneous selection* for picoeukaryotes drastically decreased from ~26% in the epipelagic to ca.

0.7% in the bathypelagic (Fig. II-2A). These patterns were slightly different in the Mediterranean Sea when compared to the tropical and subtropical open ocean. The relative weight of *selection* for the prokaryote community assembly was consistently higher than for the picoeukaryotic counterpart in the epi- (~54% vs. ~44%), meso- (~39% vs. ~25%) and bathypelagic (~32 vs. ~6%, respectively) (Fig. II-2A). The proportion of *heterogeneous selection* for prokaryotes dramatically dropped from 37% in the epipelagic to ~5% in deep waters, while the role of *homogeneous selection* increased from the epi- (~18%) to the meso- (~34%) and bathypelagic (~28%) (Fig. II-2A). For picoeukaryotes, both *heterogeneous* and *homogeneous selection* decreased from the epi- (33% and 10%) to the bathypelagic (6% and 0.2%, respectively) (Fig. II-2A).

*Dispersal limitation* was a more important driver of picoeukaryotic than prokaryotic assembly in the deep zones, especially in the mesopelagic (~60% vs. ~29%), of the open ocean. We found that, for picoeukaryotes, the proportion of *dispersal limitation* increased from ~31 % in the epi- to ~60% in the meso- and to ~38% in the bathypelagic (Fig. II-2A). In the Mediterranean Sea the relative importance of *dispersal limitation* was much higher for picoeukaryotic than for prokaryotic assembly in the epi- (~35% vs. ~22%), meso- (~52% vs. ~24%), and bathypelagic (~42 vs. ~15%). Conversely, *homogenizing dispersal* had a very limited role in the structuring of the microbiota in all depth zones of the open ocean (<2% for picoeukaryotes and <4% for prokaryotes) and the Mediterranean Sea (<5% for picoeukaryotes and <8% for prokaryotes) (Fig. II-2A). *Drift* explained a higher fraction of community turnover for prokaryotes than picoeukaryotes in the meso- (~38% vs ~7%) and bathypelagic (~37% vs ~28%) of the open ocean (Fig. II-2A). This pattern was partially observed in the Mediterranean Sea with *drift* explaining a higher proportion of community turnover for prokaryotes (~29%) and picoeukaryotes (~20%) in the mesopelagic (Fig. II-2A). While in the open ocean the percentage of turnover explained by *drift* increased with depth for prokaryotes and decreased for picoeukaryotes (Fig. II-2A), it sharply increased with depth for both prokaryotes and picoeukaryotes in the Mediterranean Sea (Fig. II-2A). When estimated using a standardized sampling-size dataset (N=39 in each depth zone) with evenly-distributed samples (Fig. II-S5A and Fig. II-S6), the different ecological processes explained fairly similar percentages of variability and the values were strongly linked ( $R^2 \sim 0.9$ ,  $p < 0.001$ ) to those found with the complete dataset (Fig. II-2).

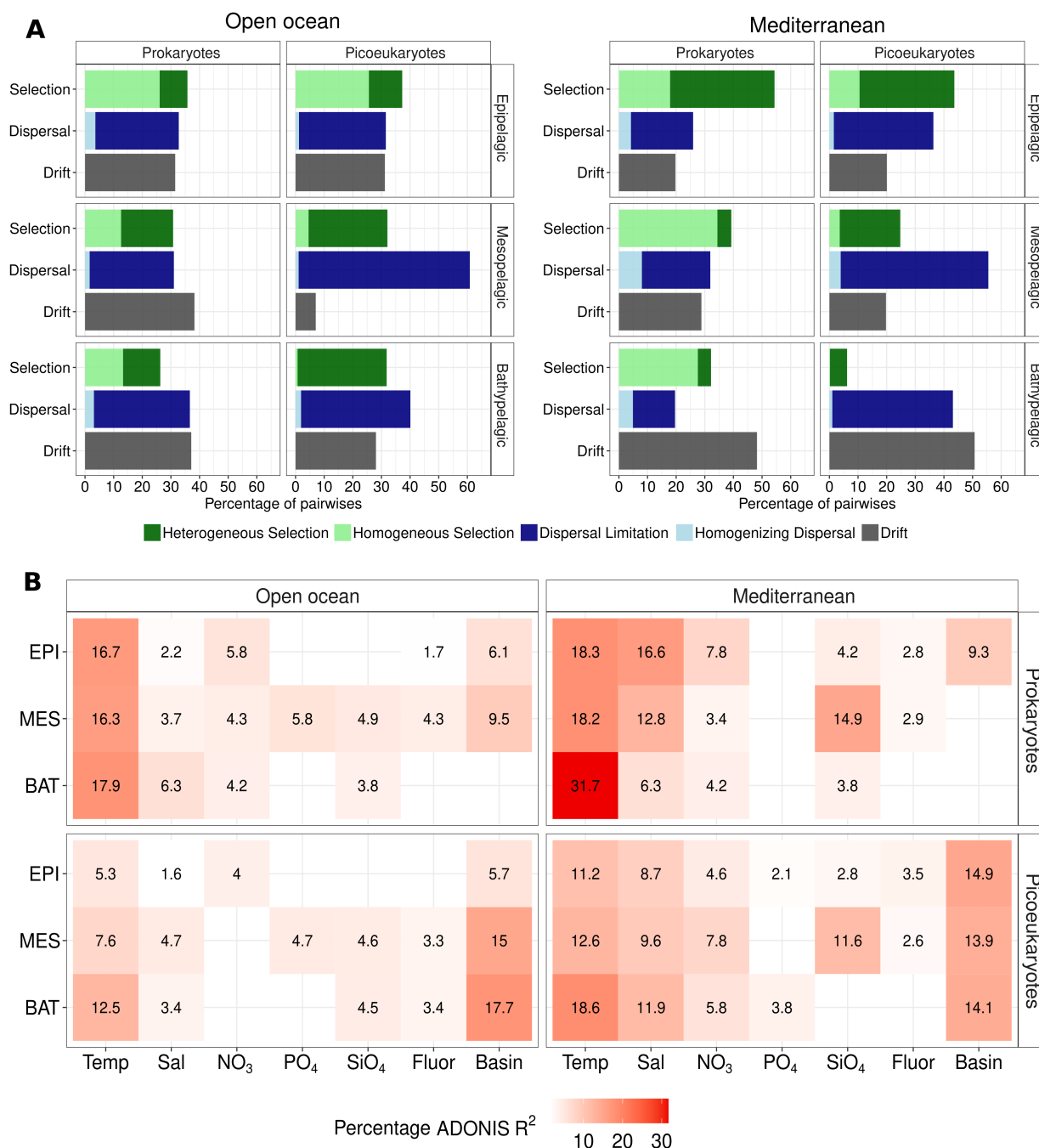




**Figure II-1. The analyzed dataset covers environmentally and biologically contrasting depth zones of the ocean.** (A) Geographic distribution of the sampled stations (N=149) from which seawater samples and environmental data were collected at different depth zones (see SI Appendix, Fig. II-S1 for sample vertical distribution) in the two cruises used in this study: *Malaspina-2010* (circumglobal expedition) and *HotMix* (trans-Mediterranean expedition). Stations for which the whole vertical profile was studied in *Malaspina* are represented by crossed squares (13 stations in *Malaspina*). Samples were separated into “open-ocean” (*Malaspina-2010* + *Hotmix* North Atlantic samples) and “Mediterranean Sea” (see reasoning in the Methods). (B) Vertical profiles of the environmental parameters: temperature, salinity, and fluorescence (Chlorophyll a proxy) that decrease with depth, while nutrient concentrations ( $\text{NO}_3$ ,  $\text{PO}_4$ , and  $\text{SiO}_2$ ) increase with depth. Higher temperature and salinity values and lower nutrient concentrations were observed in the Mediterranean Sea, especially in the meso- and bathypelagic (Fig. II-S1B). (C) dbRDA analyses (based on Bray-Curtis dissimilarities) performed on picoplankton community composition of both prokaryotic (left) and picoeukaryotic (right) samples based on 16S rRNA and 18S rRNA genes, respectively. Both communities were structured by depth zones and segregated between the tropical and subtropical open-ocean and the Mediterranean Sea. (D) Picoplankton diversity expressed as Shannon index by depth zones (SRF, surface; DCM, deep chlorophyll maxima; MES, Mesopelagic; BAT, Bathypelagic). See Fig. II-S2 for picoplankton phylogenetic diversity, gamma diversity, ASVs richness, and Pielou’s evenness index variation by depth zones and correlations with environmental variables.

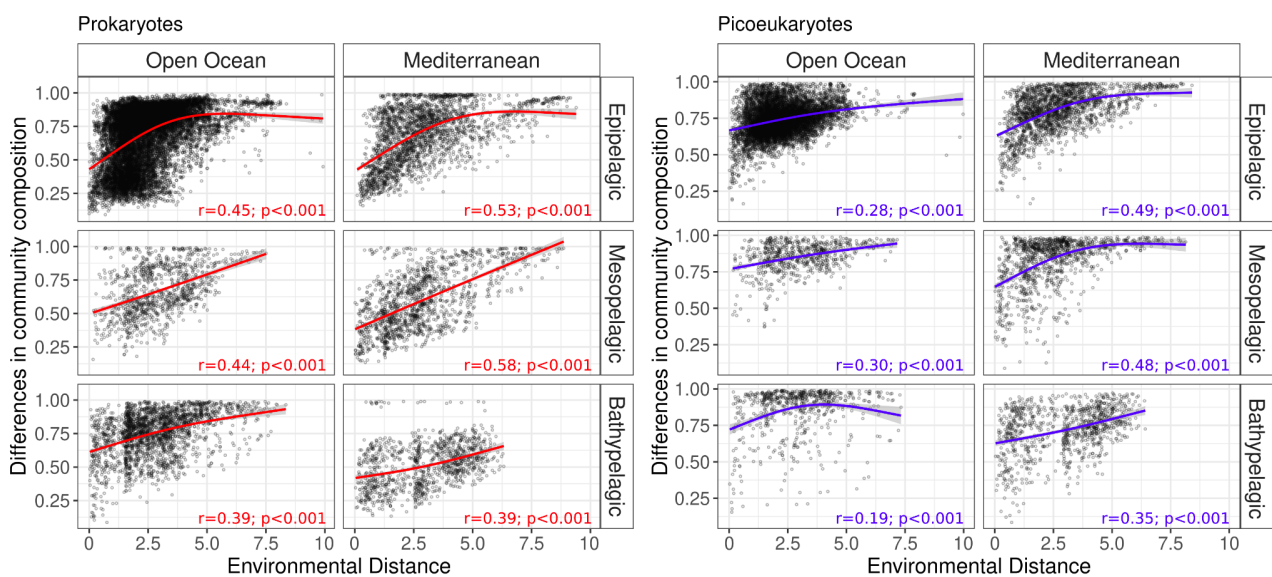


When globally estimated (all depths together), *selection* was by far the most relevant ecological process shaping both prokaryotes (~67%) and picoeukaryotes (~54%) using both datasets (Fig. II-S8). *Dispersal limitation* also tended to play a relatively more important role shaping picoeukaryotes than prokaryotes when estimated across all depth zones (Fig. II-S8). Due to the potential vertical connectivity between the surface and the deep ocean (see detailed reasoning in



**Figure II–2. Picoplankton community assembly processes and environmental drivers across ocean depth zones.** (A) Relative importance of the ecological processes structuring the communities in different depth zones of the global-ocean: Epi- (N=240), Meso- (N=97), and Bathypelagic (N=86). The results with standard evenly-distributed sampling sizes were nearly the same (Fig. II-S5). The EPI results separated by SRF and DCM are available in the (Fig. II-S7). (B) Percentage of variance (Adonis R<sup>2</sup>) in picoeukaryotic and prokaryotic community composition (Bray-Curtis dissimilarity) explained by each environmental variable and ocean basin. Blank spaces depict non-significant results ( $p>0.05$ ). Temp – temperature; Sal – salinity; Fluor – fluorescence; Basin – ocean basin.

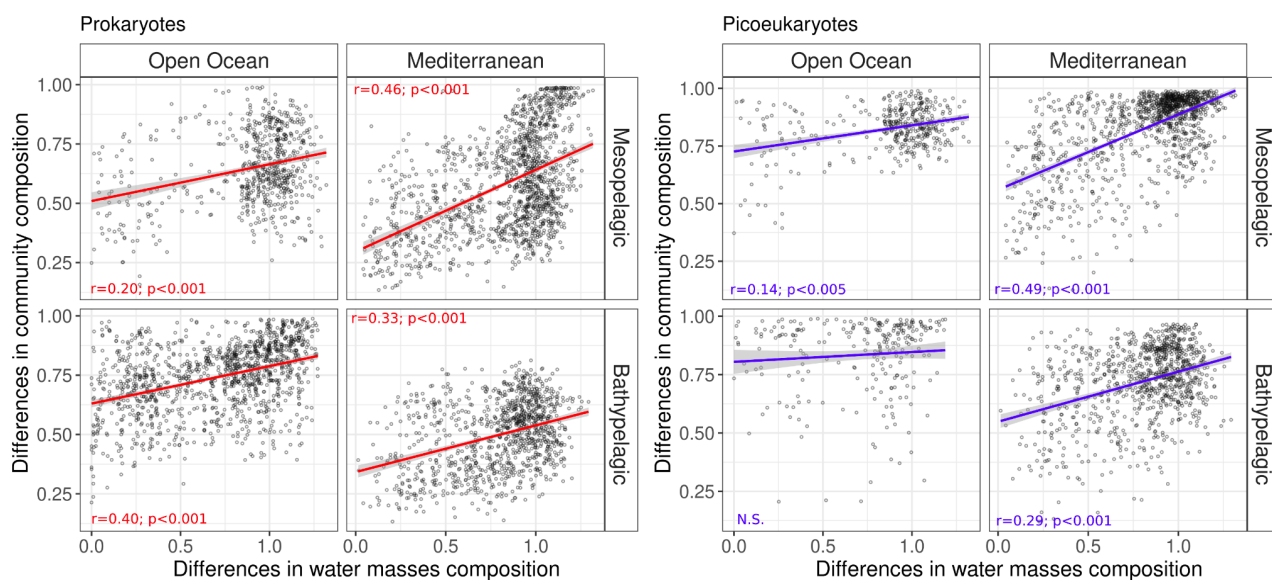
Environmental heterogeneity (average pairwise dissimilarity based on temperature, salinity, fluorescence,  $\text{PO}_4^{3-}$ ,  $\text{NO}_3^-$ , and  $\text{SiO}_2$ ) was significantly higher in the epi- than in the meso- and bathypelagic of the open ocean and the Mediterranean Sea (Fig. II-S10). We found that the picoplankton communities' dissimilarity increased with environmental distance in all depth zones (Fig. II-3). This positive relationship was always stronger in the epipelagic than in the bathypelagic (Fig. II-3). Prokaryotes displayed a stronger coupling with environmental distance than picoeukaryotes in all depth zones of both the open ocean and the Mediterranean Sea (Fig. II-3) and this coupling was stronger in the Mediterranean Sea than in the open ocean across all zones (Fig. II-3). When globally estimated (all depth zones together), the community dissimilarity correlation with environmental distance was stronger for prokaryotes than for picoeukaryotes in the open ocean ( $r=0.62$  vs.  $r=0.46$ ,  $p<0.001$ ) and nearly the same in the Mediterranean Sea ( $r=0.69$  vs.  $r=0.65$ ,  $p<0.001$ ) (Fig. II-S11). The metric used to estimate *selection* ( $\beta\text{NTI}$ ) was positively correlated, in prokaryotic and picoeukaryotic communities, with environmental distances in both the open ocean ( $r=0.55$  and  $r=0.50$ ,  $p<0.001$ ) and the Mediterranean Sea ( $r=0.55$  and  $r=0.50$ ,  $p<0.001$ ) (Fig. II-S11).



**Figure II-3. Picoplankton community composition is positively related to environmental heterogeneity.** Bray-curtis dissimilarities for all pairwise picoplankton community comparisons as a function of environmental distance for both prokaryotes and picoeukaryotes in the epi-, meso-, and bathypelagic of the open ocean and Mediterranean Sea. The solid curves illustrate the nonlinear regressions. Spearman's rank correlation coefficients are depicted on the panel. Outliers with high environmental distances ( $>10$ ) corresponding to pairwise comparisons with epipelagic samples from the Costa Rica Dome upwelling system were removed from the open ocean plot (Fig. II-S12).

### ***The role of water masses and deep sea topography in modulating picoplankton assembly***

Water masses, which were determined for the meso- and bathypelagic, were vertically structured and segregated by basins in the open ocean and the Mediterranean Sea (Fig. II-S13). We found that prokaryotic community composition (Bray-Curtis dissimilarity) was positively linked with differences in water mass composition (Euclidean distances) in the meso- and bathypelagic of the open ocean ( $r=0.2$  and  $r=0.4$ ,  $p<0.001$ ) and the Mediterranean Sea ( $r=0.46$  and  $r=0.33$ ,  $p<0.001$ ) (Fig. II-4). For picoeukaryotes, this coupling was generally weaker than for prokaryotes in both the open ocean ( $r=0.14$ ,  $p<0.001$ ) and the Mediterranean Sea ( $r=0.49$  and  $r=0.29$ ,  $p<0.001$ ) (Fig. II-4). In the Mediterranean Sea, the link between picoplankton community and water mass composition was stronger in the meso- than in the bathypelagic (Fig. II-4). Strong positive relationships between the picoplankton community and water mass' composition were also observed within most individual vertical-profile stations, with variable slopes in each station (Fig. II-S14).

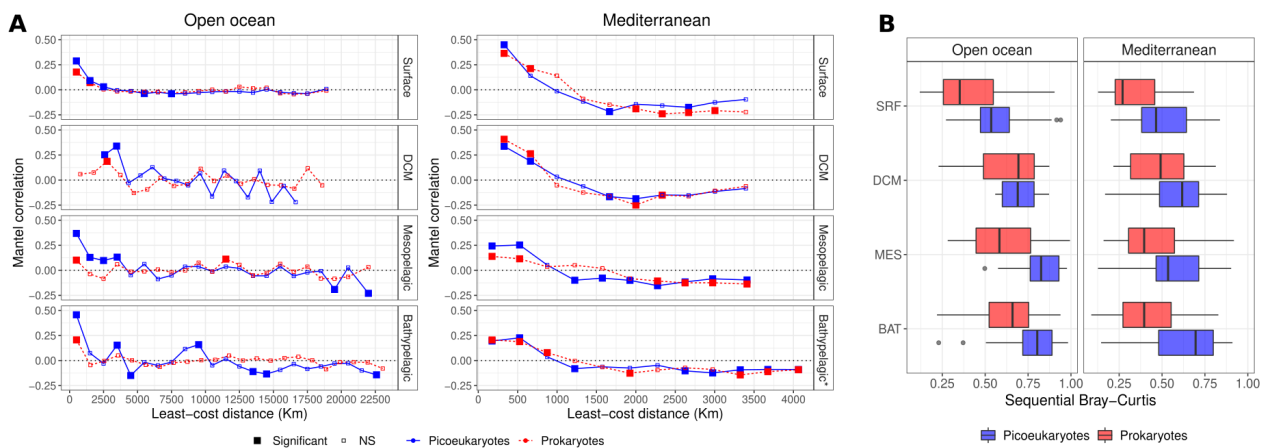


**Figure II-4. Picoplankton community composition is linked to differences in water mass composition.**

Bray-Curtis dissimilarity of pairwise picoplankton community comparisons as a function water mass composition dissimilarity (based on euclidean distances) for both prokaryotes and picoeukaryotes in the meso- and bathypelagic of the open ocean and Mediterranean Sea. The solid curves illustrate the nonlinear regressions. Spearman's rank correlation coefficients are depicted on the panel. N.S.= non significant.

In the open ocean, changes in prokaryotic and picoeukaryotic community composition ( $\beta$ -diversity) displayed positive correlations with geographic distances (distance-decay) in four depth zones (Fig. II-5A) even though correlations were weaker for prokaryotes than for picoeukaryotes in most of them. Prokaryotes displayed positive correlations with distances up to ~2,000 km in the surface and 1,000 km in the deep ocean, while picoeukaryotes showed positive correlations up to

~3,000 km in the surface and ~4,000 km in the deep ocean (Fig. II-5A). For picoeukaryotes, these positive correlations were stronger in the bathypelagic (Mantel  $r = 0.5$ ,  $p < 0.05$ ) than in the surface (Mantel  $r = 0.3$ ,  $p < 0.05$ ) (Fig. II-5A). Interestingly, picoeukaryotes also displayed negative correlations with increasing distances up to ~20,000 km across the deep zones (Fig. II-5A). In fact, picoeukaryotes had a higher variation in the spatial autocorrelations than prokaryotes in the deep ocean, especially in the bathypelagic. When evaluating these spatial autocorrelations at a regional scale as in the Mediterranean Sea, we found that prokaryotes and picoeukaryotes did not display such contrasting correlation scores as in the open ocean (Fig. II-5A). Indeed, these two domains had similar patterns of positive correlations in the first 350-850 km of the Mediterranean Sea (Fig. II-5A). Picoeukaryotes had higher mean sequential changes in communities ( $\beta$ -diversity) than prokaryotes in all depth zones (Fig. II-5B). Overall, sequential community change tended to increase with depth in picoeukaryotes, with significant differences between the surface and the meso- and bathypelagic in picoeukaryotes, but not in prokaryotes (Fig. II-5B).



**Figure II-5. Distance-decay and sequential spatial differentiation in picoplankton communities across ocean depth zones. (A)** Mantel correlograms between  $\beta$ -diversity and least-cost geographic distances featuring distance classes of 1,000 km for the open ocean and 350 km for the Mediterranean Sea. Filled squares depict significant correlations ( $p < 0.05$ ). NS – non-significant correlations. **(B)** Sequential Bray-Curtis dissimilarity values for prokaryotes and picoeukaryotes in all depth zones (means were significantly different between domains [Wilcoxon test,  $p < 0.05$ ] in all depth zones, apart from the DCM). The averages were also significantly different (ANOVA, Tukey post-hoc test;  $p < 0.001$ ) between the SRF and the deep zones (MES and BAT) for picoeukaryotes, but not for prokaryotes. See Fig. II-S15 for maps showing the sequential change in community composition across space in the surface and bathypelagic ocean. The epipelagic was here separated into surface and DCM because we aimed at evaluating only the horizontal geographic distance in each depth.

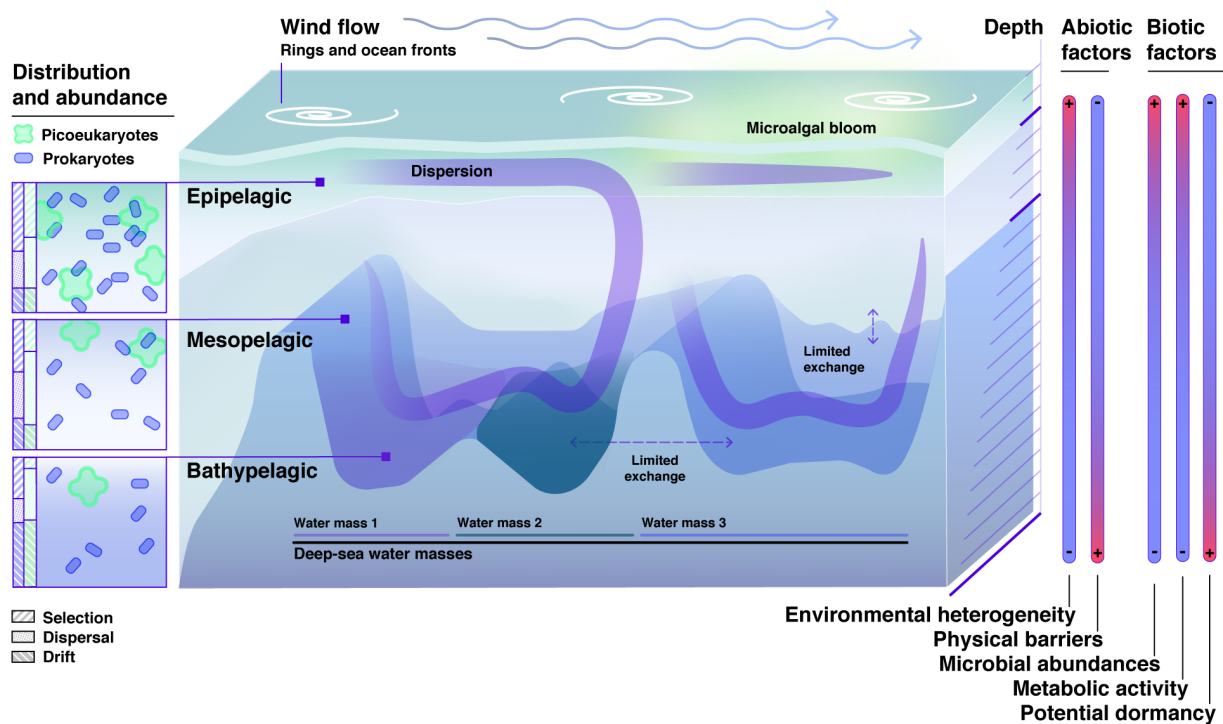
Microbial abundances and activity may also work as potential regulators of dispersal limitation and drift. Here, microbial abundances – as measured by flow-cytometry – sharply decreased with depth in both the open ocean and the Mediterranean Sea (Fig. II-S16A). Similarly, prokaryotic activity – as measured by leucine incorporation rates – drastically decreased from surface to deep ocean waters (Fig. II-S16B), with statistically significant differences between epipelagic (SRF and DCM) and deep zones (MES and BAT).

## DISCUSSION

### *Selection decreases while dispersal limitation and drift increase with depth*

Our results support our main hypothesis, indicating that a different combination of ecological processes shapes picoplankton biogeography across ocean depth zones at global and regional scales (Fig. II-6). *Selection* was the most important process shaping picoplankton in the epipelagic ocean (see also *SI Appendix*, *SI Discussion*), likely as a response to a higher overall environmental heterogeneity when compared to the deep ocean. In particular, microalgal blooms (ESTRADA et al., 2016; RUIZ-GONZÁLEZ et al., 2019), magnitude of the DCM (CORNEC et al., 2021; SUNAGAWA et al., 2015), ocean fronts and eddies (LÉVY et al., 2015; MORALES et al., 2018; RAES et al., 2018; VILLAR et al., 2015), and differences in physicochemical variables (Fig. II-1B), altogether increase environmental heterogeneity in the upper ocean (Fig. II-6). In the epipelagic, the higher relative importance of *heterogeneous selection* in the Mediterranean Sea than in the open ocean is probably linked to its environmental gradients: north-south increasing temperature (SOUKISSIAN et al., 2017), west-east increasing salinity (SOUKISSIAN et al., 2017), and west-east decreasing nutrient concentrations (SEBASTIÁN et al., 2021). This result contradicts a previous hypothesis that *homogeneous selection* should be the most important process in all ocean basins (MILKE et al., 2022). Instead, the balance between ecological processes shaping picoplankton communities will change depending on the analyzed environmental heterogeneity, circulation patterns, and geographic scale (JIE et al., 2022; MOD et al., 2020; REN et al., 2022). In our study, the overall role of *selection* decreased, for both domains, when transiting from the epipelagic into the deep waters, where there is relatively lower environmental heterogeneity in comparison to the epipelagic (Fig. II-S10). Moreover, the coupling between picoplankton community differentiation and environmental distances was stronger in the epipelagic than in the deep ocean, further indicating that the relative importance of selection raises with increasing environmental variability. *Selection* was also the most important process shaping picoplankton when ecological processes were estimated with all samples of our dataset, which captures environmental differences from surface to deep waters. This is another evidence that *selection* is

enhanced as environmental heterogeneity is increased. These findings are coherent with ecological theory and other studies that show that high environmental heterogeneity leads to higher *selection* (VELLEND, 2016) in terrestrial (DINI-ANDREOTE et al., 2015; STEGEN et al., 2013) and aquatic ecosystems (HUBER et al., 2020; LOGARES et al., 2018; VASS et al., 2020). Conversely, the sum of *dispersal limitation* and *drift* were overall higher in the deep than in the surface ocean, suggesting that factors such as microbial abundances (i.e. low population sizes) (FODELIANAKIS et al., 2021) and physical barriers (strongly differentiated water masses and deep-sea bathymetry) (VILLARINO et al., 2022) play an important role in the structuring of deep ocean picoplankton communities (Fig. II-6). *Dispersal limitation* increased with depth probably because of decreasing turbulence (stable water masses and slow currents) (REID, 1981) and the presence of straits and seamounts (YESSON et al., 2011) that work as geographical barriers for microbial dispersal in the deep ocean (Fig. II-6). Other studies have shown how strong physical barriers can limit microbial dispersal in soils (DINI-ANDREOTE et al., 2015), sediments (STEGEN et al., 2013), ponds (VASS et al., 2020) and, potentially, in the ocean (LOUCA, 2022).



**Figure II-6.** Conceptual model synthesizing the ecological processes assembling picoplankton communities across ocean depth zones. We used the main findings of this study and the knowledge available in the literature to construct this conceptual model. Vertical variation of biotic and abiotic factors, as well as geography (e.g., bathymetry), affect the ecological processes that generate community distribution patterns. The model predicts an increasing role of *dispersal limitation* with depth: *dispersal limitation* is weaker in the epipelagic than in the meso- and bathypelagic due to faster currents, and, potentially, aerial dispersal in surface waters, compared to more isolated deeper zones. Other mechanisms taking place in deep waters such as a) barriers to dispersal (e.g. water mass boundaries, deep sea topography) or b) limited random dispersal due to low species abundances, could also explain this pattern. *Selection* is the most important process structuring picoplankton communities in the epipelagic and displays a decreasing importance with depth due to higher habitat heterogeneity – driven by microalgal blooms, magnitude of the DCM and mesoscale processes (e.g.: ocean rings and fronts) – in upper than in bottom waters. The relative role of *drift* increases towards the deep, likely because of decreasing microbial abundances with depth. The importance of *dispersal limitation* is always higher in picoeukaryotes than in prokaryotes, given the smaller population sizes of picoeukaryotes and their limited capability to generate dormant stages to sustain long-range dispersal, compared to prokaryotes. Thus, a different balance of ecological processes assembles these domains, even when they share the same ocean zones.

### ***Water mass composition affects the distribution of prokaryotic communities***

Water masses may impact microbial communities in basically two ways: *a*) as a selective force – since they have different temperatures and salinity (SUN et al., 2022; ZHU et al., 2022) as

well as organic matter composition (AGOGUÉ et al., 2011; GÓMEZ-LETONA et al., 2022; MARTÍNEZ-PÉREZ et al., 2019) – or; *b*) as a physical barrier to dispersal due to sometimes strong differences in water density (GALAND et al., 2010). We found significant positive correlations between prokaryotic community structure and water mass compositions in the open ocean, which is in line with previous studies that found bacterial communities associated with specific water masses (AGOGUÉ et al., 2011; GALAND et al., 2010; RAES et al., 2018; RIGONATO et al., 2021). This relationship is likely linked to the fact that each water mass has different types of organic matter (CATALÁ et al., 2015; MARTÍNEZ-PÉREZ et al., 2019) that likely select for different prokaryotes (AGOGUÉ et al., 2011; GÓMEZ-LETONA et al., 2022). In turn, picoeukaryotes were only poorly correlated with differences in water mass in the open ocean, which implies that some of them could be able to swim across boundaries or that they are weakly linked to the composition of typical organic matter associated with each water mass. Instead, the high dispersal limitation of picoeukaryotes would be mainly regulated by their smaller population (ARÍSTEGUI et al., 2009) as well as by their limited capability to enter into dormancy when compared to prokaryotes (MASSANA; LOGARES, 2013). In the Mediterranean Sea, the coupling between community and water mass composition was significant for both prokaryotes and picoeukaryotes in the meso- and bathypelagic, which agrees with previous reports (BELLAAJ ZOUARI et al., 2018) and it is likely linked to the strong horizontal cross-basin physical separation imposed by the Straits of Sicily and Gibraltar (SEBASTIÁN et al., 2021). Our results also point out that differences in both prokaryotes and picoeukaryotic communities are coupled with differences in water mass composition in vertical profiles (Fig. II-S14). Interestingly, the slope and strength that differences in picoplankton composition was explained by differences in water masses varied among vertical profile stations (Fig. II-S14). This result indicates that local-scale events (e.g. upwelling, dense water propagation) may also regulate the impact of water mass on microbial communities in a vertical dimension (LUNA et al., 2016; NEAVE et al., 2022; SEVERIN et al., 2016).

### ***Picoplankton communities display weaker biogeography in the surface than in the deep ocean***

Our distance-decay analysis revealed that the autocorrelation in community and geographic distances is stronger in the deep than at the surface, which agrees with our sequential analysis results (Fig. II-5B) and suggests that there are more marked changes across space in the deep ocean, particularly in the picoeukaryotic community. This result agrees with a recent study that found larger eukaryotic community dissimilarity between pairs of sites in the deep than in the surface global ocean (CORDIER et al., 2022). Such changes in community composition with increasing geographic distance (that is, distance-decay) can be generated by selection and/or dispersal



limitation (HANSON et al., 2012). For picoeukaryotes, the fact that changes in community composition were better explained by geography (ocean basin) than by environmental variation (Fig. II-2B) supports that the distance-decay pattern in the deep sea is predominantly related to dispersal limitation (CORDIER et al., 2022; VILLARINO et al., 2022). On the other hand, prokaryotic community structure was predominantly explained by environmental variables rather than by geography (Fig. II-2B) which indicates that, in this domain, distance-decay is mostly driven by selection. It is important to notice that, since many prokaryotes may be in dormant state (LOCEY et al., 2020), the distance-decay could have been stronger if we had analyzed measures of the active prokaryotic community (using RNA) instead of measures of the total community (with DNA), as previously shown for bacterial communities (LOCEY et al., 2020). Another important factor that could be increasing the role of *dispersal limitation* and *drift* in the deep ocean is the decreasing microbial population sizes from surface to deep waters. Rare species with small populations are less likely to disperse (GASTON et al., 2000) and more likely to randomly collapse than species with large populations (FODELIANAKIS et al., 2021). As expected (ARÍSTEGUI et al., 2009), microbial abundances drastically decreased towards the deep ocean so that the deep ocean contains only 1% of the organisms of the surface ocean (ARÍSTEGUI et al., 2009). Overall, the depth-related patterns in ecological processes were more pronounced in the Mediterranean Sea than in the open ocean, which is partially explained by being a semi-enclosed sea, with unique oceanographic features such as limited circulation, sharp geographic barriers and strong environmental gradients (KROM et al., 1991; SEBASTIÁN et al., 2021).

### ***Differences between picoplankton members in the different depth zones***

A different balance of ecological processes shapes prokaryotic and picoeukaryotic communities in several ecosystems (BRISLAWN et al., 2019; VASS et al., 2020; VILLARINO et al., 2018), including the surface ocean (LOGARES et al., 2020; MILKE et al., 2022). Here we found that such differences between domains persist in the deep ocean. *Dispersal limitation* was always higher for picoeukaryotes than for prokaryotes, which agrees with previous studies using similar approaches conducted in Antarctic lakes (LOGARES et al., 2018) and in basin-scale oceanic regions (JIE et al., 2022). This contrast between domains in terms of dispersal rates is partially due to organismal and population size differences (DE BIE et al., 2012; GASTON et al., 2000; VILLARINO et al., 2018). Unicellular eukaryotes are on average 3 times larger than prokaryotes and, therefore, would be expected to be more limited by dispersal (DE BIE et al., 2012; VILLARINO et al., 2018). Picoeukaryotes ( $\sim 10^3$  cells mL<sup>-1</sup>) have populations that are about three orders of magnitude smaller than prokaryotes ( $\sim 10^6$  cells mL<sup>-1</sup>), which decreases their likelihood to

disperse (GASTON et al., 2000). *Homogeneous selection* was in general higher in prokaryotes than in picoeukaryotes, which is in line with previous findings in the Pacific Ocean (MILKE et al., 2022). This supports that environmental heterogeneity can act differently on prokaryotic and picoeukaryotic assembly across depths. The reason is likely due to different adaptations to the same environmental heterogeneity of prokaryotes and picoeukaryotes (MASSANA; LOGARES, 2013). For instance, a given degree of environmental heterogeneity could select for a few generalist species that have wide niches or many specialist species with narrow niches or a combination of both strategies. Moreover, the relatively higher *homogeneous selection* in prokaryotes than in picoeukaryotes suggests that dormancy could be playing an important role in modulating prokaryote assembly in the deep ocean. Dormancy is indeed a common mechanism in prokaryotes to overcome harsh environmental conditions (PEDRÓS-ALIÓ, 2021). This mechanism has been shown to affect metacommunity structure by dampening distance-decay relationships and maintaining local diversity (LENNON et al., 2021; LOCEY et al., 2020; WISNOSKI; LEIBOLD; LENNON, 2019). Many prokaryotes reach the deep ocean from the surface through vertical dispersal (MESTRE et al., 2018) or disperse as endospores from sediments (GITTINS et al., 2022). However, DNA-based community composition data includes non-active bacterial cells (ARANDIA-GOROSTIDI; PARADA; DEKAS, 2023), likely in dormancy state, to survive the very different conditions of the dark and cold deep ocean (GITTINS et al., 2022). Therefore, a relatively higher proportion of dormant bacteria can create an apparent ‘homogenization’ of prokaryotic communities in deep zones. In fact, evidence exists that bacteria decrease their activity towards the deep dark ocean [Fig. II-S16] (ARÍSTEGUI et al., 2009; HERNDL et al., 2023). As far as we know, dormancy has not been reported in picoeukaryotes (MASSANA; LOGARES, 2013), which could partially explain the negligible role of *homogeneous selection* in the assembly of this domain in the deep ocean. Finally, we found that the higher spatial turnover (sequential horizontal changes) in picoeukaryotes than in prokaryotes in the surface ocean (LOGARES et al., 2020) is also observed in the deep ocean. Furthermore, we show that this difference in spatial turnover between domains increases with depth, which is coherent with dispersal limitation being an increasingly important processes shaping picoeukaryotic communities in deeper ocean zones.

### ***Potential picoplankton responses to multiple environmental changes across ocean depths***

The global ocean is facing drastic changes in important environmental drivers such as temperature, pH, salinity, and nutrient concentrations (KWIATKOWSKI et al., 2020; SWEETMAN et al., 2017), which are very likely affecting all domains of life, their community structure, and interactions (CHAFFRON et al., 2021). Global climate change is also driving changes in ocean

currents due to shifts in wind patterns, heat balance, and freshwater inflows from glacial melting (HAYS, 2017), which may directly affect plankton dispersal rates (WARD et al., 2021). Water masses have also been modified by anthropogenic changes in temperature and salinity even in the deep ocean (SILVY et al., 2020; ZIKA et al., 2021), which may affect picoplankton community composition (AGOGUÉ et al., 2011; FRANK et al., 2016; RAES et al., 2018) by changing both selection and dispersal assembly processes. Our results suggest that the prokaryotic and eukaryotic components of the ocean's smallest plankton are likely to respond differently to environmental change as a result of the different balance of ecological processes structuring their communities. Prokaryotes seem to be relatively more sensitive to selective forces than picoeukaryotes (LOGARES et al., 2020), so that changes in important environmental drivers (e.g. temperature, organic matter composition) will have a higher potential to affect prokaryotic community composition at a global scale (GÓMEZ-LETONA et al., 2022; LOGARES et al., 2020) than changes in dispersal drivers (e.g. currents, fronts). On the other hand, picoeukaryotic community composition at global scales would be potentially more affected by changes in factors regulating horizontal and vertical dispersal processes – such as current circulation (RICHTER et al., 2022) and thermal stratification (CERMEÑO et al., 2008) – than by environmental drivers. While here we refer to the entire community, specific picoeukaryotic taxa might be strongly structured by environmental drivers (SOMMERIA-KLEIN et al., 2021). Indeed, temperature is well-known to influence relatively more heterotrophic than photosynthetic eukaryotic activity (ROSE; CARON, 2007). For instance, cosmopolitan unicellular picoeukaryotic predators (MAST-4) display clear temperature-driven niche-partitioning in the ocean (LATORRE et al., 2021). After all, in a long timescale, no matter the dispersal rate of a given species, it will eventually be selected and constrained by local abiotic and biotic factors (WARD et al., 2021). Thus, the relative effect of projected changes in environmental selection and dispersal pathways on microbial communities should be evaluated together.

Most importantly, our work suggests that the microbial communities inhabiting the deep ocean are likely to respond differently to environmental changes than those living in the surface ocean. This is particularly relevant in the context of increasing multiple stressors caused by climate change (warming, acidification, and deoxygenation) and human exploitation activities (i.e.: mining, oil and gas extraction, waste disposal) in the deep ocean (LEVIN; LE BRIS, 2015). While upper ocean picoplankton communities would be relatively more sensitive to changes in environmental selective forces (e.g. temperature and nutrient concentration), deep ocean picoplankton communities should be relatively more impacted by the removal or creation of dispersal pathways. In this regard, projected perturbations in temperature, pH, oxygen, and nutrient concentration

(KWIATKOWSKI et al., 2020) should impact relatively more the small plankton communities inhabiting the upper than those in the deep ocean. Yet, changes in air fluxes and ocean currents should also affect the surface picoplankton community (MESTRE; HÖFER, 2021), but relatively less than selective forces. On the other hand, changes in dispersion vectors should be the main factor altering the balance of ecological processes assembling picoplankton communities in the deep ocean. For example, ocean micro- and nanoplastic pollution, a widespread environmental issue (TER HALLE; GHIGLIONE, 2021; VAN SEBILLE et al., 2015a) could represent important substrates for both prokaryotes and single-cell eukaryotes colonization and work as efficient dispersion vectors (AMARAL-ZETTLER; ZETTLER; MINCER, 2020), potentially altering dispersal rates across ocean depth zones. Furthermore, changes in ocean stratification patterns are reducing nutrient exchange and expanding oligotrophic conditions in the upper ocean (POLOVINA; HOWELL; ABECASSIS, 2008). Our vertical profile results suggest that this increased stratification could affect not only microbial selective forces, but also dispersal across depth zones. These changes can ultimately impact important ocean ecosystem services such as primary productivity and nutrient cycling at a global scale (BOPP et al., 2001; CERMEÑO et al., 2008; SARMIENTO et al., 2004).

### ***A conceptual framework for the global biogeography of picoplankton across ocean depths***

Historically, many studies have focused on the effect of *selection* – also referred to as niche-modeling or environmental filtering – on marine microbial communities (AULADELL et al., 2022; GHIGLIONE et al., 2012; SARMENTO; MORANA; GASOL, 2016). Other studies aimed to model how *dispersal* influences microbial biogeography in the global surface ocean (HELLWEGER; VAN SEBILLE; FREDRICK, 2014; JÖNSSON; WATSON, 2016; VAN SEBILLE et al., 2015b; VILLARINO et al., 2018). More recently, there have been important efforts bringing together environmental selection and dispersal in the ocean (RICHTER et al., 2022; VILLARINO et al., 2022; WARD et al., 2021). Nevertheless, besides selection and dispersal, picoplankton community assembly is also ruled by ecological drift (HUBER et al., 2020; LOGARES et al., 2020). Integrating these processes into a single framework considering organism, environmental and physical differences between depth zones was still missing. By combining empirical evidence, we propose a novel conceptual framework that expands the current understanding of plankton community assembly in environmentally distinct ocean depth zones (Fig. II-6). It synthesizes how environmental heterogeneity, water mass structure, deep-sea topography, microbial abundance, and activity mediate the action of ecological processes assembling the two components of the smallest plankton communities (Fig. II-6). This framework can be used to

delineate hypothesis-driven studies to predict how plankton assemblages will respond across depths to multiple stressors in a changing ocean (CHENG et al., 2022). For instance, based on this framework, we can expect that the balance between determinism (selection) and stochasticity (dispersal limitation or ecological drift) would decrease with plankton size. Thus, nano- (3-20  $\mu\text{m}$ ), micro- (20-200  $\mu\text{m}$ ), and mesoplankton (200-2,000  $\mu\text{m}$ ) biogeography would be increasingly limited by dispersal and display more marked biogeography (BRANDÃO et al., 2021; SOMMERIA-KLEIN et al., 2021; VILLARINO et al., 2018), especially in the deep ocean (Fig. II-6). We can also foresee that particle-attached prokaryotes – which are particularly relevant in the deep ocean (SALAZAR et al., 2015) – should be more limited by dispersal than free-living prokaryotes. In general, the importance of dispersal limitation relative to that of selection should increase not only with organism and particle sizes, as expected by the size-dispersal hypothesis (FARJALLA et al., 2012), but also with ocean depth. Here we show that this dispersal-selection balance, regulated by organism size, should be more pronounced in the deep than in the upper ocean.

## **METHODS**

### ***Dataset, sampling, and analytical methods***

We compiled a dataset (Fig. II-1) composed of 451 samples from surface (3 m depth) to deep waters (up to 4,800 m), covering three depth zones of the ocean: epi- (0-200 m – including DCM), meso- (200-1,000 m), and bathypelagic (1,000-4,000 m). This dataset combines samples obtained during two oceanographic expeditions with similar sampling strategies: *i*) the *Malaspina-2010* circumglobal expedition (DUARTE, 2015; ESTRADA et al., 2016) from which we included 263 samples collected between December 2010 and July 2011 in 120 stations distributed along the tropical and subtropical portions (latitudes between 35° N and 40° S) of the Pacific, Atlantic, and Indian oceans (Fig. II-1); and *ii*) the *HotMix* trans-Mediterranean cruise (CATALÁ et al., 2015; SEBASTIÁN et al., 2021) from which we considered 188 samples collected between April and May 2014 in 29 stations distributed along the whole Mediterranean Sea (from -5° W to 33° E) and the adjacent Northeast Atlantic Ocean (Fig. II-1A). This dataset therefore allows the comparison of the tropical and subtropical ocean (samples hereafter called “open ocean”) to a semi-enclosed basin such as the Mediterranean Sea, which displays unique features such as higher temperature and salinity as well as lower nutrient concentration than the open ocean, particularly in the meso- and bathypelagic (Fig. II-1B). The *Malaspina-2010* contains 13 stations where the whole vertical profile was sampled (VP stations in Fig. II-1). A detailed vertical distribution of the samples is available in the Supplementary Material (Fig. II-S1). Due to the difference in the sampling size between depth

zones, we also generated subsets with a standardized number of samples (n=39) evenly-distributed across space (Fig. II-S7 and Fig. II-S8).

This dataset comprises a contextual database with a total of 6 standardized environmental parameters (temperature, salinity, fluorescence,  $\text{PO}_4^{3-}$ ,  $\text{NO}_3^-$ , and  $\text{SiO}_2$ ) as well as prokaryote and picoeukaryote abundances determined by flow cytometry and bacterial activity measurements. Water samples were obtained with 20L (in *Malaspina*) or 12L (in *HotMix*) Niskin bottles attached to a rosette sampler equipped with a conductivity–temperature–depth (CTD) profiler (except surface samples in *Malaspina*, that were obtained with individual 30 L bottles, not attached to the rosette). Vertical profiles of temperature, conductivity, and fluorescence were continuously recorded throughout the water column with the CTD sensors. Conductivity measurements were converted into practical salinity scale values. Inorganic nutrients ( $\text{NO}_3^-$ ,  $\text{PO}_4^{3-}$ ,  $\text{SiO}_2$ ) were measured from the Niskin bottle samples with standard spectrophotometric protocols (GRASSHOFF; KREMLING; ERHARDT, 1999), using a Skalar autoanalyzer SAN++, as described in (ESTRADA et al., 2016; MARTÍNEZ-PÉREZ et al., 2019). Missing nutrient concentration values were extracted from the World Ocean Database (BOYER et al., 2013). Prokaryotic populations and phototrophic picoeukaryotes abundances were enumerated using a FACSCalibur flow cytometer (BD Biosciences, San Jose, CA, USA) as detailed elsewhere (GASOL; MORAN, 2015). Prokaryotic heterotrophic activity was estimated using the centrifugation method and measuring  $^3\text{H}$ -leucine incorporation (SMITH; AZAM, 1992). For deep water samples we used the filtration method with a larger volume and undiluted hot leucine. Significant differences in microbial abundances and bacterial activity between depth zones were tested with an analysis of variance (ANOVA), followed by a Tukey post-hoc test.

To obtain picoplankton biomass, ~4–12 L of seawater were first pre-filtered with a 200- $\mu\text{m}$  net mesh (to remove large organisms and particles). *Malaspina* samples were then sequentially filtered through a 20  $\mu\text{m}$  nylon mesh followed by 3- $\mu\text{m}$  and 0.2- $\mu\text{m}$  polycarbonate filters (47-mm for surface and 142-mm diameter for vertical profiles, Isopore, Merck Millipore, Burlington, MA, USA) using a peristaltic pump. *HotMix* samples were sequentially filtered through 47-mm 3- $\mu\text{m}$  polycarbonate filters (Isopore, Merck Millipore) and 0.2- $\mu\text{m}$  Sterivex units. Filters were flash-frozen in liquid  $\text{N}_2$  and stored at  $-80^\circ\text{C}$  until DNA extraction. Here, only the free-living ‘picoplankton’ size-fraction (0.2–3  $\mu\text{m}$ ) was used in downstream analyses.

### ***Nucleic acid extraction, sequencing, and bioinformatics***

DNA extraction was conducted with a standard phenol-chloroform protocol (MASSANA et al., 1997) for the *Malaspina* surface samples. DNA from the *Malaspina* vertical profile samples was

extracted using the Nucleospin RNAkit (Macherey-Nagel) plus the Nucleospin RNA/DNA Buffer Set (Macherey-Nagel) procedures. *HotMix* DNA samples were extracted using the PowerWater Sterivex™ DNA isolation Kit (MO BIO Laboratories). DNA extracts were quantified with Qubit 1.0 (Thermo Fisher Scientific) and preserved at  $-80^{\circ}\text{C}$ . The same extracts were used for both the 16S and 18S rRNA-gene amplification and all samples were sequenced with the same prokaryotic and eukaryotic primers. The hypervariable V4–V5 ( $\approx 400$  bp) region of the 16S rRNA gene was PCR amplified with the primers 515F-Y (5'-GTGYCAGCMGCCGCGGTAA) -926R (5'-CCGYCAATTYMTTTRAGTTT) to target prokaryotes – both Bacteria and Archaea (PARADA; NEEDHAM; FUHRMAN, 2016). The hypervariable V4 region of the 18S rRNA gene ( $\approx 380$  bp) was PCR amplified with the primers TAREukFWD1 (5'-CCAGCASCYGC GGTAATTCC-3') and TAREukREV3 (5'-ACTTTCGTTCTTGATYRA-3') to target eukaryotes (STOECK et al., 2010). PCR amplification was carried out with a QIAGEN HotStar Taq master mix (Qiagen Inc., Valencia, CA, USA). Amplicon libraries were then paired-end sequenced on an Illumina (San Diego, CA, USA) MiSeq platform ( $2 \times 250$  bp or  $2 \times 300$  bp) at the Research and Testing Laboratory facility, Texas, USA (<https://rtlgenomics.com/>). See details about gene amplification and sequencing in (LOGARES et al., 2020; SEBASTIÁN et al., 2021).

Raw Illumina miSeq reads ( $2 \times 250$  or  $2 \times 300$ ) were processed using DADA2 (CALLAHAN et al., 2016) to determine amplicon sequence variants (ASVs). For the 16S rRNA gene, forward reads were trimmed at 220 bp and reverse reads at 200 bp, whilst for the 18S rRNA gene, we trimmed the forward reads at 240 bp and the reverse reads at 180 bp. Then, for the 16S, the maximum number of expected errors (maxEE) was set to 2 for the forward reads and to 4 for the reverse reads, while for the 18S, the maxEE was set to 7 and 8 for the forward and reverse reads respectively. Error rates for each possible nucleotide substitution type were estimated using a machine learning approach implemented in DADA2 for both the 16S and 18S. Unsurprisingly, error rates increased with decreasing quality score. Finally, DADA2 was used to estimate error rates for both the 16S and 18S genes in order to delineate the ASVs

Prokaryotic ASVs were assigned taxonomy using the naïve Bayesian classifier method (QIONG et al., 2007) alongside the SILVA v.132 database (QUAST et al., 2013) as implemented in DADA2, while Eukaryotic ASVs were BLASTed (ALTSCHUL et al., 1990) against the Protist Ribosomal Reference database [PR<sup>2</sup>, version 4.11.1; (GUILLOU et al., 2013)]. Eukaryotes, chloroplasts, and mitochondria were removed from the 16S ASVs table, while Streptophyta, Metazoa, and nucleomorphs were removed from the 18S ASVs table. Both, the 16S and 18S ASVs tables were rarefied to 20,000 reads per sample with the function *rrarefy* from the Vegan R package. To be consistent with our previous study (LOGARES et al., 2020), for the calculation of

ecological processes and associated analysis, ASVs with total abundances < 100 reads across all samples were removed to avoid PCR and sequencing depth biases. This filtering procedure removed ~5% of the total reads and ~90% of the total ASVs from both the 16S and the 18S rRNA datasets.

Computing analyses were conducted at both the MARBITS bioinformatics platform of the Institut de Ciències del Mar (ICM; <http://marbits.icm.csic.es>) and the MareNostrum (Barcelona Supercomputing Center). Sequences are publicly available at the European Nucleotide Archive (<http://www.ebi.ac.uk/ena>) under accession numbers PRJEB23913 [18S rRNA genes] & PRJEB25224 [16S rRNA genes] for the *Malaspina* expedition; PRJEB23771 [18S rRNA genes] & PRJEB45015 [16S rRNA genes] for the *Malaspina* vertical profiles; and PRJEB44683 [18S rRNA genes] & PRJEB44474 [16S rRNA genes] for the *HotMix* expedition.

### **Phylogenetics**

Phylogenetic trees were built for both the 16S and 18S rRNA gene-datasets using the ASVs full sequences. Raw ASV sequences were firstly aligned against an aligned SILVA template – for 16S rRNA – and an aligned PR<sup>2</sup> template – for 18S rRNA – using mothur (SCHLOSS et al., 2009). Poorly aligned regions or sequences were then removed using trimAl (parameters: -gt 0.3 -st 0.001) (CAPELLA-GUTIÉRREZ; SILLA-MARTÍNEZ; GABALDÓN, 2009). Aligned sequences were also visually curated with seaview v4 (GOUY; GUINDON; GASCUEL, 2010) and sequences with >=40% of gaps were removed. Finally, phylogenetic trees were inferred from the aligned quality-filtered sequences using FastTree v2.1.9 (PRICE; DEHAL; ARKIN, 2009). Additional phylogenetic analyses were carried out with the *picante* R package (KEMBEL et al., 2010).

### **Environmental heterogeneity, water masses characterization, and least-cost distance calculations**

We calculated the average pairwise dissimilarity (*EnvHt*) as an index of environmental heterogeneity based on the main standardized environmental variables: temperature, salinity, fluorescence, PO<sub>4</sub><sup>3-</sup>, NO<sub>3</sub><sup>-</sup>, and SiO<sub>2</sub>. We firstly computed an Euclidean distance matrix for each depth zone using the *vegan* R package and then determined the dissimilarity among samples by dividing the Euclidean distance matrix (*Euc*) by the maximum Euclidean distance (*Euc<sub>max</sub>*) of a given depth zone as described in (HUBER et al., 2020) and summarized here:  $EnvHt = (Euc/Euc_{max}) + 0.001$ . Finally, the mean *EnvHt* (*EnvHt*) was calculated as an estimation of environmental heterogeneity in each depth zone. Significant differences in environmental heterogeneity between depth zones were tested with a Kruskal-Wallis test, followed by a Wicoxon post-hoc test.

The presence of different water masses is an important feature to properly describe the deep dark ocean ecosystem (> 200 m depth). Water masses are well-established water bodies with unique



properties that can be characterized by their thermohaline and chemical features. A water mass is composed of different proportions of one or more water types of a given origin (TOMCZAK, 1999). Here, the percentage of different water types contributing to the water mass composition of each sample (from 200 m to the bottom) was calculated using an optimum multiparameter water mass analysis (KARSTENSEN; TOMCZAK, 1998). This method basically characterizes water types by using conservative variables such as salinity and potential temperature (see (CATALÁ et al., 2015) for details). We have identified 22 and 19 water types in the open ocean and in the Mediterranean Sea, respectively. We computed the dissimilarity (Euclidean distance) between pairwise samples based on their water mass composition (% of each water type) to use in our downstream analysis. A nonmetric multidimensional scaling (NMDS) analysis based on these euclidean distances was conducted to determine the differences among samples.

Least-cost geographical distances were calculated using the 'lc.dist()' function of the *marmap* R package (PANTE; SIMON-BOUHET, 2013). We first computed three transition matrices (using the 'trans.mat()' function) with different minimum depths, corresponding to the epi- (surface), meso- (200 m), and bathypelagic (1,000 m). Each generated transition matrix contained the probability of transition from one cell to adjacent cells of a given bathymetric grid. We used the high-resolution (15 arc-second) GEBCO bathymetric database hosted on the British Oceanographic Data Centre server (<https://www.gebco.net/>). Since the Mediterranean Sea deep waters (>400 m) are completely separated by the Strait of Sicily, the *marmap* algorithm could not calculate the horizontal distance between bathypelagic samples situated in the western and eastern Mediterranean. To deal with this issue, we simulated the vertical trajectory needed to overcome the Strait of Sicily by simply summing each sample's depth to the geographical distances between 'isolated' stations. To calculate the least-cost distances, 'marmap' sets a depth limit for geographic barriers to compute the transition matrices (PANTE; SIMON-BOUHET, 2013). For example, if the limit is set to 0, the program calculates the distance turning around the continents. However, in the case of the Mediterranean Sea, the western and eastern basins are completely isolated (at least horizontally) in depths down to 400m, so the program outputs unrealistic very long distances between western and eastern samples from the deep ocean. To deal with this issue, for these isolated samples, we computed the least-cost distances by calculating the normal geographic distances (geodesic) between samples (not considering geographic barriers) and then summed the vertical distances to theoretically overcome the Strait of Sicily. For example, a western 1,400 m depth sample (1 km deeper than the top of the Strait of Sicily) located 200 km from an eastern 1,400 m depth sample had a final least-cost distance of  $200 \text{ km} + 2 \times 1 \text{ km} = 202 \text{ km}$ .

## ***Quantification of the ecological processes***

The action of ecological processes (selection, dispersal, and drift) were here quantified using a null model approach (STEGEN et al., 2013) that has been successfully applied to microbial ecology studies in diverse aquatic environments (GAZULLA et al., 2022; HUBER et al., 2020; LOGARES et al., 2018; VASS et al., 2020). This analysis consists of two main sequential steps: 1) inference of *selection* from ASV phylogenetic turnover; and 2) inference of *dispersal* and *drift* from ASV compositional turnover (STEGEN et al., 2013). Since the existence of a phylogenetic signal (CAVENDER-BARES et al., 2009) is an assumption of the first step of this method (STEGEN et al., 2013), we first tested whether closely related taxa (based on the 16S and 18S rRNA-gene phylogeny) were more similar in terms of habitat preferences than distantly related taxa. Mantel correlograms between ASVs niche and phylogenetic distances were used to test for a phylogenetic signal in the variables that explained the highest fraction of community variance in each depth zone. We detected a phylogenetic signal within short phylogenetic distances, which is in line with the literature (HUBER et al., 2020; LOGARES et al., 2020; STEGEN et al., 2013).

Having fulfilled this assumption, we determined the phylogenetic turnover using the abundance-weighted  $\beta$ -mean nearest taxon distance ( $\beta$ MNTD) metric (STEGEN et al., 2013), which computes the mean phylogenetic distances between each ASV and its closest relative in each pair of communities (pairwise comparisons). Afterward, we run null models with 999 randomizations to simulate the community turnover by chance ( $\beta$ MNTD<sub>null</sub>), in other words, without *selection* influence (STEGEN et al., 2013). Finally, the  $\beta$ -Nearest Taxon Index ( $\beta$ NTI) was calculated from the differences between the observed  $\beta$ MNTD and the mean  $\beta$ MNTD<sub>null</sub> values. Overall,  $|\beta$ NTI| > 2 indicates that taxa are phylogenetically more or less related than expected by chance, pointing to a strong influence of selection on community assembly (STEGEN et al., 2013). More precisely,  $\beta$ NTI values higher than +2 indicate the action of heterogeneous selection, while  $\beta$ NTI values lower than -2 points out to the action of homogeneous selection (STEGEN et al., 2013).

The  $\beta$ -diversity of communities that were not governed by selection ( $|\beta$ NTI|  $\leq$  2) was evaluated in a second step, which consisted of computing ASV taxonomic turnover to calculate the influence of either dispersal or ecological drift on community structure. To do so, we calculated the Raup-Crick metric (CHASE; MYERS, 2011) based on the Bray-Curtis dissimilarities ( $RC_{\text{bray}}$ ) (STEGEN et al., 2013).  $RC_{\text{bray}}$  compares the measured  $\beta$ -diversity against the  $\beta$ -diversity obtained from null models (999 randomizations), representing a random community assembly (ecological drift). Absolute  $RC_{\text{bray}}$  values smaller than ( $|RC_{\text{bray}}| \leq 0.95$ ) indicate a community assembled by ecological drift alone (i.e., by chance). On the other hand,  $RC_{\text{bray}}$  values  $> +0.95$  or  $< -0.95$

indicate that community assembly is structured by dispersal limitation or homogenizing dispersal, respectively (CHASE; MYERS, 2011). To further investigate the community assembly patterns in each depth zone, we used the ‘betapart’ R package (BASELGA; ORME, 2012) to calculate the partitioning of  $\beta$ -diversity (Jaccard, Sorensen and Bray-Curtis) into turnover or nestedness (BASELGA, 2010).

The relative importance of ecological processes were calculated for each depth zone subset. Additionally, we globally calculated these processes by integrating all depths of both datasets (Fig. II-S5). Since there are processes taking place along the water column (vertically) that may impact the biogeography that we observe horizontally in each depth zone, we also estimated the ecological processes integrating all depths (from 3 to 4,000 m) in each of the 13 vertical profile stations (Fig. II-1A; see also Fig. II-S1 for sample vertical distribution).

### **General analysis**

Distance-based redundancy analyses (dbRDA) were performed on community composition (based on Bray-Curtis dissimilarities) of both prokaryotic (16S rRNA gene) and picoeukaryotic (18S rRNA gene) samples using the ‘capscale()’ function of the *vegan* R package (LEGENDRE; ANDERSON, 1999). Analyses of dissimilarities were conducted using the ‘adonis2()’ function of the *vegan* R package to investigate the percentage of variance in community composition explained by environmental or geographic variables (MCARDLE; ANDERSON, 2001). Classic biogeographic provinces classifications (e.g.: Longhurst provinces; (LONGHURST, 2007)) are only applied to the upper sunlit ocean (above 200 m), while deep-oceanic basins classifications (based on isolated water bodies) are only applied to the deep (bellow 3,500 m) (SALAZAR et al., 2016). Therefore, we here used the classic geographic oceanic basins (South Atlantic Ocean, North Atlantic Ocean, North Pacific Ocean, South Pacific Ocean and Indian Ocean) as a standard categorical explanatory variable to compare the effect of geography between depth zones of the open ocean. For the Mediterranean Sea, we used the sub-basin classification (Levantine Sea, Ionian Sea, Sicily Strait, Tirrenyan Sea, Sardinian Sea, Alboran Sea and Gibraltar Strait), based on Mediterranean internal circulation patterns (BERGAMASCO; MALANOTTE-RIZZOLI, 2010) as well as physico-chemical and biological features (AYATA et al., 2018).

Spearman correlations were computed between  $\beta$ -diversity (bray-curtis and  $\beta$ NTI) and environmental euclidean distances matrices using the ‘cor.test()’ function of the *stats* R package. Spearman correlations were also carried out to test the association between community (bray-curtis dissimilarity) and water masses composition (euclidean distances) in the meso- and bathypelagic. Mantel correlograms were carried out with the ‘mantel.correlog()’ function in *Vegan* to test for the

decrease in picoplankton community similarity ( $\beta$ -diversity) with increasing geographic distances (distance-decay). For the open ocean, we used distance classes of 1,000 km, while for the Mediterranean Sea we used distance classes of 350 km. Sequential differences in picoplankton  $\beta$ -diversity (bray-curtis dissimilarity) were computed in the sampling order of each project (see arrow directions in Fig. II-S15). Statistical differences between zones in sequential bray-curtis values were tested using analysis of variance (ANOVA) followed by a Tukey post-hoc test.

Pearson correlation matrices between diversity metrics and environmental variables were computed using the 'cor()' function and plotted with the *ggcorrplot* R package. Nonmetric multidimensional scaling (NMDS) based on Euclidean distances was used to visualize clustering in water mass composition among ocean depth zones and basins, followed by an analysis of similarities (ANOSIM) to test for differences among groups. The NMDS and ANOSIM were completed using the 'metaMDS()' and 'anosim()' *vegan* functions, respectively. Analysis of variance (ANOVA), followed by a Tukey post-hoc test, was used to test statistical differences in  $\beta$ -diversity metrics (Bray-Curtis,  $\beta$ NTI and  $RC_{\text{bray}}$ ). Differences in environmental heterogeneity values between zones were tested using Kruskal-Wallis, followed by a Wicoxon post-hoc test. Linear regression models were carried out to investigate the influence of water masses (euclidean distance) on community composition (bray-curtis dissimilarity) in each vertical profile. Spearman correlation was used to test correlation between the ecological processes results obtained with the total (unbalanced) dataset and the results found with a standardized sampling size dataset. All statistical analyses were conducted in the R statistical environment (R CORE TEAM, 2014) and all plots were generated using the R package *ggplot2* (WICKHAM, 2016).

### ***Data availability and resources***

DNA sequences and environmental metadata are publicly available at the European Nucleotide Archive (<http://www.ebi.ac.uk/ena>) under accession numbers PRJEB23913 [18S rRNA genes] & PRJEB25224 [16S rRNA genes] for the *Malaspina* expedition; PRJEB23771 [18S rRNA genes] & PRJEB45015 [16S rRNA genes] for the *Malaspina* vertical profiles; and PRJEB44683 [18S rRNA genes] & PRJEB44474 [16S rRNA genes] for the *HotMix* expedition. R-Scripts for calculating the  $\beta$ -NTI and the Raup-Crick metrics are available at [https://github.com/stegen/Stegen\\_etal\\_ISME\\_2013](https://github.com/stegen/Stegen_etal_ISME_2013). The r-scripts used to generate figures and statistical analysis are available at: [https://github.com/pcjunger/EcoProc\\_OceanDepths](https://github.com/pcjunger/EcoProc_OceanDepths)

## REFERENCES

- ACINAS, Silvia G. et al. Deep ocean metagenomes provide insight into the metabolic architecture of bathypelagic microbial communities. **Communications Biology**, [S. l.], v. 4, n. 1, p. 604, 2021. DOI: 10.1038/s42003-021-02112-2. Disponível em: <https://doi.org/10.1038/s42003-021-02112-2>.
- AGOGUÉ, Hélène; LAMY, Dominique; NEAL, Phillip R.; SOGIN, Mitchell L.; HERNDL, Gerhard J. Water mass-specificity of bacterial communities in the North Atlantic revealed by massively parallel sequencing. **Molecular Ecology**, [S. l.], v. 20, n. 2, p. 258–274, 2011. DOI: 10.1111/j.1365-294X.2010.04932.x. Disponível em: <https://onlinelibrary.wiley.com/doi/10.1111/j.1365-294X.2010.04932.x>.
- ALTSCHUL, Stephen F.; GISH, Warren; MILLER, Webb; MYERS, Eugene W.; LIPMAN, David J. Basic local alignment search tool. **Journal of Molecular Biology**, [S. l.], v. 215, n. 3, p. 403–410, 1990. DOI: [https://doi.org/10.1016/S0022-2836\(05\)80360-2](https://doi.org/10.1016/S0022-2836(05)80360-2). Disponível em: <https://www.sciencedirect.com/science/article/pii/S0022283605803602>.
- AMARAL-ZETTLER, Linda A.; ZETTLER, Erik R.; MINCER, Tracy J. Ecology of the plastisphere. **Nature Reviews Microbiology**, [S. l.], v. 18, n. 3, p. 139–151, 2020. DOI: 10.1038/s41579-019-0308-0. Disponível em: <https://doi.org/10.1038/s41579-019-0308-0>.
- ARANDIA-GOROSTIDI, N.; PARADA, A. E.; DEKAS, A. E. Single-cell view of deep-sea microbial activity and intracommunity heterogeneity. **The ISME Journal**, [S. l.], v. 17, n. 1, p. 59–69, 2023. DOI: 10.1038/s41396-022-01324-6. Disponível em: <https://doi.org/10.1038/s41396-022-01324-6>.
- ARÍSTEGUI, Javier; GASOL, Josep M.; DUARTE, Carlos M.; HERNDL, Gerhard J. Microbial oceanography of the dark ocean's pelagic realm. **Limnology and Oceanography**, [S. l.], v. 54, n. 5, p. 1501–1529, 2009. DOI: 10.4319/lo.2009.54.5.1501. Disponível em: <https://doi.org/10.4319/lo.2009.54.5.1501>.
- AULADELL, Adrià; BARBERÁN, Albert; LOGARES, Ramiro; GARCÉS, Esther; GASOL, Josep M.; FERRERA, Isabel. Seasonal niche differentiation among closely related marine bacteria. **The ISME Journal**, [S. l.], v. 16, n. 1, p. 178–189, 2022. DOI: 10.1038/s41396-021-01053-2. Disponível em: <https://doi.org/10.1038/s41396-021-01053-2>.
- AYATA, Sakina-Dorothee et al. Regionalisation of the Mediterranean basin, a MERMEX synthesis. **Progress in Oceanography**, [S. l.], v. 163, p. 7–20, 2018. DOI: <https://doi.org/10.1016/j.pocean.2017.09.016>. Disponível em: <https://www.sciencedirect.com/science/article/pii/S0079661117300393>.
- BALTAR, Federico; ARÍSTEGUI, Javier. Fronts at the Surface Ocean Can Shape Distinct Regions of Microbial Activity and Community Assemblages Down to the Bathypelagic Zone: The Azores Front as a Case Study. **Frontiers in Marine Science**, [S. l.], v. 4, 2017. DOI: 10.3389/fmars.2017.00252. Disponível em: <http://journal.frontiersin.org/article/10.3389/fmars.2017.00252/full>.

BAR-ON, Yinon M.; MILO, Ron. The Biomass Composition of the Oceans: A Blueprint of Our Blue Planet. **Cell**, [S. l.], v. 179, n. 7, p. 1451–1454, 2019. DOI: 10.1016/j.cell.2019.11.018. Disponível em: <https://linkinghub.elsevier.com/retrieve/pii/S0092867419312747>.

BASELGA, Andrés. Partitioning the turnover and nestedness components of beta diversity. **Global Ecology and Biogeography**, [S. l.], v. 19, n. 1, p. 134–143, 2010. DOI: <https://doi.org/10.1111/j.1466-8238.2009.00490.x>. Disponível em: <https://doi.org/10.1111/j.1466-8238.2009.00490.x>.

BASELGA, Andrés; ORME, C. David L. betapart : an R package for the study of beta diversity. **Methods in Ecology and Evolution**, [S. l.], v. 3, n. 5, p. 808–812, 2012. DOI: 10.1111/j.2041-210X.2012.00224.x. Disponível em: <http://doi.wiley.com/10.1111/j.2041-210X.2012.00224.x>. Acesso em: 25 out. 2017.

BELLAAJ ZOUARI, A.; BEL HASSEN, M.; BALAGUÉ, V.; SAHLI, E.; BEN KACEM, MY; AKROUT, F.; HAMZA, A.; MASSANA, R. Picoeukaryotic diversity in the Gulf of Gabès: variability patterns and relationships to nutrients and water masses. **Aquatic Microbial Ecology**, [S. l.], v. 81, n. 1, p. 37–53, 2018. DOI: 10.3354/ame01857. Disponível em: <https://www.int-res.com/abstracts/ame/v81/n1/p37-53/>.

BERGAMASCO, A.; MALANOTTE-RIZZOLI, P. The circulation of the Mediterranean Sea: a historical review of experimental investigations. **Advances in Oceanography and Limnology**, [S. l.], v. 1, n. 1, p. 11–28, 2010. DOI: 10.1080/19475721.2010.491656. Disponível em: <http://www.tandfonline.com/doi/abs/10.1080/19475721.2010.491656>.

BETHOUX, J. P.; GENTILI, B.; MORIN, P.; NICOLAS, E.; PIERRE, C.; RUIZ-PINO, D. The Mediterranean Sea: a miniature ocean for climatic and environmental studies and a key for the climatic functioning of the North Atlantic. **Progress in Oceanography**, [S. l.], v. 44, n. 1, p. 131–146, 1999. DOI: [https://doi.org/10.1016/S0079-6611\(99\)00023-3](https://doi.org/10.1016/S0079-6611(99)00023-3). Disponível em: <https://www.sciencedirect.com/science/article/pii/S0079661199000233>.

BOPP, Laurent; MONFRAY, Patrick; AUMONT, Olivier; DUFRESNE, Jean-Louis; LE TREUT, Hervé; MADEC, Gurvan; TERRAY, Laurent; ORR, James C. Potential impact of climate change on marine export production. **Global Biogeochemical Cycles**, [S. l.], v. 15, n. 1, p. 81–99, 2001. DOI: <https://doi.org/10.1029/1999GB001256>. Disponível em: <https://doi.org/10.1029/1999GB001256>.

BOYER, T. P. et al. World Ocean Database 2013. **NOAA Printing Office**, [S. l.], v. 72, n. NOAA Atlas NESDIS, p. 208pp, 2013. DOI: 10.25607/OBP-1454. Disponível em: <http://hdl.handle.net/11329/357%0A10.25607/OBP-1454>.

BRANDÃO, Manoela C. et al. Macroscale patterns of oceanic zooplankton composition and size structure. **Scientific Reports**, [S. l.], v. 11, n. 1, p. 15714, 2021. DOI: 10.1038/s41598-021-94615-5. Disponível em: <https://doi.org/10.1038/s41598-021-94615-5>.

BRISLAWN, Colin J. et al. Forfeiting the priority effect: turnover defines biofilm community succession. **The ISME Journal**, [S. l.], v. 13, n. 7, p. 1865–1877, 2019. DOI: 10.1038/s41396-019-0396-x. Disponível em: <https://doi.org/10.1038/s41396-019-0396-x>.

BROWN, Mark V; PHILIP, Gayle K.; BUNGE, John A.; SMITH, Matthew C.; BISSETT, Andrew; LAURO, Federico M.; FUHRMAN, Jed A.; DONACHIE, Stuart P. Microbial community structure in the North Pacific ocean. **The ISME Journal**, [S. l.], v. 3, n. 12, p. 1374–1386, 2009. DOI: 10.1038/ismej.2009.86. Disponível em: <https://doi.org/10.1038/ismej.2009.86>.

CALLAHAN, Benjamin J.; MCMURDIE, Paul J.; ROSEN, Michael J.; HAN, Andrew W.; JOHNSON, Amy Jo A.; HOLMES, Susan P. DADA2: High-resolution sample inference from Illumina amplicon data. **Nature Methods**, [S. l.], v. 13, p. 581, 2016. Disponível em: <https://doi.org/10.1038/nmeth.3869>.

CAPELLA-GUTIÉRREZ, Salvador; SILLA-MARTÍNEZ, José M.; GABALDÓN, Toni. trimAl: a tool for automated alignment trimming in large-scale phylogenetic analyses. **Bioinformatics**, [S. l.], v. 25, n. 15, p. 1972–1973, 2009. DOI: 10.1093/bioinformatics/btp348. Disponível em: <https://doi.org/10.1093/bioinformatics/btp348>.

CATALÁ, T. S. et al. Water mass age and aging driving chromophoric dissolved organic matter in the dark global ocean. **Global Biogeochemical Cycles**, [S. l.], v. 29, n. 7, p. 917–934, 2015. DOI: <https://doi.org/10.1002/2014GB005048>. Disponível em: <https://doi.org/10.1002/2014GB005048>.

CAVENDER-BARES, Jeannine; KOZAK, Kenneth H.; FINE, Paul V. A.; KEMBEL, Steven W. The merging of community ecology and phylogenetic biology. **Ecology Letters**, [S. l.], v. 12, n. 7, p. 693–715, 2009. DOI: <https://doi.org/10.1111/j.1461-0248.2009.01314.x>. Disponível em: <https://doi.org/10.1111/j.1461-0248.2009.01314.x>.

CERMEÑO, Pedro; DUTKIEWICZ, Stephanie; HARRIS, Roger P.; FOLLOWS, Mick; SCHOFIELD, Oscar; FALKOWSKI, Paul G. The role of nutricline depth in regulating the ocean carbon cycle. **Proceedings of the National Academy of Sciences**, [S. l.], v. 105, n. 51, p. 20344–20349, 2008. DOI: 10.1073/pnas.0811302106. Disponível em: <https://doi.org/10.1073/pnas.0811302106>.

CHAFFRON, Samuel et al. Environmental vulnerability of the global ocean epipelagic plankton community interactome. **Science Advances**, [S. l.], v. 7, n. 35, p. eabg1921, 2021. DOI: 10.1126/sciadv.abg1921. Disponível em: <https://doi.org/10.1126/sciadv.abg1921>.

CHASE, Jonathan M.; MYERS, Jonathan A. Disentangling the importance of ecological niches from stochastic processes across scales. **Philosophical Transactions of the Royal Society B: Biological Sciences**, 6-9 CARLTON HOUSE TERRACE, LONDON SW1Y 5AG, ENGLAND, v. 366, n. 1576, p. 2351–2363, 2011. DOI: 10.1098/rstb.2011.0063. Disponível em: <https://royalsocietypublishing.org/doi/10.1098/rstb.2011.0063>.

CHENG, Lijing et al. Past and future ocean warming. **Nature Reviews Earth & Environment**, [S. l.], v. 3, n. 11, p. 776–794, 2022. DOI: 10.1038/s43017-022-00345-1. Disponível em: <https://doi.org/10.1038/s43017-022-00345-1>.

CORDIER, Tristan et al. Patterns of eukaryotic diversity from the surface to the deep-ocean sediment. **Science Advances**, [S. l.], v. 8, n. 5, p. eabj9309, 2022. DOI: 10.1126/sciadv.abj9309. Disponível em: <https://doi.org/10.1126/sciadv.abj9309>.

CORNEC, M.; CLAUSTRE, H.; MIGNOT, A.; GUIDI, L.; LACOUR, L.; POTEAU, A.; D'ORTENZIO, F.; GENTILI, B.; SCHMECHTIG, C. Deep Chlorophyll Maxima in the Global Ocean: Occurrences, Drivers and Characteristics. **Global Biogeochemical Cycles**, [S. l.], v. 35, n. 4, p. e2020GB006759, 2021. DOI: <https://doi.org/10.1029/2020GB006759>. Disponível em: <https://doi.org/10.1029/2020GB006759>.

DE BIE, T. et al. Body size and dispersal mode as key traits determining metacommunity structure of aquatic organisms. **Ecology Letters**, [S. l.], v. 15, n. 7, p. 740–747, 2012. DOI: <https://doi.org/10.1111/j.1461-0248.2012.01794.x>. Disponível em: <https://doi.org/10.1111/j.1461-0248.2012.01794.x>.

DE VARGAS, Colomban et al. Eukaryotic plankton diversity in the sunlit ocean. **Science**, [S. l.], v. 348, n. 6237, p. 1261605–1261605, 2015. DOI: 10.1126/science.1261605. Disponível em: <http://www.sciencemag.org/cgi/doi/10.1126/science.1261605>.

DINI-ANDREOTE, Francisco; STEGEN, James C.; VAN ELSAS, Jan Dirk; SALLES, Joana Falcao. Disentangling mechanisms that mediate the balance between stochastic and deterministic processes in microbial succession. **PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA**, 2101 CONSTITUTION AVE NW, WASHINGTON, DC 20418 USA, v. 112, n. 11, p. E1326–E1332, 2015. DOI: 10.1073/pnas.1414261112.

DUARTE, Carlos M. Seafaring in the 21st Century: The Malaspina 2010 Circumnavigation Expedition. **Limnology and Oceanography Bulletin**, [S. l.], v. 24, n. 1, p. 11–14, 2015. DOI: 10.1002/lob.10008. Disponível em: <https://doi.org/10.1002/lob.10008>.

ESTRADA, Marta; DELGADO, Maximino; BLASCO, Dolors; LATASA, Mikel; CABELLO, Ana María; BENÍTEZ-BARRIOS, Verónica; FRAILE-NUEZ, Eugenio; MOZETIČ, Patricija; VIDAL, Montserrat. Phytoplankton across Tropical and Subtropical Regions of the Atlantic, Indian and Pacific Oceans. **PLOS ONE**, [S. l.], v. 11, n. 3, p. e0151699, 2016. DOI: 10.1371/journal.pone.0151699. Disponível em: <https://dx.plos.org/10.1371/journal.pone.0151699>.

FALKOWSKI, Paul G.; FENCHEL, Tom; DELONG, Edward F. The Microbial Engines That Drive Earth's Biogeochemical Cycles. **Science**, [S. l.], v. 320, n. 5879, p. 1034–1039, 2008. DOI: 10.1126/science.1153213. Disponível em: <http://www.sciencemag.org/cgi/doi/10.1126/science.1153213>.

FARJALLA, Vinicius F.; SRIVASTAVA, Diane S.; MARINO, Nicholas a C.; AZEVEDO, Fernanda D.; DIB, Viviane; LOPES, Paloma M.; ROSADO, Alexandre S.; BOZELLI, Reinaldo L.; ESTEVES, Francisco a. Ecological determinism increases with organism size. **Ecology**, [S. l.], v. 93, n. 7, p. 1752–1759, 2012. DOI: 10.1890/11-1144.1. Disponível em: <http://www.ncbi.nlm.nih.gov/pubmed/22919920>.

FODELIANAKIS, Stilianos; VALENZUELA-CUEVAS, Adriana; BAROZZI, Alan; DAFFONCHIO, Daniele. Direct quantification of ecological drift at the population level in synthetic bacterial communities. **The ISME Journal**, [S. l.], v. 15, n. 1, p. 55–66, 2021. DOI: 10.1038/s41396-020-00754-4. Disponível em: <https://doi.org/10.1038/s41396-020-00754-4>.



- FRANK, Alexander H.; GARCIA, Juan A. L.; HERNDL, Gerhard J.; REINTHALER, Thomas. Connectivity between surface and deep waters determines prokaryotic diversity in the North Atlantic Deep Water. **Environmental Microbiology**, [S. l.], v. 18, n. 6, p. 2052–2063, 2016. DOI: 10.1111/1462-2920.13237. Disponível em: <https://onlinelibrary.wiley.com/doi/10.1111/1462-2920.13237>.
- GALAND, Pierre E.; POTVIN, Marianne; CASAMAYOR, Emilio O.; LOVEJOY, Connie. Hydrography shapes bacterial biogeography of the deep Arctic Ocean. **The ISME Journal**, [S. l.], v. 4, n. 4, p. 564–576, 2010. DOI: 10.1038/ismej.2009.134. Disponível em: <https://doi.org/10.1038/ismej.2009.134>.
- GASOL, Josep M.; MORAN, Xosé Anxelu G. Flow Cytometric Determination of Microbial Abundances and Its Use to Obtain Indices of Community Structure and Relative Activity. **Springer Protocols Handbooks**, [S. l.], p. 1–29, 2015. DOI: 10.1007/8623.
- GASTON, Kevin J.; BLACKBURN, Tim M.; GREENWOOD, Jeremy J. D.; GREGORY, Richard D.; QUINN, Rachel M.; LAWTON, John H. Abundance–occupancy relationships. **Journal of Applied Ecology**, [S. l.], v. 37, n. s1, p. 39–59, 2000. DOI: <https://doi.org/10.1046/j.1365-2664.2000.00485.x>. Disponível em: <https://doi.org/10.1046/j.1365-2664.2000.00485.x>.
- GAZULLA, Carlota R.; AULADELL, Adrià; RUIZ-GONZÁLEZ, Clara; JUNGER, Pedro C.; ROYO-LLONCH, Marta; DUARTE, Carlos M.; GASOL, Josep M.; SÁNCHEZ, Olga; FERRERA, Isabel. Global diversity and distribution of aerobic anoxygenic phototrophs in the tropical and subtropical oceans. **Environmental Microbiology**, [S. l.], v. 24, n. 5, p. 2222–2238, 2022. DOI: 10.1111/1462-2920.15835. Disponível em: <https://doi.org/10.1111/1462-2920.15835>.
- GHIGLIONE, Jean-François et al. Pole-to-pole biogeography of surface and deep marine bacterial communities. **Proceedings of the National Academy of Sciences**, [S. l.], v. 109, n. 43, p. 17633–17638, 2012. DOI: 10.1073/pnas.1208160109. Disponível em: <http://www.pnas.org/content/109/43/17633.abstract>.
- GINER, Caterina R.; PERNICE, Massimo C.; BALAGUÉ, Vanessa; DUARTE, Carlos M.; GASOL, Josep M.; LOGARES, Ramiro; MASSANA, Ramon. Marked changes in diversity and relative activity of picoeukaryotes with depth in the world ocean. **The ISME Journal**, [S. l.], v. 14, n. 2, p. 437–449, 2020. DOI: 10.1038/s41396-019-0506-9. Disponível em: <https://doi.org/10.1038/s41396-019-0506-9>.
- GITTINS, Daniel A. et al. Geological processes mediate a microbial dispersal loop in the deep biosphere. **Science Advances**, [S. l.], v. 8, n. 34, 2022. DOI: 10.1126/sciadv.abn3485. Disponível em: <https://www.science.org/doi/10.1126/sciadv.abn3485>.
- GÓMEZ-LETONA, Markel et al. Deep ocean prokaryotes and fluorescent dissolved organic matter reflect the history of the water masses across the Atlantic Ocean. **Progress in Oceanography**, [S. l.], v. 205, p. 102819, 2022. DOI: 10.1016/j.pocean.2022.102819. Disponível em: <https://www.sciencedirect.com/science/article/pii/S0079661122000805>.

GOUY, Manolo; GUINDON, Stéphane; GASCUEL, Olivier. SeaView Version 4: A Multiplatform Graphical User Interface for Sequence Alignment and Phylogenetic Tree Building. **Molecular Biology and Evolution**, [S. l.], v. 27, n. 2, p. 221–224, 2010. DOI: 10.1093/molbev/msp259. Disponível em: <https://doi.org/10.1093/molbev/msp259>.

GRASSHOFF, K.; KREMLING, K.; ERHARDT, M. **Methods of seawater analysis**. 3rd. ed. Weinheim, Germany: Wiley-VCH Verlag, 1999.

GUIDI, Lionel et al. Plankton networks driving carbon export in the oligotrophic ocean. **Nature**, [S. l.], v. 532, n. 7600, p. 465–470, 2016. DOI: 10.1038/nature16942. Disponível em: <http://dx.doi.org/10.1038/nature16942>.

GUILLOU, Laure et al. The Protist Ribosomal Reference database (PR2): a catalog of unicellular eukaryote Small Sub-Unit rRNA sequences with curated taxonomy. **Nucleic Acids Research**, [S. l.], v. 41, n. D1, p. D597–D604, 2013. DOI: 10.1093/nar/gks1160. Disponível em: <https://doi.org/10.1093/nar/gks1160>.

HANSON, China A.; FUHRMAN, Jed A.; HORNER-DEVINE, M. Claire; MARTINY, Jennifer B. H. Beyond biogeographic patterns: processes shaping the microbial landscape. **NATURE REVIEWS MICROBIOLOGY**, MACMILLAN BUILDING, 4 CRINAN ST, LONDON N1 9XW, ENGLAND, v. 10, n. 7, p. 497–506, 2012. DOI: 10.1038/nrmicro2795.

HAYS, Graeme C. Ocean currents and marine life. **Current Biology**, [S. l.], v. 27, n. 11, p. R470–R473, 2017. DOI: <https://doi.org/10.1016/j.cub.2017.01.044>. Disponível em: <https://www.sciencedirect.com/science/article/pii/S0960982217300775>.

HEINO, Jani; MELO, Adriano S.; SIQUEIRA, Tadeu; SOININEN, Janne; VALANKO, Sebastian; BINI, Luis Mauricio. Metacommunity organisation, spatial extent and dispersal in aquatic systems: patterns, processes and prospects. **Freshwater Biology**, [S. l.], v. 60, n. 5, p. 845–869, 2015. DOI: <https://doi.org/10.1111/fwb.12533>. Disponível em: <https://doi.org/10.1111/fwb.12533>.

HELLWEGER, Ferdi L.; VAN SEBILLE, Erik; FREDRICK, Neil D. Biogeographic patterns in ocean microbes emerge in a neutral agent-based model. **Science**, [S. l.], v. 345, n. 6202, p. 1346–1349, 2014. DOI: 10.1126/science.1254421. Disponível em: <http://www.sciencemag.org/cgi/doi/10.1126/science.1254421>.

HERNDL, Gerhard J.; BAYER, Barbara; BALTAR, Federico; REINTHALER, Thomas. Prokaryotic Life in the Deep Ocean's Water Column. **Annual Review of Marine Science**, [S. l.], v. 15, n. 1, 2023. DOI: 10.1146/annurev-marine-032122-115655. Disponível em: <https://doi.org/10.1146/annurev-marine-032122-115655>.

HUBBELL, Stephen P. **The unified neutral theory of biodiversity and biogeography**. Princeton (NJ): Princeton University Press, 2001.

HUBER, Paula; METZ, Sebastian; UNREIN, Fernando; MAYORA, Gisela; SARMENTO, Hugo; DEVERCELLI, Melina. Environmental heterogeneity determines the ecological processes that govern bacterial metacommunity assembly in a floodplain river system. **The ISME Journal**, [S. l.],

v. 14, n. 12, p. 2951–2966, 2020. DOI: 10.1038/s41396-020-0723-2. Disponible em: <http://www.nature.com/articles/s41396-020-0723-2>.

JIE, Kong; LEI, Wang; CAI, Lin; FANGFANG, Kuang; XIWU, Zhou; A., Laws Edward; PING, Sun; HAO, Huang; BANGQIN, Huang. Contrasting Community Assembly Mechanisms Underlie Similar Biogeographic Patterns of Surface Microbiota in the Tropical North Pacific Ocean. **Microbiology Spectrum**, [S. l.], v. 10, n. 1, p. e00798-21, 2022. DOI: 10.1128/spectrum.00798-21. Disponible em: <https://doi.org/10.1128/spectrum.00798-21>.

JÖNSSON, Bror F.; WATSON, James R. The timescales of global surface-ocean connectivity. **Nature Communications**, [S. l.], v. 7, n. 1, p. 11239, 2016. DOI: 10.1038/ncomms11239. Disponible em: <https://doi.org/10.1038/ncomms11239>.

KARSTENSEN, Johannes; TOMCZAK, Matthias. Age determination of mixed water masses using CFC and oxygen data. **Journal of Geophysical Research: Oceans**, [S. l.], v. 103, n. C9, p. 18599–18609, 1998. DOI: <https://doi.org/10.1029/98JC00889>. Disponible em: <https://doi.org/10.1029/98JC00889>.

KEMBEL, Steven W.; COWAN, Peter D.; HELMUS, Matthew R.; CORNWELL, William K.; MORLON, Helene; ACKERLY, David D.; BLOMBERG, Simon P.; WEBB, Campbell O. Picante: R tools for integrating phylogenies and ecology. **Bioinformatics**, [S. l.], v. 26, n. 11, p. 1463–1464, 2010. DOI: 10.1093/bioinformatics/btq166. Disponible em: <https://doi.org/10.1093/bioinformatics/btq166>.

KROM, M. D.; KRESS, N.; BRENNER, S.; GORDON, L. I. Phosphorus limitation of primary productivity in the eastern Mediterranean Sea. **Limnology and Oceanography**, [S. l.], v. 36, n. 3, p. 424–432, 1991. DOI: <https://doi.org/10.4319/lo.1991.36.3.0424>. Disponible em: <https://doi.org/10.4319/lo.1991.36.3.0424>.

KWIATKOWSKI, Lester et al. Twenty-first century ocean warming, acidification, deoxygenation, and upper-ocean nutrient and primary production decline from CMIP6 model projections. **Biogeosciences**, [S. l.], v. 17, n. 13, p. 3439–3470, 2020. DOI: 10.5194/bg-17-3439-2020. Disponible em: <https://bg.copernicus.org/articles/17/3439/2020/>.

LATORRE, Francisco et al. Niche adaptation promoted the evolutionary diversification of tiny ocean predators. **Proceedings of the National Academy of Sciences**, [S. l.], v. 118, n. 25, p. e2020955118, 2021. DOI: 10.1073/pnas.2020955118. Disponible em: <https://doi.org/10.1073/pnas.2020955118>.

LEGENDRE, Pierre; ANDERSON, Marti J. Distance-based redundancy analysis: testing multispecies response in multifactorial ecological experiments. **Ecological Monographs**, [S. l.], v. 69, n. 1, p. 1–24, 1999. DOI: /10.1890/0012-9615(1999)069[0001:DBRATM]2.0.CO;2. Disponible em: [https://doi.org/10.1890/0012-9615\(1999\)069\[0001:DBRATM\]2.0.CO](https://doi.org/10.1890/0012-9615(1999)069[0001:DBRATM]2.0.CO).

LENNON, Jay T.; DEN HOLLANDER, Frank; WILKE-BERENGUER, Maite; BLATH, Jochen. Principles of seed banks and the emergence of complexity from dormancy. **Nature**

**Communications**, [S. l.], v. 12, n. 1, p. 4807, 2021. DOI: 10.1038/s41467-021-24733-1. Disponível em: <https://doi.org/10.1038/s41467-021-24733-1>.

LEVIN, Lisa A.; LE BRIS, Nadine. The deep ocean under climate change. **Science**, [S. l.], v. 350, n. 6262, p. 766–768, 2015. DOI: 10.1126/science.aad0126. Disponível em: <https://doi.org/10.1126/science.aad0126>.

LÉVY, Marina; JAHN, Oliver; DUTKIEWICZ, Stephanie; FOLLOWS, Michael J.; D’OVIDIO, Francesco. The dynamical landscape of marine phytoplankton diversity. **Journal of The Royal Society Interface**, [S. l.], v. 12, n. 111, p. 20150481, 2015. DOI: 10.1098/rsif.2015.0481. Disponível em: <https://doi.org/10.1098/rsif.2015.0481>.

LOCEY, K. J.; MUSCARELLA, M. E.; LARSEN, M. L.; BRAY, S. R.; JONES, S. E.; LENNON, J. T. Dormancy dampens the microbial distance–decay relationship. **Philosophical Transactions of the Royal Society B: Biological Sciences**, [S. l.], v. 375, n. 1798, p. 20190243, 2020. DOI: 10.1098/rstb.2019.0243. Disponível em: <https://doi.org/10.1098/rstb.2019.0243>.

LOGARES, Ramiro et al. Disentangling the mechanisms shaping the surface ocean microbiota. **Microbiome**, [S. l.], v. 8, n. 1, p. 55, 2020. DOI: 10.1186/s40168-020-00827-8. Disponível em: <https://microbiomejournal.biomedcentral.com/articles/10.1186/s40168-020-00827-8>.

LOGARES, Ramiro; TESSON, Sylvie V. M.; CANBÄCK, Björn; PONTARP, Mikael; HEDLUND, Katarina; RENGEFORS, Karin. Contrasting prevalence of selection and drift in the community structuring of bacteria and microbial eukaryotes. **Environmental Microbiology**, [S. l.], v. 20, n. 6, p. 2231–2240, 2018. DOI: 10.1111/1462-2920.14265. Disponível em: <https://onlinelibrary.wiley.com/doi/10.1111/1462-2920.14265>.

LONGHURST, Alan Reece. **Ecological Geography of the Sea**. Second ed. Burlington, MA: Academic Press, 2007.

LOUCA, Stilianos. The rates of global bacterial and archaeal dispersal. **The ISME Journal**, [S. l.], v. 16, n. 1, p. 159–167, 2022. DOI: 10.1038/s41396-021-01069-8. Disponível em: <https://doi.org/10.1038/s41396-021-01069-8>.

LUNA, Gian Marco; CHIGGIATO, Jacopo; QUERO, Grazia Marina; SCHROEDER, Katrin; BONGIORNI, Lucia; KALENITCHENKO, Dimitri; GALAND, Pierre E. Dense water plumes modulate richness and productivity of deep sea microbes. **Environmental Microbiology**, [S. l.], v. 18, n. 12, p. 4537–4548, 2016. DOI: <https://doi.org/10.1111/1462-2920.13510>. Disponível em: <https://doi.org/10.1111/1462-2920.13510>.

MARTÍNEZ-PÉREZ, Alba María; CATALÁ, Teresa S.; NIETO-CID, Mar; OTERO, Jaime; ÁLVAREZ, Marta; EMELIANOV, Mikhail; RECHE, Isabel; ÁLVAREZ-SALGADO, Xosé Antón; ARÍSTEGUI, Javier. Dissolved organic matter (DOM) in the open Mediterranean Sea. II: Basin-wide distribution and drivers of fluorescent DOM. **Progress in Oceanography**, [S. l.], v. 170, p. 93–106, 2019. DOI: <https://doi.org/10.1016/j.pocean.2018.10.019>. Disponível em: <https://www.sciencedirect.com/science/article/pii/S0079661117303579>.

- MASSANA, R.; MURRAY, A. E.; PRESTON, C. M.; DELONG, E. F. Vertical distribution and phylogenetic characterization of marine planktonic Archaea in the Santa Barbara Channel. **Applied and Environmental Microbiology**, [S. l.], v. 63, n. 1, p. 50–56, 1997. DOI: 10.1128/aem.63.1.50-56.1997. Disponível em: <https://journals.asm.org/doi/10.1128/aem.63.1.50-56.1997>.
- MASSANA, Ramon. Eukaryotic Picoplankton in Surface Oceans. **Annual Review of Microbiology**, [S. l.], v. 65, n. 1, p. 91–110, 2011. DOI: 10.1146/annurev-micro-090110-102903. Disponível em: <https://www.annualreviews.org/doi/10.1146/annurev-micro-090110-102903>.
- MASSANA, Ramon; LOGARES, Ramiro. Eukaryotic versus prokaryotic marine picoplankton ecology. **Environmental Microbiology**, [S. l.], v. 15, n. 5, p. 1254–1261, 2013. DOI: 10.1111/1462-2920.12043. Disponível em: <https://doi.org/10.1111/1462-2920.12043>.
- MAYOL, Eva et al. Long-range transport of airborne microbes over the global tropical and subtropical ocean. **Nature Communications**, [S. l.], v. 8, n. 1, p. 201, 2017. DOI: 10.1038/s41467-017-00110-9. Disponível em: <https://doi.org/10.1038/s41467-017-00110-9>.
- MCARDLE, Brian H.; ANDERSON, Marti J. Fitting multivariate models to community data: A comment on distance-based redundancy analysis. **Ecology**, [S. l.], v. 82, n. 1, p. 290–297, 2001. DOI: [https://doi.org/10.1890/0012-9658\(2001\)082\[0290:FMMTCD\]2.0.CO;2](https://doi.org/10.1890/0012-9658(2001)082[0290:FMMTCD]2.0.CO;2). Disponível em: [https://doi.org/10.1890/0012-9658\(2001\)082\[0290:FMMTCD\]2.0.CO](https://doi.org/10.1890/0012-9658(2001)082[0290:FMMTCD]2.0.CO).
- MESTRE, Mireia; HÖFER, Juan. The Microbial Conveyor Belt: Connecting the Globe through Dispersion and Dormancy. **Trends in Microbiology**, [S. l.], v. 29, n. 6, p. 482–492, 2021. DOI: <https://doi.org/10.1016/j.tim.2020.10.007>. Disponível em: <https://www.sciencedirect.com/science/article/pii/S0966842X20302699>.
- MESTRE, Mireia; RUIZ-GONZÁLEZ, Clara; LOGARES, Ramiro; DUARTE, Carlos M.; GASOL, Josep M.; SALA, M. Montserrat. Sinking particles promote vertical connectivity in the ocean microbiome. **Proceedings of the National Academy of Sciences**, [S. l.], v. 115, n. 29, p. E6799 LP-E6807, 2018. DOI: 10.1073/pnas.1802470115. Disponível em: <http://www.pnas.org/content/115/29/E6799.abstract>.
- MILKE, Felix; WAGNER-DOEBLER, Irene; WIENHAUSEN, Gerrit; SIMON, Meinhard. Selection, drift and community interactions shape microbial biogeographic patterns in the Pacific Ocean. **The ISME Journal**, [S. l.], v. 16, n. 12, p. 2653–2665, 2022. DOI: 10.1038/s41396-022-01318-4. Disponível em: <https://doi.org/10.1038/s41396-022-01318-4>.
- MOD, Heidi K.; CHEVALIER, Mathieu; LUOTO, Miska; GUIBAN, Antoine. Scale dependence of ecological assembly rules: Insights from empirical datasets and joint species distribution modelling. **Journal of Ecology**, [S. l.], v. 108, n. 5, p. 1967–1977, 2020. DOI: <https://doi.org/10.1111/1365-2745.13434>. Disponível em: <https://doi.org/10.1111/1365-2745.13434>.
- MORALES, Sergio E.; MEYER, Moana; CURRIE, Kim; BALTAR, Federico. Are oceanic fronts ecotones? Seasonal changes along the subtropical front show fronts as bacterioplankton transition zones but not diversity hotspots. **Environmental Microbiology Reports**, [S. l.], v. 10, n. 2, p. 184–

189, 2018. DOI: 10.1111/1758-2229.12618. Disponible em:  
<https://onlinelibrary.wiley.com/doi/10.1111/1758-2229.12618>.

NEAVE, Erika F.; SEIM, Harvey; GIFFORD, Scott M.; TORANO, Olivia; JOHNSON, Zackary I.; PÁEZ-ROSAS, Diego; MARCHETTI, Adrian. Protistan plankton communities in the Galápagos Archipelago respond to changes in deep water masses resulting from the 2015/16 El Niño. **Environmental Microbiology**, [S. l.], v. 24, n. 4, p. 1746–1759, 2022. DOI:  
<https://doi.org/10.1111/1462-2920.15863>. Disponible em: <https://doi.org/10.1111/1462-2920.15863>.

NEMERGUT, Diana R. et al. Patterns and Processes of Microbial Community Assembly. **MICROBIOLOGY AND MOLECULAR BIOLOGY REVIEWS**, 1752 N ST NW, WASHINGTON, DC 20036-2904 USA, v. 77, n. 3, p. 342–356, 2013. DOI:  
10.1128/MMBR.00051-12.

NIHOUL, Jacques C. J. Oceanography of Semi-Enclosed Seas: Medalpex : an international field experiment in the Western Mediterranean. In: NIHOUL, Jacques C. J. B. T. Elsevier Oceanography Series (org.). **Hydrodynamics of Semi-Enclosed Seas**. [s.l.] : Elsevier, 1982. v. 34p. 1–12. DOI:  
[https://doi.org/10.1016/S0422-9894\(08\)71236-4](https://doi.org/10.1016/S0422-9894(08)71236-4). Disponible em:  
<https://www.sciencedirect.com/science/article/pii/S0422989408712364>.

PANTE, Eric; SIMON-BOUHET, Benoit. marmap: A Package for Importing, Plotting and Analyzing Bathymetric and Topographic Data in R. **PLOS ONE**, [S. l.], v. 8, n. 9, p. e73051, 2013. Disponible em: <https://doi.org/10.1371/journal.pone.0073051>.

PARADA, Alma E.; NEEDHAM, David M.; FUHRMAN, Jed A. Every base matters: assessing small subunit rRNA primers for marine microbiomes with mock communities, time series and global field samples. **Environmental Microbiology**, [S. l.], v. 18, n. 5, p. 1403–1414, 2016. DOI:  
10.1111/1462-2920.13023. Disponible em: <https://doi.org/10.1111/1462-2920.13023>.

PEDRÓS-ALIÓ, Carlos. Time travel in microorganisms. **Systematic and applied microbiology**, Department of Systems Biology, Centro Nacional de Biotecnología (CSIC), c/ Darwin 3, 28049 Madrid, Spain. Electronic address: cpedros@cnb.csic.es., v. 44, n. 4, p. 126227, 2021. DOI:  
10.1016/j.syapm.2021.126227. Disponible em: <http://europepmc.org/abstract/MED/34252729>.

PERNICE, Massimo C.; GINER, Caterina R.; LOGARES, Ramiro; PERERA-BEL, Júlia; ACINAS, Silvia G.; DUARTE, Carlos M.; GASOL, Josep M.; MASSANA, Ramon. Large variability of bathypelagic microbial eukaryotic communities across the world's oceans. **The ISME Journal**, [S. l.], v. 10, n. 4, p. 945–958, 2016. DOI: 10.1038/ismej.2015.170. Disponible em:  
<https://doi.org/10.1038/ismej.2015.170>.

POLOVINA, Jeffrey J.; HOWELL, Evan A.; ABECASSIS, Melanie. Ocean's least productive waters are expanding. **Geophysical Research Letters**, [S. l.], v. 35, n. 3, 2008. DOI:  
<https://doi.org/10.1029/2007GL031745>. Disponible em: <https://doi.org/10.1029/2007GL031745>.

PRICE, Morgan N.; DEHAL, Paramvir S.; ARKIN, Adam P. FastTree: Computing Large Minimum Evolution Trees with Profiles instead of a Distance Matrix. **Molecular Biology and Evolution**, [S.

*l.*], v. 26, n. 7, p. 1641–1650, 2009. DOI: 10.1093/molbev/msp077. Disponível em: <https://doi.org/10.1093/molbev/msp077>.

QIONG, Wang; M., Garrity George; M., Tiedje James; R., Cole James. Naïve Bayesian Classifier for Rapid Assignment of rRNA Sequences into the New Bacterial Taxonomy. **Applied and Environmental Microbiology**, [*S. l.*], v. 73, n. 16, p. 5261–5267, 2007. DOI: 10.1128/AEM.00062-07. Disponível em: <https://doi.org/10.1128/AEM.00062-07>.

QUAST, Christian; PRUESSE, Elmar; YILMAZ, Pelin; GERKEN, Jan; SCHWEER, Timmy; YARZA, Pablo; PEPLIES, Jörg; GLÖCKNER, Frank Oliver. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. **Nucleic Acids Research**, [*S. l.*], v. 41, n. D1, p. D590–D596, 2013. DOI: 10.1093/nar/gks1219. Disponível em: <https://doi.org/10.1093/nar/gks1219>.

R CORE TEAM. **R: A Language and Environment for Statistical Computing**. R Foundation for Statistical Computing Vienna, Austria, 2014. Disponível em: <http://www.r-project.org/>.

RAES, Eric J.; BODROSSY, Levente; VAN DE KAMP, Jodie; BISSETT, Andrew; OSTROWSKI, Martin; BROWN, Mark V.; SOW, Swan L. S.; SLOYAN, Bernadette; WAITE, Anya M. Oceanographic boundaries constrain microbial diversity gradients in the South Pacific Ocean. **Proceedings of the National Academy of Sciences**, [*S. l.*], v. 115, n. 35, p. E8266–E8275, 2018. DOI: 10.1073/pnas.1719335115. Disponível em: <http://www.pnas.org/lookup/doi/10.1073/pnas.1719335115>.

REID, J. L. On the mid-depth circulation of the world ocean. **Evolution of Physical Oceanography**, [*S. l.*], v. 623, p. 70–111, 1981.

REN, Yanhu; LUO, Zhenhao; LIU, Qian; WEI, Bin; WU, Yue-Hong; SHU, Wen-Sheng; XU, Xue-Wei. Insights into community assembly mechanisms, biogeography, and metabolic potential of particle-associated and free-living prokaryotes in tropical oligotrophic surface oceans. **Frontiers in Marine Science**, [*S. l.*], v. 9, 2022. DOI: 10.3389/fmars.2022.923295. Disponível em: <https://www.frontiersin.org/articles/10.3389/fmars.2022.923295/full>.

RICHTER, Daniel J. et al. Genomic evidence for global ocean plankton biogeography shaped by large-scale current systems. **eLife**, [*S. l.*], v. 11, p. e78129, 2022. DOI: 10.7554/eLife.78129. Disponível em: <https://doi.org/10.7554/eLife.78129>.

RIGONATO, Janaina et al. Insights into biotic and abiotic modulation of ocean mesopelagic communities. **bioRxiv**, [*S. l.*], p. 2021.02.26.433055, 2021. DOI: 10.1101/2021.02.26.433055. Disponível em: <http://biorxiv.org/content/early/2021/02/27/2021.02.26.433055.abstract>.

ROSE, Julie M.; CARON, David A. Does low temperature constrain the growth rates of heterotrophic protists? Evidence and implications for algal blooms in cold waters. **Limnology and Oceanography**, [*S. l.*], v. 52, n. 2, p. 886–895, 2007. DOI: <https://doi.org/10.4319/lo.2007.52.2.0886>. Disponível em: <https://doi.org/10.4319/lo.2007.52.2.0886>.

- RUIZ-GONZÁLEZ, Clara et al. Higher contribution of globally rare bacterial taxa reflects environmental transitions across the surface ocean. **Molecular Ecology**, [S. l.], v. 28, n. 8, p. 1930–1945, 2019. DOI: 10.1111/mec.15026. Disponível em: <https://onlinelibrary.wiley.com/doi/10.1111/mec.15026>.
- SALAZAR, Guillem et al. Particle-association lifestyle is a phylogenetically conserved trait in bathypelagic prokaryotes. **Molecular Ecology**, [S. l.], v. 24, n. 22, 2015. DOI: 10.1111/mec.13419.
- SALAZAR, Guillem; CORNEJO-CASTILLO, Francisco M.; BENÍTEZ-BARRIOS, Verónica; FRAILE-NUEZ, Eugenio; ÁLVAREZ-SALGADO, X. Antón; DUARTE, Carlos M.; GASOL, Josep M.; ACINAS, Silvia G. Global diversity and biogeography of deep-sea pelagic prokaryotes. **The ISME Journal**, [S. l.], v. 10, n. 3, 2016. DOI: 10.1038/ismej.2015.137.
- SAMMARTINO, S.; GARCÍA LAFUENTE, J.; NARANJO, C.; SÁNCHEZ GARRIDO, J. C.; SÁNCHEZ LEAL, R.; SÁNCHEZ ROMÁN, A. Ten years of marine current measurements in Espartel Sill, Strait of Gibraltar. **Journal of Geophysical Research: Oceans**, [S. l.], v. 120, n. 9, p. 6309–6328, 2015. DOI: <https://doi.org/10.1002/2014JC010674>. Disponível em: <https://doi.org/10.1002/2014JC010674>.
- SARMENTO, Hugo; MORANA, Cédric; GASOL, Josep M. Bacterioplankton niche partitioning in the use of phytoplankton-derived dissolved organic carbon: quantity is more important than quality. **The ISME Journal**, [S. l.], v. 10, n. 11, p. 2582–2592, 2016. DOI: 10.1038/ismej.2016.66. Disponível em: <https://doi.org/10.1038/ismej.2016.66>.
- SARMIENTO, J. L. et al. Response of ocean ecosystems to climate warming. **Global Biogeochemical Cycles**, [S. l.], v. 18, n. 3, 2004. DOI: <https://doi.org/10.1029/2003GB002134>. Disponível em: <https://doi.org/10.1029/2003GB002134>.
- SCHLOSS, Patrick D. et al. Introducing mothur: Open-Source, Platform-Independent, Community-Supported Software for Describing and Comparing Microbial Communities. **Applied and Environmental Microbiology**, [S. l.], v. 75, n. 23, p. 7537–7541, 2009. DOI: 10.1128/AEM.01541-09. Disponível em: <https://doi.org/10.1128/AEM.01541-09>.
- SEBASTIÁN, Marta; ORTEGA-RETUERTA, Eva; GÓMEZ-CONSARNAU, Laura; ZAMANILLO, Marina; ÁLVAREZ, Marta; ARÍSTEGUI, Javier; GASOL, Josep M. Environmental gradients and physical barriers drive the basin-wide spatial structuring of Mediterranean Sea and adjacent eastern Atlantic Ocean prokaryotic communities. **Limnology and Oceanography**, [S. l.], v. 66, n. 12, p. 4077–4095, 2021. DOI: 10.1002/lno.11944. Disponível em: <https://onlinelibrary.wiley.com/doi/10.1002/lno.11944>.
- SEVERIN, Tatiana et al. Impact of an intense water column mixing (0–1500 m) on prokaryotic diversity and activities during an open-ocean convection event in the NW Mediterranean Sea. **Environmental Microbiology**, [S. l.], v. 18, n. 12, p. 4378–4390, 2016. DOI: <https://doi.org/10.1111/1462-2920.13324>. Disponível em: <https://doi.org/10.1111/1462-2920.13324>.
- SHERR, Evelyn B.; SHERR, Barry F. Understanding roles of microbes in marine pelagic food webs: a brief history. In: **Microbial Ecology of the Oceans**. [s.l.: s.n.]. p. 27–44.



SILVY, Yona; GUILYARDI, Eric; SALLÉE, Jean-Baptiste; DURACK, Paul J. Human-induced changes to the global ocean water masses and their time of emergence. **Nature Climate Change**, [S. l.], v. 10, n. 11, p. 1030–1036, 2020. DOI: 10.1038/s41558-020-0878-x. Disponível em: <https://doi.org/10.1038/s41558-020-0878-x>.

SMITH, David C.; AZAM, Farooq. A simple, economical method for measuring bacterial protein synthesis rates in seawater using 3H-leucine. **Marine Microbial Food Webs**, [S. l.], v. 6, n. 2, p. 107–114, 1992.

SOMMERIA-KLEIN, Guilhem; WATTEAUX, Romain; IBARBALZ, Federico M.; KARLUSICH, Juan José Pierella; IUDICONE, Daniele; BOWLER, Chris; MORLON, Hélène. Global drivers of eukaryotic plankton biogeography in the sunlit ocean. **Science**, [S. l.], v. 374, n. 6567, p. 594–599, 2021. DOI: 10.1126/science.abb3717. Disponível em: <http://biorxiv.org/content/early/2020/12/24/2020.09.08.287524.abstract>.

SOUKISSIAN, Takvor; DENAXA, Dimitra; KARATHANASI, Flora; PROSPATHOPOULOS, Aristides; SARANTAKOS, Konstantinos; IONA, Athanasia; GEORGANTAS, Konstantinos; MAVRAKOS, Spyridon. Marine Renewable Energy in the Mediterranean Sea: Status and Perspectives. **Energies**, [S. l.], v. 10, n. 10, p. 1512, 2017. DOI: 10.3390/en10101512. Disponível em: <http://www.mdpi.com/1996-1073/10/10/1512>.

STEGEN, James C.; LIN, Xueju; FREDRICKSON, Jim K.; CHEN, Xingyuan; KENNEDY, David W.; MURRAY, Christopher J.; ROCKHOLD, Mark L.; KONOPKA, Allan. Quantifying community assembly processes and identifying features that impose them. **The ISME Journal**, [S. l.], v. 7, n. 11, p. 2069–2079, 2013. DOI: 10.1038/ismej.2013.93. Disponível em: <http://dx.doi.org/10.1038/ismej.2013.93>.

STOECK, Thorsten; BASS, David; NEBEL, Markus; CHRISTEN, Richard; JONES, Meredith D. M.; BREINER, Hans-Werner; RICHARDS, Thomas A. Multiple marker parallel tag environmental DNA sequencing reveals a highly complex eukaryotic community in marine anoxic water. **Molecular Ecology**, [S. l.], v. 19, n. s1, p. 21–31, 2010. DOI: 10.1111/j.1365-294X.2009.04480.x. Disponível em: <https://doi.org/10.1111/j.1365-294X.2009.04480.x>.

SUN, Ping; WANG, Ying; HUANG, Xin; HUANG, Bangqin; WANG, Lei. Water masses and their associated temperature and cross-domain biotic factors co-shape upwelling microbial communities. **Water Research**, [S. l.], v. 215, p. 118274, 2022. DOI: <https://doi.org/10.1016/j.watres.2022.118274>. Disponível em: <https://www.sciencedirect.com/science/article/pii/S0043135422002378>.

SUNAGAWA, Shinichi et al. Structure and function of the global ocean microbiome. **Science**, [S. l.], v. 348, n. 6237, p. 1261359, 2015. DOI: 10.1126/science.1261359. Disponível em: <https://www.science.org/doi/10.1126/science.1261359>.

SWEETMAN, Andrew K. et al. Major impacts of climate change on deep-sea benthic ecosystems. **Elementa: Science of the Anthropocene**, [S. l.], v. 5, p. 4, 2017. DOI: 10.1525/elementa.203. Disponível em: <https://doi.org/10.1525/elementa.203>.

TER HALLE, Alexandra; GHIGLIONE, Jean François. Nanoplastics: A Complex, Polluting Terra Incognita. **Environmental Science & Technology**, [S. l.], v. 55, n. 21, p. 14466–14469, 2021. DOI: 10.1021/acs.est.1c04142. Disponível em: <https://doi.org/10.1021/acs.est.1c04142>.

TOMCZAK, Matthias. Some historical, theoretical and applied aspects of quantitative water mass analysis. **Journal of Marine Research**, [S. l.], v. 57, n. 2, p. 275–303, 1999. DOI: 10.1357/002224099321618227. Disponível em: <http://www.ingentaeselect.com/rpsv/cgi-bin/cgi?ini=xref&body=linker&reqdoi=10.1357/002224099321618227>.

VAN SEBILLE, Erik et al. A global inventory of small floating plastic debris. **Environmental Research Letters**, [S. l.], v. 10, n. 12, p. 124006, 2015. a. DOI: 10.1088/1748-9326/10/12/124006. Disponível em: <https://iopscience.iop.org/article/10.1088/1748-9326/10/12/124006>.

VAN SEBILLE, Erik; SCUSSOLINI, Paolo; DURGADOO, Jonathan V; PEETERS, Frank J. C.; BIASTOCH, Arne; WEIJER, Wilbert; TURNEY, Chris; PARIS, Claire B.; ZAHN, Rainer. Ocean currents generate large footprints in marine palaeoclimate proxies. **Nature Communications**, [S. l.], v. 6, n. 1, p. 6521, 2015. b. DOI: 10.1038/ncomms7521. Disponível em: <https://doi.org/10.1038/ncomms7521>.

VASS, Máté; SZÉKELY, Anna J.; LINDSTRÖM, Eva S.; LANGENHEDER, Silke. Using null models to compare bacterial and microeukaryotic metacommunity assembly under shifting environmental conditions. **Scientific Reports**, [S. l.], v. 10, n. 1, p. 2455, 2020. DOI: 10.1038/s41598-020-59182-1. Disponível em: <https://doi.org/10.1038/s41598-020-59182-1>.

VELLEND, Mark. **The Theory of Ecological Communities. Monographs in Population Biology**, 2016. DOI: 10.1016/B978-0-7234-5558-5.00001-4.

VILLAR, Emilie et al. Environmental characteristics of Agulhas rings affect interocean plankton transport. **Science**, [S. l.], v. 348, n. 6237, p. 1261447, 2015. DOI: 10.1126/science.1261447. Disponível em: <https://doi.org/10.1126/science.1261447>.

VILLARINO, Ernesto et al. Large-scale ocean connectivity and planktonic body size. **Nature Communications**, [S. l.], v. 9, n. 1, p. 142, 2018. DOI: 10.1038/s41467-017-02535-8. Disponível em: <https://doi.org/10.1038/s41467-017-02535-8>.

VILLARINO, Ernesto et al. Global beta diversity patterns of microbial communities in the surface and deep ocean. **Global Ecology and Biogeography**, [S. l.], v. 31, n. 11, p. 2323–2336, 2022. DOI: 10.1111/geb.13572. Disponível em: <https://onlinelibrary.wiley.com/doi/10.1111/geb.13572>.

WARD, Ben A.; CAEL, B. B.; COLLINS, Sinead; YOUNG, C. Robert. Selective constraints on global plankton dispersal. **Proceedings of the National Academy of Sciences**, [S. l.], v. 118, n. 10, p. e2007388118, 2021. DOI: 10.1073/pnas.2007388118. Disponível em: <https://doi.org/10.1073/pnas.2007388118>.

WICKHAM, Hadley. **Ggplot2: Elegant graphics for data analysis**. 2. ed. Cham, Switzerland: Springer International Publishing, 2016. Disponível em: <https://ggplot2.tidyverse.org>.

WISNOSKI, Nathan I.; LEIBOLD, Mathew A.; LENNON, Jay T. Dormancy in Metacommunities. **The American Naturalist**, [S. l.], v. 194, n. 2, p. 135–151, 2019. DOI: 10.1086/704168. Disponível em: <https://doi.org/10.1086/704168>.

WOESE, C. R. Bacterial evolution. **Microbiological Reviews**, [S. l.], v. 51, n. 2, p. 221–271, 1987. DOI: 10.1128/mr.51.2.221-271.1987. Disponível em: <https://journals.asm.org/doi/10.1128/mr.51.2.221-271.1987>.

WORDEN, Alexandra Z.; FOLLOWS, Michael J.; GIOVANNONI, Stephen J.; WILKEN, Susanne; ZIMMERMAN, Amy E.; KEELING, Patrick J. Rethinking the marine carbon cycle: Factoring in the multifarious lifestyles of microbes. **Science**, [S. l.], v. 347, n. 6223, 2015. DOI: 10.1126/science.1257594. Disponível em: <https://www.science.org/doi/10.1126/science.1257594>.

YESSON, Chris; CLARK, Malcolm R.; TAYLOR, Michelle L.; ROGERS, Alex D. The global distribution of seamounts based on 30 arc seconds bathymetry data. **Deep Sea Research Part I: Oceanographic Research Papers**, [S. l.], v. 58, n. 4, p. 442–453, 2011. DOI: <https://doi.org/10.1016/j.dsr.2011.02.004>. Disponível em: <https://www.sciencedirect.com/science/article/pii/S0967063711000392>.

ZHOU, Jizhong; NING, Daliang. Stochastic Community Assembly: Does It Matter in Microbial Ecology? **Microbiology and Molecular Biology Reviews**, [S. l.], v. 81, n. 4, p. e00002-17, 2017. DOI: 10.1128/MMBR.00002-17. Disponível em: <http://www.ncbi.nlm.nih.gov/pubmed/29021219>. Acesso em: 20 nov. 2017.

ZHU, Wentao; ZHU, Ming; LIU, Xiangbo; XIA, Jingquan; YIN, Hongyang; LI, Xiubao. Different Responses of Bacteria and Microeukaryote to Assembly Processes and Co-occurrence Pattern in the Coastal Upwelling. **Microbial Ecology**, [S. l.], 2022. DOI: 10.1007/s00248-022-02093-7. Disponível em: <https://doi.org/10.1007/s00248-022-02093-7>.

ZIKA, Jan D.; GREGORY, Jonathan M.; MCDONAGH, Elaine L.; MARZOCCHI, Alice; CLÉMENT, Louis. Recent Water Mass Changes Reveal Mechanisms of Ocean Warming. **Journal of Climate**, [S. l.], v. 34, n. 9, p. 3461–3479, 2021. DOI: 10.1175/JCLI-D-20-0355.1. Disponível em: <https://journals.ametsoc.org/view/journals/clim/34/9/JCLI-D-20-0355.1.xml>.



# CAPÍTULO 3

ECOLOGICAL PROCESSES SHAPING  
MICROBIAL ASSOCIATIONS OVER TIME IN  
CONTRASTING LATITUDES OF THE OCEAN

## ECOLOGICAL PROCESSES SHAPING MICROBIAL ASSOCIATIONS OVER TIME IN CONTRASTING LATITUDES OF THE OCEAN

### ABSTRACT

Marine microbes establish diverse and complex communities whose ecological interactions are essential to sustain ecosystem services for planetary health. Global oceanographic expeditions have lately revealed large-scale spatial patterns of microbial diversity, ecological associations, and the mechanisms underpinning microbial biogeography. However, we still lack comparative studies using globally distributed time-series to fully understand the ecological processes structuring the ocean microbiome. Most long-term microbial observatories with molecular data are restricted to mid-latitudes ( $\sim 22^\circ$  to  $50^\circ$ ) and present methodological differences that undermine their inter-comparison. Here, we investigated microbial communities from two contrasting (Atlantic  $6^\circ\text{S}$ , Mediterranean  $42^\circ\text{N}$ ) coastal marine observatories using monthly samples from April 2013 to August 2016. We analyzed 16S- and 18S-rRNA-gene amplicon sequence variants of two size-fractions ( $0.22\text{-}3\ \mu\text{m}$  and  $>3\ \mu\text{m}$ ). We applied ecological models and network analysis to compare the ecological processes shaping these communities over time. Our results indicate that the relative importance of determinism (selection) was consistently larger in the temperate than in the tropical observatory. Our network analysis revealed that the tropical microbial communities were less connected but displayed more stable associations over time than the temperate ones. We conclude that, as we move from high to low latitudes, the influence of deterministic factors such as day length and temperature drastically reduces while stochastic factors – related to biological interactions – emerge as the main drivers of microbial community dynamics. This study reinforces the importance of comparative studies using globally distributed time-series to understand the ecological processes structuring the ocean microbiome.

**Keywords:** Equatorial Atlantic, Mediterranean Sea, marine microbiota, microbial observatory, network analysis

## INTRODUCTION

Marine microbial communities bear an important share of the ocean's biodiversity (DE VARGAS et al., 2015; SUNAGAWA et al., 2015) and their interactions are fundamental for Earth's ecosystem functioning and biogeochemical cycles (FALKOWSKI; FENCHEL; DELONG, 2008; GUIDI et al., 2016). Comprehending the factors and mechanisms that drive microbial dynamics is important to predict how undergoing environmental changes would impact their community composition, interactions and functions (CHAFFRON et al., 2021; DONEY et al., 2012). Global oceanographic expeditions (i.e.: TARA and Malaspina) have lately revealed large-scale spatial patterns of microbial diversity (GINER et al., 2020; IBARBALZ et al., 2019; SALAZAR et al., 2016; SOMMERIA-KLEIN et al., 2021; SUNAGAWA et al., 2015) and their ecological associations (CHAFFRON et al., 2021; DEUTSCHMANN et al., 2022; LIMA-MENDEZ et al., 2015). The ecological processes shaping microbial communities have recently been shown to change between biological domains (prokaryotes vs. eukaryotes) as well as with depth and with spatial scale of the ocean (JUNGER et al., 2023; LOGARES et al., 2020). However, these space-for-time studies lack the temporal dimension, which is essential to fully understand the patterns of microbial diversity and the underpinning mechanisms shaping ocean microbial communities (BUTTIGIEG et al., 2018; MOREIRA; LÓPEZ-GARCÍA, 2019).

Most long-term microbial observatories with molecular data are still restricted to northern mid-latitudes ( $\sim 22^\circ$  to  $50^\circ$ ) and present methodological differences (e.g., primers, sequencing technology) that limit their inter-comparison. Most of the world's ocean surface is under warm oligotrophic tropical conditions (BEHRENFELD et al., 2006), where primary production is usually dominated by picoplankton ( $< 3 \mu\text{m}$ ), both prokaryotes (*Synechococcus* and *Prochlorococcus*) and tiny single-cell eukaryotes (ALVAIN et al., 2008). In this context, we lack comprehensive comparative studies of ocean time-series using the same standard methods to assess microbial diversity and associations in the low-latitude parts of the global ocean, specially considering understudied areas such as the South Atlantic Ocean. There are some remarkable exceptions, though, such as the Australian Microbiome initiative, that generated a dataset – conceived with standard methods and protocols – of seven coastal microbial time-series covering a latitudinal gradient ( $\sim 12^\circ$  to  $42^\circ$ ) at continental scale (BROWN et al., 2018).

Efforts using ecological models have paved the way to understanding the ecological processes (selection, dispersal, and drift) structuring microbial communities (HUBER et al., 2020; JUNGER et al., 2023; LOGARES et al., 2020; VASS et al., 2020). Several studies have found prokaryotes to be relatively better explained by environmental selection than by dispersal or drift, while single-cell eukaryotes more determined with the latter factors (JUNGER et al., 2023;

LOGARES et al., 2018, 2020; VASS et al., 2020), mainly due to differences in organism and population sizes (DE BIE et al., 2012; FODELIANAKIS et al., 2021; VILLARINO et al., 2018), as well because of the dormancy capacity of bacteria (LOCEY et al., 2020). The balance of these mechanisms also changes with ocean depth due to differences in microbial abundances, environmental heterogeneity, and barriers to dispersal (JUNGER et al., 2023). However, these ecological models do not take into consideration biological interactions, which also exert an important influence on species distributions at macroecological scales (ARAÚJO; LUOTO, 2007). As an alternative, network topological metrics have been used to capture the ecological characteristics of plankton communities (FUHRMAN; CRAM; NEEDHAM, 2015), to investigate community resilience (MOORE; GREWAR; CUMMING, 2016; SOLÉ; MONTOYA, 2001), as well as potential responses to environmental changes (CHAFFRON et al., 2021). The application of network analyses to omics data has improved our understanding of the range of potential ecological associations between microbes, the role of keystone species, and the likely response of these communities to environmental changes in the ocean (CHAFFRON et al., 2021; DEUTSCHMANN et al., 2021; KRABBERØD et al., 2022; LIMA-MENDEZ et al., 2015).

Here we aimed to compare the ecological processes as well as the topological metrics of microbial co-occurrence networks in two coastal marine observatories located in contrasting latitudes. We determined which processes (selection or stochasticity) temporally structure free-living (0.22-3  $\mu\text{m}$ ) and particle-attached (>3  $\mu\text{m}$ ) prokaryote as well as small (<3  $\mu\text{m}$ ) and large (3-200  $\mu\text{m}$ ) protists in each time-series. To do so, we sequenced 16S and 18S rRNA gene amplicons from DNA samples collected monthly for four years in two microbial observatories, one tropical site located in the Western Equatorial Atlantic (6°S), and one temperate site located in the Northwestern Mediterranean Sea (42°N). We hypothesized temperate microbial communities would be relatively more structured by selection than tropical microbial communities, due to the larger temporal environmental heterogeneity in intermediate latitudes when compared to low-latitude sites. On the other hand, stochastic factors possibly related to biological associations (as estimated by network analysis) would arise as the main structuring factor of microbial communities in the tropics.

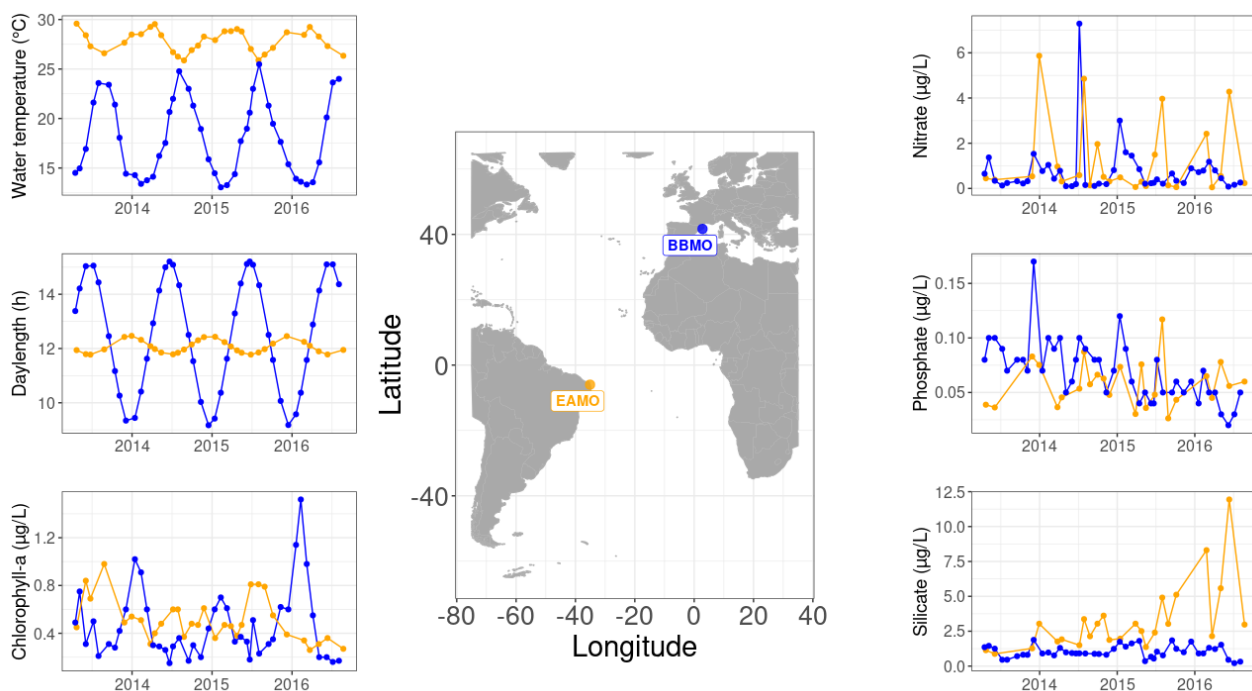
## **MATERIAL AND METHODS**

### ***Study sites and sampling design***

Surface seawater (~1 m depth) samples were collected monthly from April 2013 to August 2016 at two coastal marine observatories: the Equatorial Atlantic Microbial Observatory – EAMO (-5.99°, -35.08°) located in the western coast of the Atlantic, 30 km away from the city of Natal

(Brazil); and the LTER Blanes Bay Microbial Observatory – BBMO (41.66°, 2.80°) located in the Northwestern Mediterranean Sea (Fig. III-1). BBMO is a temperate oligotrophic coastal site ~1 km offshore with little riverine or human influence (GASOL et al., 2016), while EAMO is a tropical oligotrophic site ~3 km from the Brazilian coastline. Both coastal sites are on average 20 m deep. To our knowledge, EAMO is the first microbial observatory located in the South Atlantic Ocean and one of the very few observatories in low-latitudes (0-10°) with available amplicon sequencing data. Daylength (hours of light) was calculated for both sites based on coordinates and sampling dates.

In each site, 20 L sub-surface (~1 m depth) seawater samples were passed through a 200- $\mu\text{m}$  mesh net and transported to the lab in 20-L in polycarbonate carboys, under dim light, and processed within 1.5 h. To obtain microbial biomass, 2 to 6 L sub-surface seawater was first filtered through a 20 $\mu\text{m}$  filter nylon mesh and then sequentially filtered through 3- $\mu\text{m}$  polycarbonate filters ("nanoplankton"; 3-20  $\mu\text{m}$  size-fraction) and 0.22  $\mu\text{m}$ -Sterivex "cartridges" (Millipore) ("picoplankton"; 0.22-3  $\mu\text{m}$  size-fraction), using a peristaltic pump. The filters were embedded in a lysis buffer solution and maintained in the ultra-freezer (-80 °C) until DNA extraction.



**Figure III-1.** Geographic location of the two contrasting coastal marine microbial observatories sampled in this study: the Equatorial Atlantic Microbial Observatory (EAMO) and the Blanes Bay Microbial Observatory (BBMO). Temporal variability in temperature, day-length, chlorophyll-a as well as inorganic nutrients in these observatories during the sampling period of this study (April 2013 to August 2016). Fig. III-S1 shows the differences in all environmental and biological variables between observatories.



## ***Analytical methods***

Water transparency was determined with a Secchi disk. Water temperature, conductivity, and dissolved oxygen were measured in situ using CTDs model SAIV A/S SD204. Chlorophyll-a concentration was obtained by filtering the seawater in 3 µm polycarbonate filters and then GF/F (Whatman), and extracted with acetone (90% acetone, 4°C, overnight), and determined by fluorescence measured with calibrated Turner Designs fluorometers. Dissolved inorganic nutrient concentrations were determined from GF/F (Whatman) filtered seawater samples in an autoanalyzer following standard procedures (GRASSHOFF; KREMLING; ERHARDT, 1999).

Seawater samples for flow cytometry counts were preserved with 1% paraformaldehyde + 0.05% glutaraldehyde (final concentration). Bacterial abundance was analyzed by flow cytometry in a BD FACSCalibur flow cytometer with a blue (488 nm) laser and SybrGreen I staining, according to (GASOL; DEL GIORGIO, 2000). Picocyanobacteria were subtracted in independent counts of non-stained samples in a plot of side light scatter versus red and orange fluorescences. Bacterial size was estimated with the relationship, and bacterial biomass was calculated using the volume-to-carbon relationship where  $\text{pgC cell}^{-1} = 0.12 \text{ pg } (\mu\text{m}^3 \text{ cell}^{-1})^{0.7}$  (NORLAND; FAGERBAKKE; HELDAL, 1995).

## ***DNA extraction, sequencing, and bioinformatics***

DNA extraction was carried out with a phenol-chloroform protocol, cutting the filters into small pieces (LOGARES et al., 2014), and subsequent purification using Amicon column (Millipore® 100KDa/100.000MWCO). DNA extracts were quantified with a Qubit 1.0 (Thermo Fisher Scientific) and preserved at -80 °C. PCR amplification was performed using the primers 515F-Y (5'-GTGYCAGCMGCCGCGGTAA) and -926R (5'-CCGYCAATTYMTTTRAGTTT) for the 16S rRNA gene hypervariable V4-V5 region ( $\approx 400$  bp) to target prokaryotes – both Bacteria and Archaea (PARADA; NEEDHAM; FUHRMAN, 2016). For eukaryotes, the primers used were TAREukFWD1 (5'-CCAGCASCYGC GGTAATTCC-3') and TAREukREV3 (5'-ACTTTCGTTCTTGATYRA-3') of the 18S rRNA gene hypervariable V4 region ( $\approx 380$  bp) (STOECK et al., 2010).

Samples were sequenced in an Illumina MiSeq platform, and raw reads were processed using DADA2 (CALLAHAN et al., 2016) to determine amplicon sequence variants (ASVs). For the 16S rRNA gene, we trimmed the forward reads at 210 bp and the reverse reads at 180 bp, while for the 18S rRNA gene, forward reads were trimmed at 220 bp and the reverse reads at 190 bp. Then, for the 16S rRNA gene, the maximum number of expected errors (maxEE) was set to 5 for the forward and reverse reads, while for the 18S rRNA gene, the maxEE was set to 5 and 6 for the

forward and reverse reads, respectively. Finally, error rates were estimated using DADA2 for both the 16S and 18S genes to delineate the ASVs.

ASVs taxonomy was assigned with DADA2 using the naïve Bayesian classifier method (QIONG et al., 2007) together with the SILVA v.138 database (QUAST et al., 2013) for prokaryotes, and the Protist Ribosomal Reference database (PR<sup>2</sup>, version 4.14, (GUILLOU et al., 2013)), for eukaryotes. Eukaryotes, chloroplasts, and mitochondria were removed from the 16S ASVs table, while Holozoan (Metazoa and Fungi), Streptophyta, and nucleomorphs were removed from the 18S ASVs table. The samples' prokaryotic and eukaryotic taxonomic composition is shown in the supplementary material (Fig. III-S2 and Fig. III-S3). The prokaryotic and eukaryotic community compositions were segregated both by observatory and size-fraction (Fig. III-S4).

For ecological models and most statistical analyses (apart from network analysis), prokaryotic and eukaryotic ASVs tables were rarefied to 8,536 reads and 9,568 reads per sample, respectively, with the function *rrarefy* from the Vegan R package. Rare ASVs with an abundance sum <50 reads and prevalence <15% were removed from each ASV table to reduce PCR and sequencing depth biases. This filtering procedure kept ~95% of the total reads and ~25% of the ASVs. The raw DNA sequences obtained in this study were deposited in the European Nucleotide Archive (<http://www.ebi.ac.uk/ena>) under accession numbers PRJEB48035 for BBMO and PRJNA414763 for EAMO.

### **General statistical analyses**

We used the R software v 4.0.3 (R CORE TEAM, 2014) with the packages *vegan* (OKSANEN et al., 2015), *MASS* (VENABLES; RIPLEY, 2002), and *BiodiversityR* (KINDT; COE, 2005) for data processing and statistical analyses. Analyses of dissimilarities were conducted using the 'adonis2()' function of the *vegan* R package to investigate the percentage of variance in community composition explained by environmental variables (MCARDLE; ANDERSON, 2001).

Phylogenetic trees were built for both the 16S and 18S rRNA gene datasets. First, we aligned raw ASV sequences against an aligned SILVA template – for 16S rRNA – and an aligned PR<sup>2</sup> template – for 18S rRNA – using *mothur* (SCHLOSS et al., 2009). Poorly aligned regions or sequences were then removed using *trimAl* (parameters: -gt 0.3 -st 0.001) (CAPELLA-GUTIÉRREZ; SILLA-MARTÍNEZ; GABALDÓN, 2009). The alignment was visually curated with *seaview v4* (GOUY; GUINDON; GASCUEL, 2010), and sequences with >=40% of gaps were removed. Finally, phylogenetic trees were inferred from the curated alignment using *FastTree v2.1.9* (PRICE; DEHAL; ARKIN, 2009).

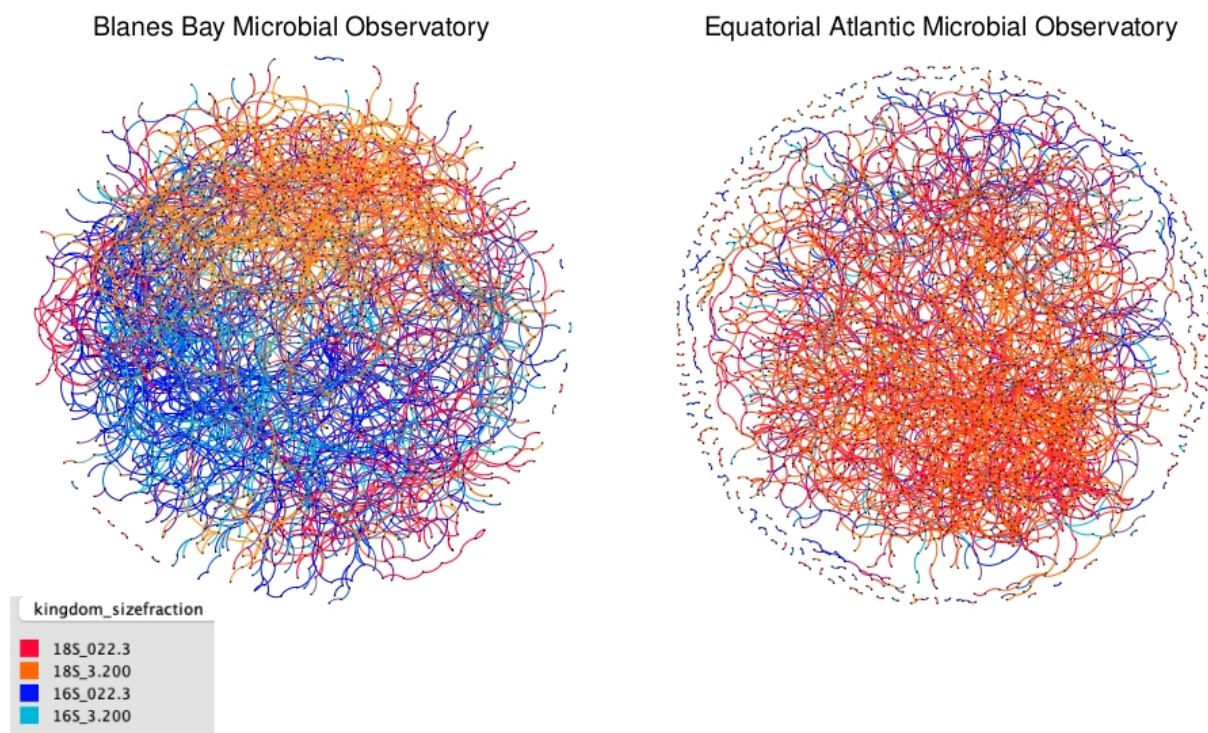
### ***Computation of determinism and stochasticity***

The relative importance of determinism (selection) and stochasticity was estimated using a null model approach (STEGEN et al., 2013) that has been widely used in ecological studies (HUBER et al., 2020; JUNGER et al., 2023; LOGARES et al., 2020; VASS et al., 2020). This analysis consists of inferring selection from ASV phylogenetic turnover. First, we determined the phylogenetic turnover using the abundance-weighted  $\beta$ -mean nearest taxon distance ( $\beta$ MNTD) metric (STEGEN et al., 2013), which computes the mean phylogenetic distances between each ASV and its closest relative in each pair of communities (pairwise comparisons). Second, we run null models with 999 randomizations to simulate the community turnover by chance ( $\beta$ MNTD<sub>null</sub>), in other words, stochasticity (STEGEN et al., 2013). Finally, the  $\beta$ -Nearest Taxon Index ( $\beta$ NTI) was calculated from the differences between the observed  $\beta$ MNTD and the mean  $\beta$ MNTD<sub>null</sub> values. Overall,  $|\beta$ NTI| > 2 indicates that taxa are phylogenetically more related or less related than expected by chance, pointing to a strong influence of selection on community assembly (STEGEN et al., 2013). More precisely,  $\beta$ NTI values higher than +2 indicate the action of heterogeneous selection, while  $\beta$ NTI values lower than -2 points indicate the action of homogeneous selection (STEGEN et al., 2013). The fraction of  $\beta$ -diversity of the communities that was not explained by selection ( $|\beta$ NTI|  $\leq$  2) was considered to be ruled by stochasticity.

### ***Network construction***

For network construction, we used only samples that have both 16S and 18S data, and pico- (0.2–3  $\mu$ m) and nano- (>3  $\mu$ m) size fractions. First, to control for data compositionality in network construction (GLOOR et al., 2017), we applied a centered-log-ratio transformation separately to the prokaryotic and eukaryotic ASV tables of both size fractions and locations. Second, we merged the four data tables corresponding to each primer-size fraction (16S 0.2-3  $\mu$ m, 16S 3-20  $\mu$ m, 18S 0.2-3  $\mu$ m and 18S 3-20  $\mu$ m) into a single matrix for each observatory. Then, we constructed one preliminary network for each observatory (BBMO and EAMO) using FlashWeave (TACKMANN; MATIAS RODRIGUES; VON MERING, 2019), selecting the options “heterogeneous” and “sensitive”. The resulting preliminary BBMO network contained 2311 nodes and 3403 edges (3061 or 90% positive, and 342 or 10% negative), while the preliminary EAMO network contained 2773 nodes and 3243 edges (2712, 84% positive, and 531, 16% negative) (Fig. III-2). The nodes represent microorganisms, while edges stand for potential interactions in the network. Finally, we approximated the temporal networks via monthly subnetworks (27 in BBMO and 22 in EAMO) from each static network (DEUTSCHMANN et al., 2021). Shortly, each sub-network contains a node and an edge subset of the static network. An edge is present in a subnetwork of a particular

month if both nodes correspond to microorganisms detected in that month. A microorganism is determined as detected if the sequence abundance is above zero.



**Figure III–2.** Visualization of the static networks from the Blanes Bay Microbial Observatory (N = 27 samples) and the Equatorial Atlantic Microbial Observatory (N = 22 samples). Edges are colored according to biological domain (prokaryotes and eukaryotes) and size-fraction (pico- and nanoplankton).

### **Network analysis**

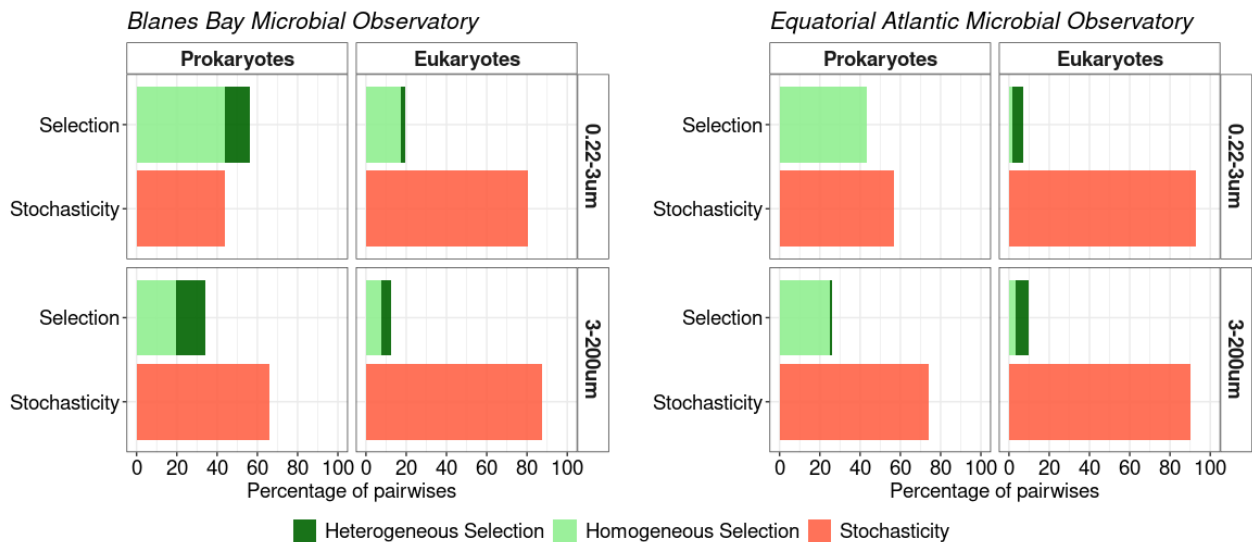
We computed global network metrics to characterize the single static network and each monthly subnetwork using the igraph R-package (CSARDI, G. NEPUSZ, 2006) and adapted code from (DEUTSCHMANN et al., 2021). We computed the following metrics: mean degree, edge density, average path length, transitivity, and assortativity based on node degree. We also computed the average strength of positive associations between microorganisms, and assortativity based on the nominal classification of nodes based on the domain (prokaryotes vs. eukaryotes) and size-fraction (small vs. large). Mean degree is the number of edges that connect the focal node to other nodes. It captures the average number of links per species. Edge density is the ratio between the number of edges and the number of possible edges, which measures how well the graph is connected. It is a connectivity index capturing the proportion of possible links between species. Average path length is the average length of all possible shortest paths in the graph. A small average

path length is interpreted to increase the speed of the network's response to perturbations. Transitivity measures how well nodes in a graph cluster together (community clustering). It is also a connectivity index capturing the tendency of links between species to cluster together. Assortativity quantifies whether nodes tend to be connected to nodes of the same or similar characteristics (numerical or categorical). Assortativity (bacteria vs. eukaryotes) is positive if bacteria tend to connect with bacteria, and eukaryotes tend to connect with eukaryotes. It is negative if bacteria tend to connect to eukaryotes, and vice versa. Assortativity (small vs. large) is positive if plankton tend to connect with those of the same size-fraction, while it is negative if they tend to connect with those of different size-fraction. Spearman correlations were computed between global network metrics and environmental data using the Holm's multiple test correction to adjust p-values (HOLM, 1979), with the function `corr.test` in the `psych` R-package (REVELLE, 2022). We used Gephi (BASTIAN; HEYMANN; JACOMY, 2009), v.0.9.2, and the Fruchterman Reingold Layout (FRUCHTERMAN; REINGOLD, 1991) for network visualizations.

## **RESULTS**

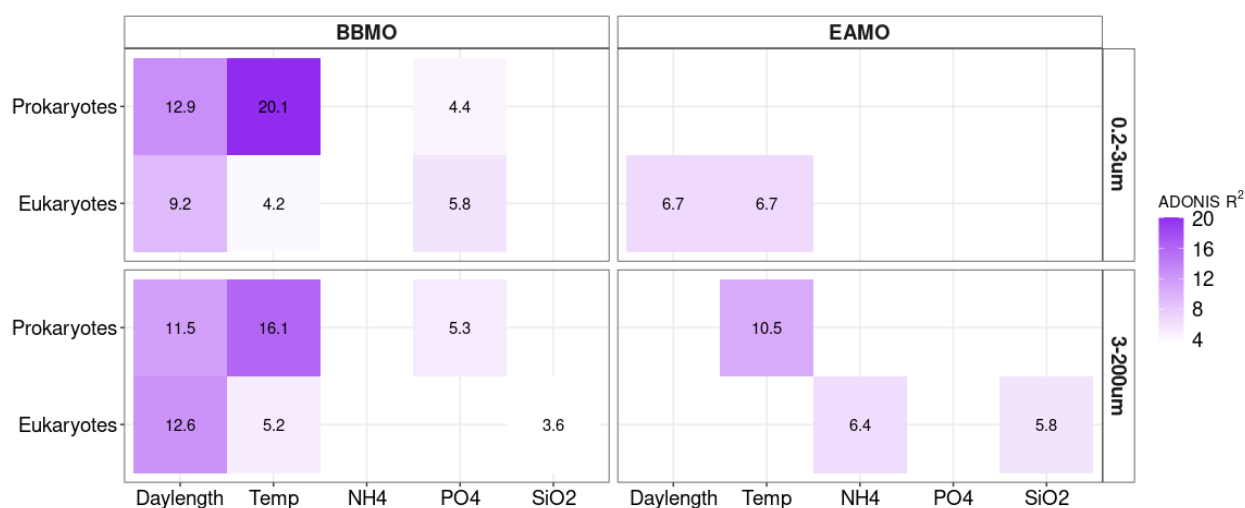
### ***Ecological mechanisms and factors shaping the microbial community structure***

We found differences in the balance between deterministic (selection) vs. stochasticity among sites (Fig. III-3). Selection always explained a higher percentage of the turnover of prokaryotes as compared to eukaryotes in both size-fractions (Fig. III-3). Selection always explained a higher percentage of prokaryotes' turnover than eukaryotes in both size-fractions (~56% vs. ~34%) and EAMO (~43% vs. ~26%). These striking differences in the percentage explained by selection between smaller and larger size-fractions were not observed for eukaryotes (~20% vs. ~13% in BBMO and ~7% vs. ~9% in EAMO) (Fig. III-3). Selection was relatively more important than other processes in the temperate site compared to the tropical site, regardless of the biological domain and size-fraction (Fig. III-3).



**Figure III-3.** Microbial community assembly processes in contrasting time-series. Relative importance of the different ecological mechanisms (homogeneous or heterogeneous Selection vs. Stochasticity) structuring the microbial communities in the Blanes Bay Microbial Observatory (BBMO, 42°N) and the Equatorial Atlantic Microbial Observatory (EAMO, 6°S).

To investigate the environmental factors determining the microbial communities in contrasting latitudes, we used dissimilarity analysis (ADONIS). The temperate site free-living prokaryotic community turnover was mainly explained by temperature (~20%), day length (~13%), and phosphate (~4%) (Fig. III-4). Conversely, the tropical site free-living prokaryotic community turnover was not explained by any of the measured environmental variables (Fig. III-4). The temperate site particle-attached prokaryotic community turnover was also explained by temperature (~16%), day length (~11%), and phosphate (~5%), while the tropical site particle-attached prokaryotic community was explained by temperature (~10%) (Fig 4). The picoeukaryotes (0.2-3µm) were mainly explained by day length (9%), phosphate (6%), and temperature (4%) in the temperate site and by day length (7%), and temperature (7%) in the tropical site (Fig. III-4). The turnover in temperate nanoeukaryotic (3-20 µm) communities was explained by day length (~13%) temperature (~5%), and silicate (~4%), while ammonia (~6%) and silicate (~6%) explained only a limited fraction of the turnover in the tropical site for the same size-fraction (Fig. III-4).



**Figure III-4.** Environmental drivers of microbial communities in the two contrasting time-series. Percentage of variance (Adonis  $R^2$ ) in eukaryotic and prokaryotic community composition (Bray-Curtis dissimilarity) explained by the environmental variables with significant values ( $p < 0.05$ ) in at least one of the subsets. Blank spaces depict non-significant results ( $p > 0.05$ ). Temp – temperature. Salinity,  $\text{NO}_3$ ,  $\text{NO}_2$  and chlorophyll-a are not shown because they did not explain a significant portion of community turnover in any of the two observatories. BBMO – Blanes Bay Microbial Observatory. EAMO – Equatorial Atlantic Microbial Observatory.

### Network topological metrics

Most network topological metrics were significantly different between BBMO and EAMO temporal networks (Fig. III-4). Mean degree ( $t=7.14$ ,  $df=46.99$ ,  $p < 0.001$ ) and edge density ( $t=4.87$ ,  $df=36.7$ ,  $p < 0.001$ ) were significantly higher in BBMO than in EAMO (Fig. III-4). In turn, mean positive association strength ( $t=-36.85$ ,  $df=45.14$ ,  $p < 0.001$ ), average path length ( $t=-4.78$ ,  $df=31.632$ ,  $p < 0.001$ ), assortativity degree ( $t=-3.35$ ,  $df=37.68$ ,  $p < 0.001$ ) and assortativity based on size-fractions ( $t=-5.6$ ,  $df=46.50$ ,  $p < 0.001$ ) were significantly lower in BBMO than in EAMO (Fig. III-4). The metrics extracted from each static network analysis are shown in the supplementary material (Fig. III-S5).

Overall, the network topological metrics were significantly associated with day length and temperature in the temperate site BBMO (Fig. III-5A). The number of nodes and edges negatively correlated with day length ( $r=-0.7$ ) and temperature ( $r=-0.45$ ). Conversely, edge density positively correlated with day length ( $r=0.7$ ) and temperature ( $r=0.43$ ). Average positive association positively correlated with day length ( $r=0.79$ ) and negatively correlated with nitrate ( $r=-0.39$ ). Average path length positively correlated with day length ( $r=0.74$ ) and temperature ( $r=0.54$ ) and negatively correlated with nitrate ( $r=-0.42$ ). Transitivity negatively correlated with temperature ( $r=-0.59$ ) and

positively correlated with nitrite ( $r=0.48$ ), nitrate ( $r=0.44$ ), and silicate ( $r=0.41$ ). On the other hand, in the tropical site EAMO, most network topological metrics displayed no correlations with day length and temperature (Fig. III-5B). Assortativity (prokaryotes vs. eukaryotes) positively correlated with nitrite ( $r=0.56$ ). The number of nodes and edges negatively correlated with silicate ( $r=-0.49$ ) (Fig. III-5B). Edge density and average positive association positively correlated with silicate concentration ( $r=0.5$  and  $r=0.52$ ).

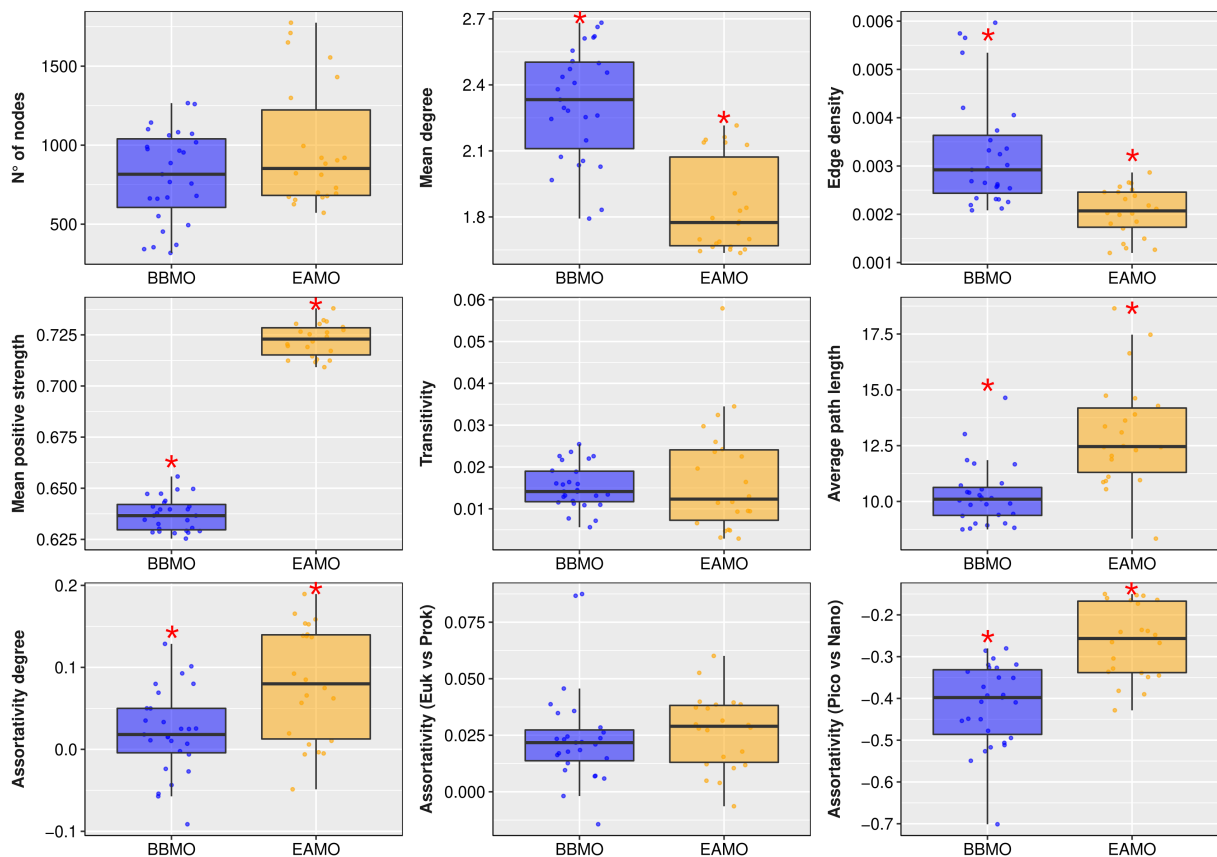
## DISCUSSION

Our results support our main hypothesis that the turnover of microbial communities would be relatively more explained by selection in the temperate (BBMO) rather than in the tropical site (EAMO) due to larger environmental heterogeneity in intermediate latitudes when compared to low-latitude sites. This result is coherent with a recent Lagrangian study that found selection to increase with increasing environmental heterogeneity in the ocean (JUNGER et al., 2023). Similarly, other studies conducted over time have found the same pattern for microbial communities inhabiting both terrestrial (DINI-ANDREOTE et al., 2015; STEGEN et al., 2013) and freshwater ecosystems (HUBER et al., 2020; VASS et al., 2020). These results are further supported by the generally higher percentage of turnover explained by environmental variables in BBMO compared to EAMO (Fig. III-3).

Our results also point out that the balance between determinism (selection) and stochasticity changes between prokaryotic and eukaryotic microbial communities. Prokaryotes were generally more explained by selection than microbial eukaryotes. This finding agrees with previous Lagrangian studies that showed a relatively stronger selection for prokaryotes than for picoeukaryotes in different depth zones of the ocean (JUNGER et al., 2023; LOGARES et al., 2020) as well in lacustrine ecosystems (LOGARES et al., 2018; VASS et al., 2020). These differences between domains are probably associated with factors (i.e., organism and population sizes) regulating stochastic processes such as dispersal limitation and ecological drift (DE BIE et al., 2012; FODELIANAKIS et al., 2021; VILLARINO et al., 2018). Unicellular eukaryotes are larger and present smaller populations (MASSANA; LOGARES, 2013) than prokaryotes, displaying lower dispersal rates (VILLARINO et al., 2018). Besides, the relatively stronger action of selection on prokaryotes than on eukaryotes could also be related to the fact that dormancy is a common mechanism for prokaryotes but not for eukaryotes (LOCEY et al., 2020; WISNOSKI; LEIBOLD; LENNON, 2019). Dormancy keeps local bacterial diversity stable over time (LENNON et al., 2021) and, therefore, it can influence how we perceive the action of homogeneous selection on prokaryotic communities (JUNGER et al., 2023). These patterns have previously been observed



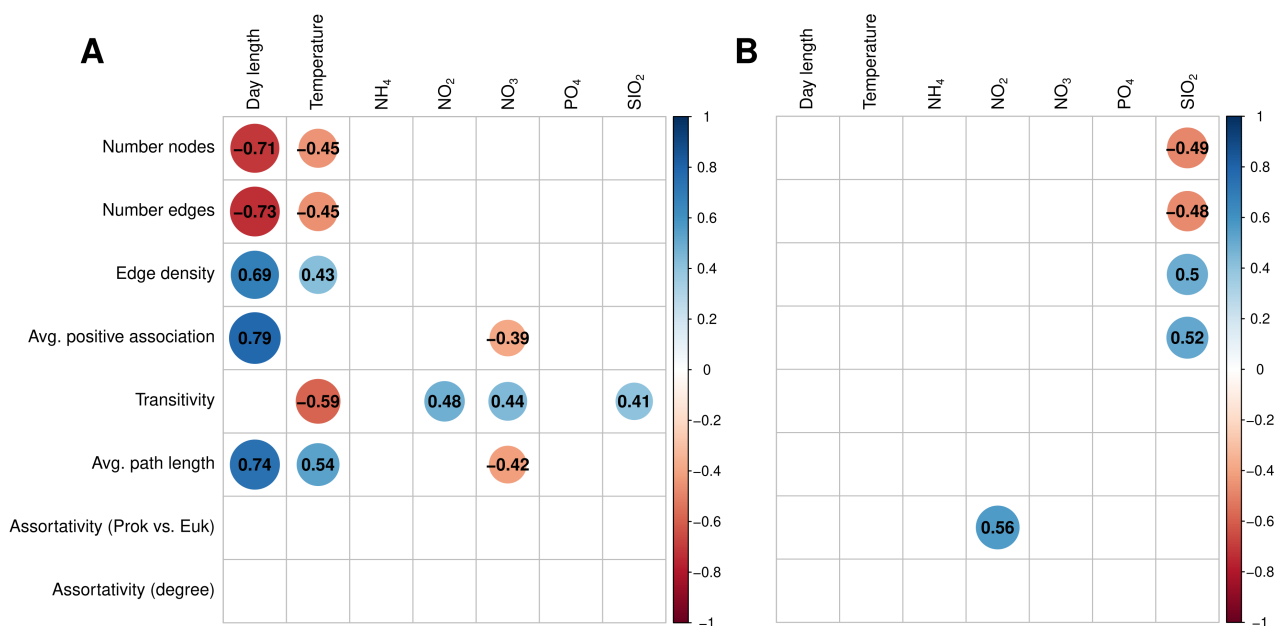
across different spatial scales (JUNGER et al., 2023), and the current study suggests that such patterns persist over time at different latitudes of the ocean.



**Figure III-5.** Dynamic temporal network topological metrics differ between the Blanes Bay Microbial Observatory (BBMO) and the Equatorial Atlantic Microbial Observatory (EAMO). Red asterisks depict statistically significant differences (t-test,  $p < 0.005$ ). Each point represents one monthly sample. Topological metrics from the static networks are available in the supplementary material (Fig. III-S4).

We also found that the importance of selection relative to that of stochasticity (dispersal limitation or ecological drift) was usually higher for the 0.22-3 $\mu\text{m}$  (free-living prokaryotes or picoeukaryotes) than for the  $>3\ \mu\text{m}$  (particle-attached prokaryotes or nanoeukaryotes) size-fraction. This result not only supports previous reports of increasing dispersal limitation and biogeography structure with increasing plankton size (FARJALLA et al., 2012; JUNGER et al., 2023; SOMMERIA-KLEIN et al., 2021), but it also demonstrates that the importance of stochastic processes for particle-attached prokaryotes should increase with the size of the particle (or organism) they are associated with (JUNGER et al., 2023). Recent studies using a multi-fractionation approach (from 0.2 to 200  $\mu\text{m}$ ) have shown that the size of the particles that

prokaryotes inhabit determines their community composition and diversity over time and space (MESTRE et al., 2017a, 2017b, 2020). Here, we show that these different dynamics could be driven by differences in the relative importance of ecological processes – such as selection and dispersal limitation – acting on the prokaryoplankton communities associated with particles drifting in the ocean. Determining the processes underlying the biogeographical patterns of particle-attached prokaryotes is particularly important because such particles contribute to the biological pump essential to the global carbon cycling (GUIDI et al., 2016; SIMON et al., 2002).



**Figure III-6.** Spearman correlation matrices between network topological metrics and environmental variables at **(A)** Blanes Bay Microbial Observatory – BBMO and **(B)** Equatorial Atlantic Microbial Observatory – EAMO. Empty boxes represent non-significant correlations ( $p > 0.05$ ).

We found significant differences in topology between the temperate and tropical networks. The edge density values were within the range found in static and dynamic plankton networks built from the TARA Oceans dataset (CHAFFRON et al., 2021; LIMA-MENDEZ et al., 2015). Mean degree and edge density were significantly higher in BBMO than in EAMO, which suggests a larger number of potential associations per species and stronger connectivity (DEUTSCHMANN et al., 2021, 2022) in the temperate than in the tropical observatory. This result suggests that either there are more microbial interactions in temperate than tropical coastal sites (DEUTSCHMANN et al., 2022) or that the strong environmental selection exerted by light and temperature leads to higher microbial recurrence, which ultimately translates into higher detectable connectivity in the temperate site (AULADELL et al., 2022; CHAFFRON et al., 2021; GINER et al., 2019). Edge

density positively correlated with day length and temperature in BBMO but not in EAMO. This finding agrees with the hypothesis that the microbial dynamics driven by the wider seasonal environmental fluctuation explain the stronger network connectivity in the temperate station. These results are coherent with our finding of higher relative importance of selection in temperate microbial communities' assembly compared to the tropical ones. Accordingly, classic studies have shown that the amplitude of fluctuations in tropical plankton populations is lower, with no clear ecological succession or phenology (CONNELL; ORIAS, 1964; DUNBAR, 1960), when compared with temperate populations (MARGALEF, 1978). In fact, several reports have found that seasonal patterns of both prokaryotes and eukaryotes are mainly driven by temperature and light fluctuation in temperate sites (AULADELL et al., 2022; CARACCILO et al., 2022; GINER et al., 2019; MESTRE et al., 2020). Although inconclusive, the higher mean degree may indicate potentially more keystone species (BERRY; WIDDER, 2014) in BBMO than in EAMO (KRABBERØD et al., 2022). In this case, microbial communities inhabiting temperate coastal systems would be likely more vulnerable to global environmental changes than their tropical counterpart (CHAFFRON et al., 2021).

The EAMO network was significantly more assortative than the BBMO network, which means that microbes tended to be more connected to those with similar mean degree (average number of links per microbe) in the tropical site. Assortativity (prokaryotes vs. eukaryotes) was mostly positive in both observatories, but there were no significant differences between them. This result means that nodes of the same domain tended to connect, which agrees with previous reports showing a trend of cyanobacteria to connect primarily with other prokaryotes (DEUTSCHMANN et al., 2021; LIMA-MENDEZ et al., 2015). Conversely, assortativity based on size-fraction was mainly negative in both observatories yet significantly lower in BBMO. This result indicates that connections between plankton of different size-fractions tended to be more common in the temperate than in the tropical site. Altogether, the difference between observatories regarding assortativity seems to be driven more by the size-fraction than by the biological domain (prokaryotes vs. eukaryotes). This finding could reflect the overall higher abundance of larger eukaryotes, such as diatoms in temperate regions, where the broader seasonal fluctuation results in typical phytoplankton successions, with a diatom winter/spring bloom (CARACCILO et al., 2022; MARGALEF, 1978), which is not common in the constantly warm waters of the tropics (ALVAIN et al., 2008). Diatoms are indeed known to display important biological associations with bacterial lineages such as Bacteroidetes and Gammaproteobacteria (AMIN; PARKER; ARMBRUST, 2012; CHAFFRON et al., 2021; LIMA-MENDEZ et al., 2015). Conversely, diatoms are relatively less abundant in tropical waters, where picoplankton – such as *Synechococcus* – typically represents a

larger fraction of the phytoplankton biomass (ALVAIN et al., 2008).

Finally, the tropical network had significantly stronger positive associations than the temperate network, which indicates relatively higher co-occurrence than mutual exclusion and likely more ‘stability’ in the predicted associations than in the temperate site. This result contradicts a recent global space-for-time study that reported a similar difference in association strength between temperate (Westerlies) and tropical (Trades) regions of the epipelagic ocean (CHAFFRON et al., 2021). Since association strength may also indicate high niche overlap (positive association) or divergence (negative association) between microorganisms (HERNANDEZ et al., 2021), these divergent results suggest that Eulerian sampling is essential to fully capture the temporal variability (or the lack of it) in environmental factors that are driving community structure and interactions. Another possible explanation for this difference is that coastal sites are more hydrodynamic and thus more affected by idiosyncrasies than the open ocean (LONGHURST, 2007). The tropical network also displayed a significantly larger average path length than the temperate, which may indicate a slower response to environmental perturbations when compared to the temperate station (ZHOU et al., 2010). In graph theory, networks with small average path lengths are known as ‘small-world networks,’ where most nodes can reach other nodes through a small number of steps (WATTS; STROGATZ, 1998). This feature would allow a quick ‘communication’ among components of a given microbial co-occurrence network, and it may be interpreted as an indication of low ecosystem resilience (DEUTSCHMANN et al., 2021; ZHOU et al., 2010). Accordingly, a recent report has found stronger associations and higher average path length in the deep ocean (DEUTSCHMANN et al., 2022), which is environmentally more homogeneous than the surface ocean (JUNGER et al., 2023). These results also indicate that increasing temperature variability seems to decrease network stability (CHAFFRON et al., 2021). Thus, the low amplitude of temperature variation recorded in tropical surface waters and in the deep ocean would lead to relatively more stable networks. Furthermore, average path length positively correlated with day length and temperature in the temperate but not in the tropical site, where it varies little. Altogether, these results suggest more stable ecological associations in the tropics, which is coherent with our finding that stochasticity was relatively more important in the tropical than in the temperate observatory.

## **CONCLUSION**

In summary, our results indicate that the relative importance of determinism (selection) was consistently more prominent in the temperate than in the tropical observatory. Conversely, the relative importance of stochasticity (dispersal limitation or drift) was higher in the tropical than in

the temperate site. Our network analysis revealed that the tropical microbes were less connected but displayed more stable associations over time – which is coherent with our ecological model findings. We can infer that, as we move from high to low latitudes, the influence of deterministic factors, such as day-length and temperature, is drastically reduced while stochastic factors related to biological interactions emerge as the main drivers of microbial community dynamics. We also found that selection was always larger for prokaryotes than eukaryotes, which agrees with several previous studies conducted in diverse aquatic ecosystems. Selection was also larger for the 0.22-3 µm (free-living or picoplankton) than for the >3 µm (particle attached or nanoplankton) fraction, which supports the ecological hypothesis of increasing dispersal limitation with increasing organism (or particle) size. This study also reinforces the importance of comparative studies using globally distributed time-series in order to understand the ecological processes structuring the ocean microbiome.

## REFERENCES

ALVAIN, S.; MOULIN, C.; DANDONNEAU, Y.; LOISEL, H. Seasonal distribution and succession of dominant phytoplankton groups in the global ocean: A satellite view. **Global Biogeochemical Cycles**, [S. l.], v. 22, n. 3, p. 1–15, 2008. DOI: 10.1029/2007GB003154.

AMIN, Shady A.; PARKER, Micaela S.; ARMBRUST, E. Virginia. Interactions between Diatoms and Bacteria. **Microbiology and Molecular Biology Reviews**, [S. l.], v. 76, n. 3, p. 667–684, 2012. DOI: 10.1128/MMBR.00007-12. Disponível em: <https://doi.org/10.1128/MMBR.00007-12>.

ARAÚJO, Miguel B.; LUOTO, Miska. The importance of biotic interactions for modelling species distributions under climate change. **Global Ecology and Biogeography**, [S. l.], v. 16, n. 6, p. 743–753, 2007. DOI: <https://doi.org/10.1111/j.1466-8238.2007.00359.x>. Disponível em: <https://doi.org/10.1111/j.1466-8238.2007.00359.x>.

AULADELL, Adrià; BARBERÁN, Albert; LOGARES, Ramiro; GARCÉS, Esther; GASOL, Josep M.; FERRERA, Isabel. Seasonal niche differentiation among closely related marine bacteria. **The ISME Journal**, [S. l.], v. 16, n. 1, p. 178–189, 2022. DOI: 10.1038/s41396-021-01053-2. Disponível em: <https://doi.org/10.1038/s41396-021-01053-2>.

BASTIAN, Mathieu; HEYMANN, Sebastien; JACOMY, Mathieu. Gephi: An Open Source Software for Exploring and Manipulating Networks. **Proceedings of the International AAAI Conference on Web and Social Media**, [S. l.], v. 3, n. 1, p. 361–362, 2009. DOI: 10.1609/icwsm.v3i1.13937. Disponível em: <https://ojs.aaai.org/index.php/ICWSM/article/view/13937>.

BEHRENFELD, Michael J. et al. Climate-driven trends in contemporary ocean productivity. **Nature**, [S. l.], v. 444, n. 7120, p. 752–755, 2006. DOI: 10.1038/nature05317. Disponível em: <https://doi.org/10.1038/nature05317>.

BERRY, David; WIDDER, Stefanie. Deciphering microbial interactions and detecting keystone species with co-occurrence networks. **Frontiers in Microbiology**, [S. l.], v. 5, 2014. DOI: 10.3389/fmicb.2014.00219. Disponível em: <https://www.frontiersin.org/articles/10.3389/fmicb.2014.00219>.

BUTTIGIEG, Pier Luigi; FADEEV, Eduard; BIENHOLD, Christina; HEHEMANN, Laura; OFFRE, Pierre; BOETIUS, Antje. Marine microbes in 4D—using time series observation to assess the dynamics of the ocean microbiome and its links to ocean health. **Current Opinion in Microbiology**, [S. l.], v. 43, p. 169–185, 2018. DOI: <https://doi.org/10.1016/j.mib.2018.01.015>. Disponível em: <https://www.sciencedirect.com/science/article/pii/S1369527417302035>.

CALLAHAN, Benjamin J.; MCMURDIE, Paul J.; ROSEN, Michael J.; HAN, Andrew W.; JOHNSON, Amy Jo A.; HOLMES, Susan P. DADA2: High-resolution sample inference from Illumina amplicon data. **Nature Methods**, [S. l.], v. 13, p. 581, 2016. Disponível em: <https://doi.org/10.1038/nmeth.3869>.

CAPELLA-GUTIÉRREZ, Salvador; SILLA-MARTÍNEZ, José M.; GABALDÓN, Toni. trimAl: a tool for automated alignment trimming in large-scale phylogenetic analyses. **Bioinformatics**, [S. l.], v. 25, n. 15, p. 1972–1973, 2009. DOI: 10.1093/bioinformatics/btp348. Disponível em: <https://doi.org/10.1093/bioinformatics/btp348>.

CARACCILO, Mariarita et al. Seasonal dynamics of marine protist communities in tidally mixed coastal waters. **Molecular Ecology**, [S. l.], v. n/a, n. n/a, 2022. DOI: <https://doi.org/10.1111/mec.16539>. Disponível em: <https://doi.org/10.1111/mec.16539>.

CHAFFRON, Samuel et al. Environmental vulnerability of the global ocean epipelagic plankton community interactome. **Science Advances**, [S. l.], v. 7, n. 35, p. eabg1921, 2021. DOI: 10.1126/sciadv.abg1921. Disponível em: <https://doi.org/10.1126/sciadv.abg1921>.

CONNELL, Joseph H.; ORIAS, Eduardo. The Ecological Regulation of Species Diversity. **The American Naturalist**, [S. l.], v. 98, n. 903, p. 399–414, 1964. DOI: 10.1086/282335. Disponível em: <https://doi.org/10.1086/282335>.

CSARDI, G. NEPUSZ, T. The igraph software package for complex network research. **InterJournal**, [S. l.], p. Complex Systems:1695, 2006.

DE BIE, T. et al. Body size and dispersal mode as key traits determining metacommunity structure of aquatic organisms. **Ecology Letters**, [S. l.], v. 15, n. 7, p. 740–747, 2012. DOI: <https://doi.org/10.1111/j.1461-0248.2012.01794.x>. Disponível em: <https://doi.org/10.1111/j.1461-0248.2012.01794.x>.

DE VARGAS, Colomban et al. Eukaryotic plankton diversity in the sunlit ocean. **Science**, [S. l.], v. 348, n. 6237, p. 1261605–1261605, 2015. DOI: 10.1126/science.1261605. Disponível em: <http://www.sciencemag.org/cgi/doi/10.1126/science.1261605>.

DEUTSCHMANN, Ina Maria et al. Disentangling microbial networks across pelagic zones in the global ocean. **bioRxiv**, [S. l.], p. 2021.07.12.451729, 2022. DOI: 10.1101/2021.07.12.451729. Disponível em: <http://biorxiv.org/content/early/2022/09/02/2021.07.12.451729.abstract>.

DEUTSCHMANN, Ina Maria; LIMA-MENDEZ, Gipsi; KRABBERØD, Anders K.; RAES, Jeroen; VALLINA, Sergio M.; FAUST, Karoline; LOGARES, Ramiro. Disentangling environmental effects in microbial association networks. **Microbiome**, [S. l.], v. 9, n. 1, p. 232, 2021. DOI: 10.1186/s40168-021-01141-7. Disponível em: <https://doi.org/10.1186/s40168-021-01141-7>.

DINI-ANDREOTE, Francisco; STEGEN, James C.; VAN ELSAS, Jan Dirk; SALLES, Joana Falcao. Disentangling mechanisms that mediate the balance between stochastic and deterministic processes in microbial succession. **PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA**, 2101 CONSTITUTION AVE NW, WASHINGTON, DC 20418 USA, v. 112, n. 11, p. E1326–E1332, 2015. DOI: 10.1073/pnas.1414261112.

DONEY, Scott C. et al. Climate Change Impacts on Marine Ecosystems. **Annual Review of Marine Science**, [S. l.], v. 4, n. 1, p. 11–37, 2012. DOI: 10.1146/annurev-marine-041911-111611. Disponível em: <https://www.annualreviews.org/doi/10.1146/annurev-marine-041911-111611>.

DUNBAR, M. J. The Evolution of Stability in Marine Environments Natural Selection at the Level of the Ecosystem. **The American Naturalist**, [S. l.], v. 94, n. 875, p. 129–136, 1960. DOI: 10.1086/282114. Disponível em: <https://doi.org/10.1086/282114>.

FALKOWSKI, Paul G.; FENCHEL, Tom; DELONG, Edward F. The Microbial Engines That Drive Earth's Biogeochemical Cycles. **Science**, [S. l.], v. 320, n. 5879, p. 1034–1039, 2008. DOI: 10.1126/science.1153213. Disponível em: <http://www.sciencemag.org/cgi/doi/10.1126/science.1153213>.

FARJALLA, Vinicius F.; SRIVASTAVA, Diane S.; MARINO, Nicholas a C.; AZEVEDO, Fernanda D.; DIB, Viviane; LOPES, Paloma M.; ROSADO, Alexandre S.; BOZELLI, Reinaldo L.; ESTEVES, Francisco a. Ecological determinism increases with organism size. **Ecology**, [S. l.], v. 93, n. 7, p. 1752–1759, 2012. DOI: 10.1890/11-1144.1. Disponível em: <http://www.ncbi.nlm.nih.gov/pubmed/22919920>.

FODELIANAKIS, Stilianos; VALENZUELA-CUEVAS, Adriana; BAROZZI, Alan; DAFFONCHIO, Daniele. Direct quantification of ecological drift at the population level in synthetic bacterial communities. **The ISME Journal**, [S. l.], v. 15, n. 1, p. 55–66, 2021. DOI: 10.1038/s41396-020-00754-4. Disponível em: <https://doi.org/10.1038/s41396-020-00754-4>.

FRUCHTERMAN, Thomas M. J.; REINGOLD, Edward M. Graph drawing by force-directed placement. **Software: Practice and Experience**, [S. l.], v. 21, n. 11, p. 1129–1164, 1991. DOI: 10.1002/spe.4380211102. Disponível em: <https://doi.org/10.1002/spe.4380211102>.

FUHRMAN, Jed A.; CRAM, Jacob A.; NEEDHAM, David M. Marine microbial community dynamics and their ecological interpretation. **Nature Reviews Microbiology**, [S. l.], v. 13, n. 3, p. 133–146, 2015. DOI: 10.1038/nrmicro3417. Disponível em: <http://www.nature.com/doi/10.1038/nrmicro3417>.

GASOL, Josep M. et al. Seasonal patterns in phytoplankton photosynthetic parameters and primary production at a coastal NW Mediterranean site. **Scientia Marina**, [S. l.], v. 80, n. S1, p. 63–77,

2016. DOI: 10.3989/scimar.04480.06E. Disponible em:

<https://scientiamarina.revistas.csic.es/index.php/scientiamarina/article/view/1658>.

GASOL, Josep M.; DEL GIORGIO, Paul A. Using flow cytometry for counting natural planktonic bacteria and understanding the structure of planktonic bacterial communities. **Scientia Marina**, [S. l.], v. 64, n. 2, p. 197–224, 2000. DOI: 10.3989/scimar.2000.64n2197. Disponible em:

<http://links.isiglobalnet2.com/gateway/Gateway.cgi?>

<http://links.isiglobalnet2.com/gateway/Gateway.cgi?GWVersion=2&SrcAuth=mekentosj&SrcApp=Papers&DestLinkType=FullRecord&DestApp=WOSS&KeyUT=000088019800007%5Cnpapers2://publication/uuid/D6D95D52-6E18-4CF1-BFA5-B1A9C65CA5FC>.

GINER, Caterina R.; BALAGUÉ, Vanessa; KRABBERØD, Anders K.; FERRERA, Isabel; REÑÉ, Albert; GARCÉS, Esther; GASOL, Josep M.; LOGARES, Ramiro; MASSANA, Ramon.

Quantifying long-term recurrence in planktonic microbial eukaryotes. **Molecular Ecology**, [S. l.], v. 28, n. 5, p. 923–935, 2019. DOI: 10.1111/mec.14929. Disponible em:

<https://doi.org/10.1111/mec.14929>.

GINER, Caterina R.; PERNICE, Massimo C.; BALAGUÉ, Vanessa; DUARTE, Carlos M.;

GASOL, Josep M.; LOGARES, Ramiro; MASSANA, Ramon. Marked changes in diversity and relative activity of picoeukaryotes with depth in the world ocean. **The ISME Journal**, [S. l.], v. 14, n. 2, p. 437–449, 2020. DOI: 10.1038/s41396-019-0506-9. Disponible em:

<https://doi.org/10.1038/s41396-019-0506-9>.

GLOOR, Gregory B.; MACKLAIM, Jean M.; PAWLOWSKY-GLAHN, Vera; EGOZCUE, Juan J. Microbiome Datasets Are Compositional: And This Is Not Optional. **Frontiers in Microbiology**,

[S. l.], v. 8, 2017. DOI: 10.3389/fmicb.2017.02224. Disponible em:

<https://www.frontiersin.org/articles/10.3389/fmicb.2017.02224>.

GOUY, Manolo; GUINDON, Stéphane; GASCUEL, Olivier. SeaView Version 4: A Multiplatform Graphical User Interface for Sequence Alignment and Phylogenetic Tree Building. **Molecular Biology and Evolution**, [S. l.], v. 27, n. 2, p. 221–224, 2010. DOI: 10.1093/molbev/msp259.

Disponible em: <https://doi.org/10.1093/molbev/msp259>.

GRASSHOFF, K.; KREMLING, K.; ERHARDT, M. **Methods of seawater analysis**. 3rd. ed. Weinheim, Germany: Wiley-VCH Verlag, 1999.

GUIDI, Lionel et al. Plankton networks driving carbon export in the oligotrophic ocean. **Nature**, [S. l.], v. 532, n. 7600, p. 465–470, 2016. DOI: 10.1038/nature16942. Disponible em:

<http://dx.doi.org/10.1038/nature16942>.

GUILLOU, Laure et al. The Protist Ribosomal Reference database (PR2): a catalog of unicellular eukaryote Small Sub-Unit rRNA sequences with curated taxonomy. **Nucleic Acids Research**, [S. l.], v. 41, n. D1, p. D597–D604, 2013. DOI: 10.1093/nar/gks1160. Disponible em:

<https://doi.org/10.1093/nar/gks1160>.

HERNANDEZ, Damian J.; DAVID, Aaron S.; MENGES, Eric S.; SEARCY, Christopher A.; AFKHAMI, Michelle E. Environmental stress destabilizes microbial networks. **The ISME**



**Journal**, [S. l.], v. 15, n. 6, p. 1722–1734, 2021. DOI: 10.1038/s41396-020-00882-x. Disponível em: <https://doi.org/10.1038/s41396-020-00882-x>.

HOLM, Sture. A Simple Sequentially Rejective Multiple Test Procedure. **Scandinavian Journal of Statistics**, [S. l.], v. 6, n. 2, p. 65–70, 1979. Disponível em: <http://www.jstor.org/stable/4615733>.

HUBER, Paula; METZ, Sebastian; UNREIN, Fernando; MAYORA, Gisela; SARMENTO, Hugo; DEVERCELLI, Melina. Environmental heterogeneity determines the ecological processes that govern bacterial metacommunity assembly in a floodplain river system. **The ISME Journal**, [S. l.], v. 14, n. 12, p. 2951–2966, 2020. DOI: 10.1038/s41396-020-0723-2. Disponível em: <http://www.nature.com/articles/s41396-020-0723-2>.

IBARBALZ, Federico M. et al. Global Trends in Marine Plankton Diversity across Kingdoms of Life. **Cell**, [S. l.], v. 179, n. 5, p. 1084–1097.e21, 2019. DOI: 10.1016/j.cell.2019.10.008. Disponível em: <https://doi.org/10.1016/j.cell.2019.10.008>.

JUNGER, Pedro C. et al. Global biogeography of the smallest plankton across ocean depths. **bioRxiv**, [S. l.], p. 2023.01.13.523743, 2023. DOI: 10.1101/2023.01.13.523743. Disponível em: <http://biorxiv.org/content/early/2023/01/15/2023.01.13.523743.abstract>.

KINDT, R.; COE, R. **Tree diversity analysis; A manual and software for common statistical methods for ecological and biodiversity studies**. Nairobi (Kenya): World Agroforestry Centre (ICRAF), 2005. Disponível em: <http://www.worldagroforestry.org/output/tree-diversity-analysis>.

KRABBERØD, Anders K. et al. Long-term patterns of an interconnected core marine microbiota. **Environmental Microbiome**, [S. l.], v. 17, n. 1, p. 22, 2022. DOI: 10.1186/s40793-022-00417-1. Disponível em: <https://doi.org/10.1186/s40793-022-00417-1>.

LENNON, Jay T.; DEN HOLLANDER, Frank; WILKE-BERENGUER, Maite; BLATH, Jochen. Principles of seed banks and the emergence of complexity from dormancy. **Nature Communications**, [S. l.], v. 12, n. 1, p. 4807, 2021. DOI: 10.1038/s41467-021-24733-1. Disponível em: <https://doi.org/10.1038/s41467-021-24733-1>.

LIMA-MENDEZ, Gipsi et al. Determinants of community structure in the global plankton interactome. **Science**, [S. l.], v. 348, n. 6237, p. 1–10, 2015. DOI: 10.1126/science.1262073. Disponível em: <https://www.science.org/doi/10.1126/science.1262073>.

LOCEY, K. J.; MUSCARELLA, M. E.; LARSEN, M. L.; BRAY, S. R.; JONES, S. E.; LENNON, J. T. Dormancy dampens the microbial distance–decay relationship. **Philosophical Transactions of the Royal Society B: Biological Sciences**, [S. l.], v. 375, n. 1798, p. 20190243, 2020. DOI: 10.1098/rstb.2019.0243. Disponível em: <https://doi.org/10.1098/rstb.2019.0243>.

LOGARES, Ramiro et al. Metagenomic 16S rDNA Illumina tags are a powerful alternative to amplicon sequencing to explore diversity and structure of microbial communities. **Environmental Microbiology**, [S. l.], v. 16, n. 9, p. 2659–2671, 2014. DOI: 10.1111/1462-2920.12250.

- LOGARES, Ramiro et al. Disentangling the mechanisms shaping the surface ocean microbiota. **Microbiome**, [S. l.], v. 8, n. 1, p. 55, 2020. DOI: 10.1186/s40168-020-00827-8. Disponível em: <https://microbiomejournal.biomedcentral.com/articles/10.1186/s40168-020-00827-8>.
- LOGARES, Ramiro; TESSON, Sylvie V. M.; CANBÄCK, Björn; PONTARP, Mikael; HEDLUND, Katarina; RENGEFORS, Karin. Contrasting prevalence of selection and drift in the community structuring of bacteria and microbial eukaryotes. **Environmental Microbiology**, [S. l.], v. 20, n. 6, p. 2231–2240, 2018. DOI: 10.1111/1462-2920.14265. Disponível em: <https://onlinelibrary.wiley.com/doi/10.1111/1462-2920.14265>.
- LONGHURST, Alan Reece. **Ecological Geography of the Sea**. Second ed. Burlington, MA: Academic Press, 2007.
- MARGALEF, Ramon. Life-forms of phytoplankton as survival alternatives in an unstable environment. **Oceanologica acta**, [S. l.], v. 1, n. 4, p. 493–509, 1978.
- MASSANA, Ramon; LOGARES, Ramiro. Eukaryotic versus prokaryotic marine picoplankton ecology. **Environmental Microbiology**, [S. l.], v. 15, n. 5, p. 1254–1261, 2013. DOI: 10.1111/1462-2920.12043. Disponível em: <https://doi.org/10.1111/1462-2920.12043>.
- MCARDLE, Brian H.; ANDERSON, Marti J. Fitting multivariate models to community data: A comment on distance-based redundancy analysis. **Ecology**, [S. l.], v. 82, n. 1, p. 290–297, 2001. DOI: [https://doi.org/10.1890/0012-9658\(2001\)082\[0290:FMMTCD\]2.0.CO;2](https://doi.org/10.1890/0012-9658(2001)082[0290:FMMTCD]2.0.CO;2). Disponível em: [https://doi.org/10.1890/0012-9658\(2001\)082\[0290:FMMTCD\]2.0.CO](https://doi.org/10.1890/0012-9658(2001)082[0290:FMMTCD]2.0.CO).
- MESTRE, Mireia; BORRULL, Encarna; SALA, Mmontserrat; GASOL, Josep M. Patterns of bacterial diversity in the marine planktonic particulate matter continuum. **ISME Journal**, [S. l.], v. 11, n. 4, p. 999–1010, 2017. a. DOI: 10.1038/ismej.2016.166.
- MESTRE, Mireia; FERRERA, Isabel; BORRULL, Encarna; ORTEGA-RETUERTA, Eva; MBEDI, Susan; GROSSART, Hans-Peter; GASOL, Josep M.; SALA, M. Montserrat. Spatial variability of marine bacterial and archaeal communities along the particulate matter continuum. **Molecular Ecology**, [S. l.], v. 26, n. 24, p. 6827–6840, 2017. b. DOI: 10.1111/mec.14421. Disponível em: <https://onlinelibrary.wiley.com/doi/10.1111/mec.14421>.
- MESTRE, Mireia; HÖFER, Juan; SALA, M. Montserrat; GASOL, Josep M. Seasonal Variation of Bacterial Diversity Along the Marine Particulate Matter Continuum. **Frontiers in Microbiology**, [S. l.], v. 11, 2020. DOI: 10.3389/fmicb.2020.01590. Disponível em: <https://www.frontiersin.org/articles/10.3389/fmicb.2020.01590>.
- MOORE, Christine; GREWAR, John; CUMMING, Graeme S. Quantifying network resilience: comparison before and after a major perturbation shows strengths and limitations of network metrics. **Journal of Applied Ecology**, [S. l.], v. 53, n. 3, p. 636–645, 2016. DOI: <https://doi.org/10.1111/1365-2664.12486>. Disponível em: <https://doi.org/10.1111/1365-2664.12486>.
- MOREIRA, David; LÓPEZ-GARCÍA, Purificación. Time series are critical to understand microbial plankton diversity and ecology. **Molecular Ecology**, [S. l.], v. 28, n. 5, p. 920–922, 2019. DOI: 10.1111/mec.15015. Disponível em: <https://doi.org/10.1111/mec.15015>.

NORLAND, S.; FAGERBAKKE, K. M.; HELDAL, M. Light element analysis of individual bacteria by x-ray microanalysis . These include : Light Element Analysis of Individual Bacteria by X-Ray Microanalysis. [*S. l.*], v. 61, n. 4, p. 1357–1362, 1995.

OKSANEN, Jari et al. vegan: Community Ecology Package. **R package version 2.3-2**, [*S. l.*], p. <https://CRAN.R-project.org/package=vegan>, 2015. Disponível em: <https://github.com/vegandevs/vegan/issues%0Ahttps://github.com/vegandevs/vegan>.

PARADA, Alma E.; NEEDHAM, David M.; FUHRMAN, Jed A. Every base matters: assessing small subunit rRNA primers for marine microbiomes with mock communities, time series and global field samples. **Environmental Microbiology**, [*S. l.*], v. 18, n. 5, p. 1403–1414, 2016. DOI: 10.1111/1462-2920.13023. Disponível em: <https://doi.org/10.1111/1462-2920.13023>.

PRICE, Morgan N.; DEHAL, Paramvir S.; ARKIN, Adam P. FastTree: Computing Large Minimum Evolution Trees with Profiles instead of a Distance Matrix. **Molecular Biology and Evolution**, [*S. l.*], v. 26, n. 7, p. 1641–1650, 2009. DOI: 10.1093/molbev/msp077. Disponível em: <https://doi.org/10.1093/molbev/msp077>.

QIONG, Wang; M., Garrity George; M., Tiedje James; R., Cole James. Naïve Bayesian Classifier for Rapid Assignment of rRNA Sequences into the New Bacterial Taxonomy. **Applied and Environmental Microbiology**, [*S. l.*], v. 73, n. 16, p. 5261–5267, 2007. DOI: 10.1128/AEM.00062-07. Disponível em: <https://doi.org/10.1128/AEM.00062-07>.

QUAST, Christian; PRUESSE, Elmar; YILMAZ, Pelin; GERKEN, Jan; SCHWEER, Timmy; YARZA, Pablo; PEPLIES, Jörg; GLÖCKNER, Frank Oliver. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. **Nucleic Acids Research**, [*S. l.*], v. 41, n. D1, p. D590–D596, 2013. DOI: 10.1093/nar/gks1219. Disponível em: <https://doi.org/10.1093/nar/gks1219>.

R CORE TEAM. **R: A Language and Environment for Statistical Computing**. R Foundation for Statistical Computing Vienna, Austria, 2014. Disponível em: <http://www.r-project.org/>.

REVELLE, William. **psych: Procedures for Psychological, Psychometric, and Personality Research**. Evanston, Illinois Northwestern University, , 2022. Disponível em: <https://cran.r-project.org/package=psych>.

SALAZAR, Guillem; CORNEJO-CASTILLO, Francisco M.; BENÍTEZ-BARRIOS, Verónica; FRAILE-NUEZ, Eugenio; ÁLVAREZ-SALGADO, X. Antón; DUARTE, Carlos M.; GASOL, Josep M.; ACINAS, Silvia G. Global diversity and biogeography of deep-sea pelagic prokaryotes. **The ISME Journal**, [*S. l.*], v. 10, n. 3, 2016. DOI: 10.1038/ismej.2015.137.

SCHLOSS, Patrick D. et al. Introducing mothur: Open-Source, Platform-Independent, Community-Supported Software for Describing and Comparing Microbial Communities. **Applied and Environmental Microbiology**, [*S. l.*], v. 75, n. 23, p. 7537–7541, 2009. DOI: 10.1128/AEM.01541-09. Disponível em: <https://doi.org/10.1128/AEM.01541-09>.

SIMON, Meinhard; GROSSART, HP; SCHWEITZER, Bernd; PLOUG, Helle. Microbial ecology of organic aggregates in aquatic ecosystems. **Aquatic Microbial Ecology**, [*S. l.*], v. 28, n. 2, p.

175–211, 2002. DOI: 10.3354/ame028175. Disponível em:  
<https://www.int-res.com/abstracts/ame/v28/n2/p175-211>.

SOLÉ, Ricard V; MONTOYA, M. Complexity and fragility in ecological networks. **Proceedings of the Royal Society of London. Series B: Biological Sciences**, [S. l.], v. 268, n. 1480, p. 2039–2045, 2001. DOI: 10.1098/rspb.2001.1767. Disponível em: <https://doi.org/10.1098/rspb.2001.1767>.

SOMMERIA-KLEIN, Guilhem; WATTEAUX, Romain; IBARBALZ, Federico M.; KARLUSICH, Juan José Pierella; IUDICONE, Daniele; BOWLER, Chris; MORLON, Hélène. Global drivers of eukaryotic plankton biogeography in the sunlit ocean. **Science**, [S. l.], v. 374, n. 6567, p. 594–599, 2021. DOI: 10.1126/science.abb3717. Disponível em:  
<http://biorxiv.org/content/early/2020/12/24/2020.09.08.287524.abstract>.

STEGEN, James C.; LIN, Xueju; FREDRICKSON, Jim K.; CHEN, Xingyuan; KENNEDY, David W.; MURRAY, Christopher J.; ROCKHOLD, Mark L.; KONOPKA, Allan. Quantifying community assembly processes and identifying features that impose them. **The ISME Journal**, [S. l.], v. 7, n. 11, p. 2069–2079, 2013. DOI: 10.1038/ismej.2013.93. Disponível em:  
<http://dx.doi.org/10.1038/ismej.2013.93>.

STOECK, Thorsten; BASS, David; NEBEL, Markus; CHRISTEN, Richard; JONES, Meredith D. M.; BREINER, Hans-Werner; RICHARDS, Thomas A. Multiple marker parallel tag environmental DNA sequencing reveals a highly complex eukaryotic community in marine anoxic water. **Molecular Ecology**, [S. l.], v. 19, n. s1, p. 21–31, 2010. DOI: 10.1111/j.1365-294X.2009.04480.x. Disponível em: <https://doi.org/10.1111/j.1365-294X.2009.04480.x>.

SUNAGAWA, Shinichi et al. Structure and function of the global ocean microbiome. **Science**, [S. l.], v. 348, n. 6237, p. 1261359, 2015. DOI: 10.1126/science.1261359. Disponível em:  
<https://www.science.org/doi/10.1126/science.1261359>.

TACKMANN, Janko; MATIAS RODRIGUES, João Frederico; VON MERING, Christian. Rapid Inference of Direct Interactions in Large-Scale Ecological Networks from Heterogeneous Microbial Sequencing Data. **Cell Systems**, [S. l.], v. 9, n. 3, p. 286–296.e8, 2019. DOI: 10.1016/j.cels.2019.08.002. Disponível em:  
<https://www.sciencedirect.com/science/article/pii/S2405471219302716>.

VASS, Máté; SZÉKELY, Anna J.; LINDSTRÖM, Eva S.; LANGENHEDER, Silke. Using null models to compare bacterial and microeukaryotic metacommunity assembly under shifting environmental conditions. **Scientific Reports**, [S. l.], v. 10, n. 1, p. 2455, 2020. DOI: 10.1038/s41598-020-59182-1. Disponível em: <https://doi.org/10.1038/s41598-020-59182-1>.

VENABLES, W. N.; RIPLEY, B. D. **Statistics Complements to Modern applied statistics with S**. [s.l: s.n.]. Disponível em: <http://discount-hardcover-bestsellers.info/wp-content/uploads/pdfs/Modern Applied Statistics with S by BD Ripley - Great Reference Book.pdf%5Cnpapers2://publication/uuid/F4B9BEB2-0A5B-4653-A395-3D9342AC0C5E>.

VILLARINO, Ernesto et al. Large-scale ocean connectivity and planktonic body size. **Nature Communications**, [S. l.], v. 9, n. 1, p. 142, 2018. DOI: 10.1038/s41467-017-02535-8. Disponível em: <https://doi.org/10.1038/s41467-017-02535-8>.

WATTS, Duncan J.; STROGATZ, Steven H. Collective dynamics of ‘small-world’ networks. **Nature**, [S. l.], v. 393, n. 6684, p. 440–442, 1998. DOI: 10.1038/30918. Disponível em: <https://doi.org/10.1038/30918>.

WISNOSKI, Nathan I.; LEIBOLD, Mathew A.; LENNON, Jay T. Dormancy in Metacommunities. **The American Naturalist**, [S. l.], v. 194, n. 2, p. 135–151, 2019. DOI: 10.1086/704168. Disponível em: <https://doi.org/10.1086/704168>.

ZHOU, Jizhong; DENG, Ye; LUO, Feng; HE, Zhili; TU, Qichao; ZHI, Xiaoyang. Functional Molecular Ecological Networks. **mBio**, [S. l.], v. 1, n. 4, p. e00169-10, 2010. DOI: 10.1128/mBio.00169-10. Disponível em: <https://doi.org/10.1128/mBio.00169-10>.



A dense field of small, rod-shaped bacteria, likely Rhodospirillaceae, viewed under a microscope. The bacteria are scattered across the frame, appearing as numerous small, dark, elongated structures against a lighter background.

## CAPÍTULO 4

NICHE-PARTITIONING OF RHODOSPIRILLACEAE  
AN UBIQUITOUS, ABUNDANT AND  
PREVIOUSLY OVERLOOKED MARINE BACTERIA

## NICHE-PARTITIONING OF *RHODOSPIRILLACEAE* AN UBIQUITOUS, ABUNDANT AND PREVIOUSLY OVERLOOKED MARINE BACTERIA

### ABSTRACT

Marine bacteria stand for a great part of the global biodiversity and play critical roles in the ocean. Recently, modern genomic data obtained during large-scale oceanographic cruises has founded a new era of discovery of bacterial diversity and ecology in marine ecosystems. Temperature has been found as the strongest selective driver on bacterial communities in the global ocean, which in turn is rapidly warming due to climate change. Niche-partitioning is a process in which different species coexisting in the same ecosystem evolve to occupy different ecological niches to reduce competition for limited resources. Here, we investigated temperature-driven niche-partitioning patterns in *Rhodospirillaceae*, an ubiquitous, abundant, but previously overlooked group of marine bacteria. We brought together classic ecological theory, state-of-the-art molecular techniques, and culture-independent methods to explore the ecology of a potentially important marine bacterial group (*Rhodospirillaceae*) in the global ocean. Using data from Malaspina, we found that *Rhodospirillaceae* is a free-living bacterial group more abundant in epipelagic than deep waters. We were able to identify at least four temperature niches among the most abundant *Rhodospirillaceae*: very warm (20-30°C), warm (15-20°), cold (5-15°C) and very cold (0-5°C) waters. We also assembled 5 single amplified genomes (SAGs) from Tara Oceans and retrieved 44 SAGs and 14 metagenome assembled genomes (MAGs) with high quality (>70% completeness, <10% contamination) from the literature to conduct comparative genomic analysis. The average amino acid identity (AAI) varied from 46% to 96.8% for the SAGs and from 49.2 to 99.6% for the MAGs, while the orthologous fraction (OF) varied from 20% to 87.5% for the SAGs and 18.2% to 80% for the MAGs. We found a significant inverse relationship ( $R^2=0.28$ ,  $p<0.001$ ) between mean AAI and temperature differences, indicating that *Rhodospirillaceae* genomes tended to be more similar in environments featuring similar temperature ranges. Furthermore, we found a strong negative correlation ( $r=-0.47$ ,  $p<0.001$ ) between genome size and temperature for *Rhodospirillaceae* MAGs, reinforcing that temperature is an important selective force driving bacterial niche-partitioning.

**Keywords:** Community ecology, marine microbiota, amplicon sequencing, MAGs, SAGs

## INTRODUCTION

Marine bacterial communities represent a great proportion of the biodiversity on Earth (LOCEY; LENNON, 2016) and play essential roles in global biogeochemical cycles (FALKOWSKI; FENCHEL; DELONG, 2008) as well as in trophic interactions (SHERR; SHERR, 2008). Having a representative sampling of the marine microbiome to fully understand its distribution and functionality on a global scale was very challenging since the ocean covers more than 70% of the planet's surface (~361 million km<sup>2</sup>). However, important large-scale oceanographic cruises such as the *Tara Oceans* (PESANT et al., 2015) and Malaspina (DUARTE, 2015; SALAZAR et al., 2016) have taken place during the last decade, helping the scientific community to assess the global ocean's planktonic diversity. These global oceanographic cruises, together with the technological revolution in molecular and bioinformatic tools (SALAZAR; SUNAGAWA, 2017), allowed scientists to adequately address scientific questions regarding the ecology, diversity, and evolution of microbes in the oceans (GREGORY et al., 2019; IBARBALZ et al., 2019; LOGARES et al., 2018; SALAZAR et al., 2016, 2019).

Ecological communities are structured by four main ecological processes: selection, dispersion, drift, and diversification (VELLEND, 2016). Selection is basically a reformulation of the ecological niche theory (HUTCHINSON, 1957) and it is defined as a set of deterministic factors that alter the communities by changing the species' fitness (VELLEND, 2016). A recent large-scale study has shown that selection, dispersion, and drift have a balanced role (~33%) in shaping prokaryotic communities in surface waters of the tropical and sub-tropical oceans (LOGARES et al., 2020). Among many environmental parameters, temperature has been determined as the strongest selective pressure on microbial communities in the global ocean (SUNAGAWA et al., 2015). This finding is particularly important given that the rate of ocean warming has increased by at least two times over the last two centuries (BINDOFF et al., 2019; CHENG et al., 2020) and has reached a record high in 2019 (CHENG et al., 2020). Moreover, recent climate change reports project a further ocean heating of 2 to 4 times considering the low emissions scenario or 5 to 7 times in the high emissions scenario by 2100 (BINDOFF et al., 2019). Therefore, investigating how microbial populations will respond to ocean warming is crucial to understand potential feedback mechanisms affecting ecosystem functioning.

The 'ecological niche' has been defined by Hutchinson (1957) as a hyper-dimensional space where the dimensions are the environmental conditions/resources required by an individual or population to persist. In other words, the niche concept establishes the relationship between a species' population and its biotic and abiotic environment (HUTCHINSON, 1957). According to Hutchinson's concept, two species cannot coexist with identical niches (*a.k.a.* competitive exclusion



principle) and, therefore, there must be a differentiation in at least one of the niche's dimensions to avoid extinction (HUTCHINSON, 1957). Niche-partitioning refers to the mechanism through which natural selection leads competing species into different niches (MACARTHUR, 1958). Such mechanism is fundamental for promoting co-existence between species and maintaining biological and functional diversity (LEVINE; HILLERISLAMBERS, 2009).

There are classic examples of niche-partitioning in nature, such as the case of the *Anolis* lizards from the Bimini island (SCHOENER, 1968), the Darwin's finches in the Galápagos islands (DE LEÓN et al., 2014) and the plant-resource partitioning among *Bombus* bees with different proboscises lengths (PYKE, 1982). There are also many examples of niche-partitioning in prokaryotes (SARMENTO; MORANA; GASOL, 2016), often referred to in the literature as 'ecotype diversification'. For instance, depth-related diversification among sympatric microbial populations has been described in the oceans for the archaeal phylum *Thaumarchaeota* (REJI et al., 2019) and several bacterial groups such as *Prochlorococcus* (JOHNSON et al., 2006), *Nitrospinae* (NGUGI et al., 2016), *Bacteroidetes* (DÍEZ-VIVES et al., 2019) and the ubiquitous SAR86 clade (HOARFROST et al., 2020). The important SAR11 clade also has ecotypes driven by niche-partitioning based on depth, season, and biome (SALTER et al., 2015; VERGIN et al., 2013).

*Rhodospirillales* is an order of bacteria within the subclass *Alphaproteobacteria*, which places it in the same taxonomic level as the well-known SAR11 clade, the most abundant bacteria (~25% of the total bacterial cells) in marine systems (GIOVANNONI, 2017). According to the up-to-date SILVA database (LSU r138), the *Rhodospirillales* currently contains six families: *Rhodospirillaceae*, *Magnetospiraceae*, *Magnetospirillaceae*, *Terasakiellaceae*, *Thalassospiraceae*, and AEGEAN-169 marine group. These last five families were previously classified as genera within the *Rhodospirillaceae*, the most known family within the order *Rhodospirillales*. Members of this family are Gram-negative and form rod-shaped to spirillum-formed cells (BALDANI et al., 2014). They have been generally described as purple non-sulfur bacteria and, thus, can perform a wide range of metabolisms (BALDANI et al., 2014). While some genera may shift from photoheterotrophic growth under anoxic conditions in the light to chemoheterotrophic growth in the dark, others grow only heterotrophically under aerobic conditions (BALDANI et al., 2014). Such wide metabolic capacities likely confer them the ability to habit a wide range of environments, from surface waters to the deep ocean, regardless of temperature and trophic state. Despite being constantly cited among the most abundant taxa in the ocean (AULADELL et al., 2022; CRAM et al., 2015; REINTJES et al., 2019), there is still very minimal information about this bacterial group, especially those within the newly classified families, because most of them are so far uncultured.

The use of culture-independent methods such as co-assembled metagenomes (metagenomic assembled genomes; MAGs) and single-cell genomics (single amplified genomes; SAGs) has been rapidly growing in the field of microbial ecology (PACHIADAKI et al., 2019; SWAN et al., 2013). These techniques are compelling alternatives that allow the retrieval of uncultured genomes that can be used to assess genetic, functional, and evolutionary differences among closely related microbes and identify ecological roles and biotechnology potential of uncultured microbial groups (PACHIADAKI et al., 2019). MAGs and SAGs results have recently been shown to strongly correlate and, thus, generate precise genomic information about not-yet-cultured bacterial populations (ALNEBERG et al., 2018), proving that the combination of these two methods is a powerful approach to expand our knowledge on the majority part of the microbial diversity. Most SAG-based pangenome analyses in marine systems have focused on the link between the environment and hyper-variable genomic islands from *Prochlorococcus* and SAR11 populations (DELMONT; EREN, 2018; THOMPSON et al., 2019). However, a recent study has successfully used this technology to describe novel species and their ecological and functional capacities (ROYO-LLONCH et al., 2020). Single-cell genomics has also enabled the functional annotation to lower taxonomic resolutions, from genus (PACHIADAKI et al., 2019) to species-level (ROYO-LLONCH et al., 2020), which represents a breakthrough in the study of microbial diversity.

Very little is known about the biology, functionality, and ecology of the *Rhodospirillaceae* groups in the ocean, particularly the AEGEAN-169 marine group, despite being among the most abundant marine bacteria in the ocean (CRAM et al., 2015; REINTJES et al., 2019). This work brings together classic ecological theory and state-of-the-art molecular techniques, including culture-independent methods (e.g., SAGs, MAGs), to explore the biology and ecology of a potentially important but previously overlooked marine bacterial group (*Rhodospirillaceae*) in the global ocean. Our specific aims were: 1) to investigate niche-partitioning patterns of marine bacteria belonging to the *Rhodospirillaceae* order in the global ocean. To achieve this goal, we combined spatial data from a global oceanographic cruise (Malaspina) and one regional cruise (HotMix Mediterranean Cruise), thereby covering the spatial (horizontal and vertical) dimension in different size-fractions of the planktonic community and; 2) to investigate the genomic basis of this niche-segregation pattern by using comparative genomics of single-cell amplified genomes (SAGs) and metagenome amplified genomes (MAGs) of the *Rhodospirillaceae* bacteria.

## METHODOLOGY

### *Datasets*

To evaluate the general distribution and niche-partitioning patterns of *Rhodospirillaceae*, we used a compiled dataset hereafter called *MalaMix* (Malaspina Global Expedition + HotMix Mediterranean Expedition) with a total of 688 samples (500 in Malaspina and 188 in HotMix) in 148 sampling stations (119 in Malaspina and 29 in Hotmix) divided in different size fractions covering the epi- (0 - 200m), meso- (200 - 1000m) and bathypelagic (1000-4000m) regions of the tropical and sub-tropical global ocean (Fig. II-1). To evaluate these patterns in different size fractions of the planktonic community, we used part of the Malaspina dataset with the following size-fractions: 0.2–0.8, 0.8–3.0, 3.0–5.0, 5.0–10, 10–20 and 20–200  $\mu\text{m}$  (MESTRE et al., 2018). To obtain information about the adaptation mechanisms of *Rhodospirillaceae* to different temperatures, which ultimately leads to the observed niche partitioning patterns, we used both SAGs and MAGs (see details below).

### *Single-amplified genomes (SAGs)*

Single-amplified genomes were generated from seawater samples from the global ocean during the circumnavigation expedition *Tara Oceans* (PESANT et al., 2015). Five out of 35 SAGs with the 16S rRNA gene assigned to *Rhodospirillaceae* were selected for complete genome sequencing based on a preliminary phylogenetical tree to identify SAGs representative of abundant clusters in contrasting temperatures of the ocean (Table 1). These SAGs were sequenced in one batch by Illumina HiSeq 2 x 250 bp technology at the Centre Nacional d'anàlisi Genòmica (CNAG, Barcelona). First, we conducted the quality assessment of the raw reads using Bowtie2 v2.2.9 (LANGMEAD; SALZBERG, 2012) and Samtools v1.3.1 (LI et al., 2009). Then, reads were individually normalized by coverage for each SAG with DOW JGI's Bbnorm and the normalized paired-end libraries were trimmed and filtered using Trimmomatic (BOLGER; LOHSE; USADEL, 2014) and merged with PEAR v0.9.6 (ZHANG et al., 2013). These genomes were co-assembled with the assembler Ray v2.2.0 and then combined using assembler SPAdes v3.10 (BANKEVICH et al., 2012), which is a strategy demonstrated to reduce gene redundancy and genome fragmentation (ROYO-LLONCH et al., 2020). Finally, gene prediction and functional annotation of the co-assembled contigs were conducted using the software Prokka v1.12 (SEEMANN, 2014).

Two of the five SAGs had excellent quality (which were used in downstream analysis), one medium quality, and two were classified as bad quality (Table 1). In order to have more genomes for further analysis, we also retrieved *Rhodospirillaceae* SAGs from a recently published marine SAGs database (PACHIADAKI et al., 2019). We selected only high-quality SAGs with >70%

completeness and <10% contamination. We found, in total, 44 SAGs belonging to the *Rhodospirillaceae* that were within our quality criteria. We managed to gather a total of 46 good-quality SAGs with >70% completeness and <10% contamination.

**Table IV–1.** List of the *Rhodospirillaceae* SAGs that were sequenced for this study with information on the *Tara Oceans* station and respective ocean region where they were retrieved from as well as the taxonomy (genus level classification from 16S rRNA gene) and correspondent temperature niche. Completeness and contamination values were obtained using *compareM*.

SAG code	Tara Station	Ocean Region	Temperature Niche	Genera	Completeness (%)	Contamination (%)
AAA536_F16+AAA682_D05	023	Mediterranean Sea	High temperature (Summer/Fall in Blanes)	<i>Telmatospirillum</i>	90.53	5.42
AAA536_L03+AAA682_F08	023	Mediterranean Sea	Low temperature (Winter in Blanes)	<i>Telmatospirillum</i>	84.94	24.7
AAA536_M11+AAA682_B10	023	Mediterranean Sea	Medium temperature (Mid fall in Blanes)	<i>Telmatospirillum</i>	41.29	0
AD-623_O19+AF-152_A06	078	South Atlantic Ocean	Medium temperature	<i>Nisaea</i>	35.34	1.72
AH-341_L07+AK-140_E03	194	Pacific Arctic (Beaufort Gyre)	Low temperature	<i>Magnetovibrio</i>	88.45	2.69

### ***Metagenomic assembled genomes (MAGs)***

We obtained 14 *Rhodospirillaceae* MAGs binned from the OceanDNA MAG catalog (NISHIMURA; YOSHIZAWA, 2022), including *TARA Oceans* MAGs. We also obtained 3 MAGs from the Blanes Bay Microbial Observatory (BBMO) database (not yet published). We have selected 17 *Rhodospirillaceae* MAGs classified as ‘good quality’ according to our criteria (>70% completeness and <10% contamination).

## **Comparative genomics of MAGs and SAGs**

Comparative genomics analysis of *Rhodospirillaceae* genomes (MAGs and SAGs) was performed using compareM workflow (<https://github.com/dparks1134/CompareM>). We calculated the average amino acid identity (AAI) and the orthologous fraction (OF) between genomes to identify those representing the same or different ecotypes.

## **RESULTS**

### ***General patterns of distribution***

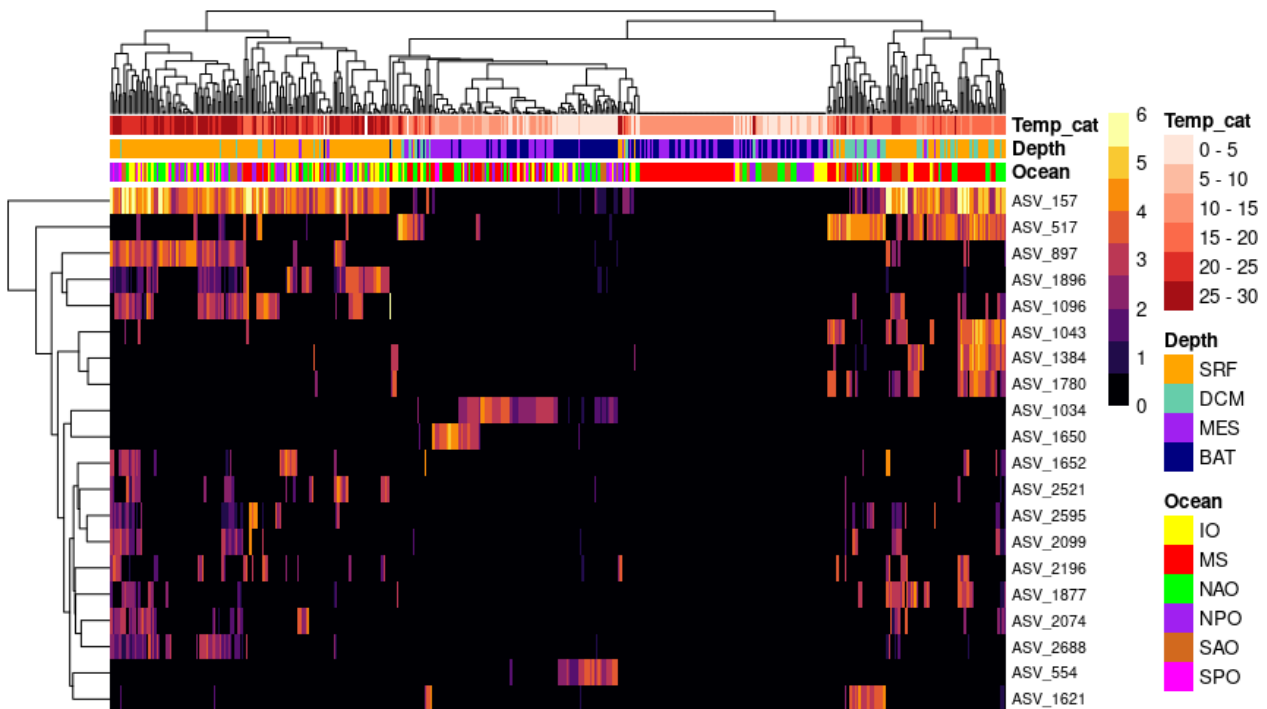
We found that the *Rhodospirillaceae* has a free-living life-style, as pointed out by their significantly higher abundance (n° of reads) in the 0.2-3µm size-fraction than in the other large size-fractions (Fig. IV-S1A). We also found that the free-living *Rhodospirillaceae* bacteria are more abundant in the epipelagic, especially at the surface (Fig. IV-S1B). They displayed more reads in the North Atlantic Ocean (NAO) and the Mediterranean Sea (MS) than in the other ocean basins (Fig. IV-S1B). We also explored the correlation between *Rhodospirillaceae* reads and the environmental variables (Fig. IV-S2). This group seems relatively more abundant in warm rather than cold waters, reaching its largest n° of reads at around 15-25°C (Fig. IV-S2). They also displayed larger n° of reads in productive waters with relatively high nutrients and chlorophyll-a concentration (Fig. IV-S2).

### ***Niche-partitioning pattern***

We have used a database (see a more detailed description in methods) covering a wide temperature range (from 0 to 30°C). We found a clear niche-partitioning pattern driven by temperature in *Rhodospirillaceae*, which are among the most abundant taxa in the global ocean (4 to 12% of the total bacterial community reads). The most abundant *Rhodospirillaceae* ASVs displayed a temperature-driven niche-partitioning pattern (Fig. IV-1). We were able to identify at least four temperature ASV clusters:

- (a) Very warm (20-30°C) surface waters dominated by ASV\_157, ASV\_897, ASV\_1896, ASV\_1096, among others. Here ASV\_157 seems to be more generalist, inhabiting waters ranging from 15 to 30°C;
- (b) Epipelagic waters with intermediate temperatures (15-20°C) dominated by ASV\_517, ASV\_1043, ASV\_1384, ASV\_1780, ASV\_1621, among others. Here ASV\_1621 was very characteristic of the DCM;

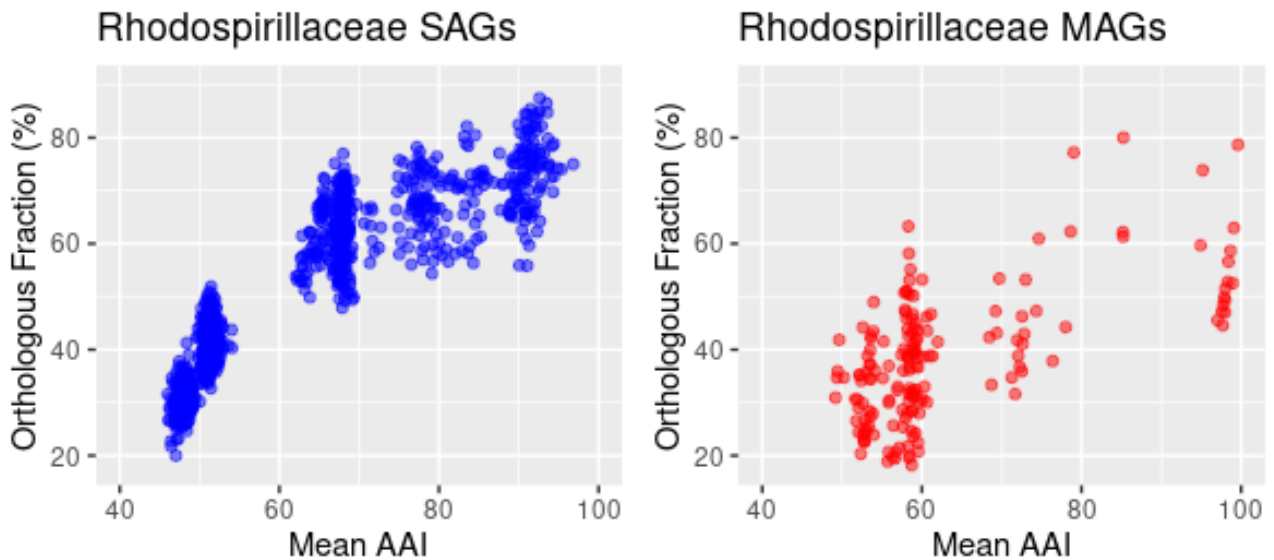
- (c) Cold meso- and bathypelagic waters (5-15 °C) dominated by ASV\_1034 and ASV\_1650. Here ASV\_1034 had a wider distribution, also inhabiting bathypelagic waters, while ASV\_1650 was more restricted to the mesopelagic;
- (d) Very cold bathypelagic waters (0-5°C) dominated only by ASV\_554.



**Figure IV-1.** Heatmap showing the distribution of the 20 most abundant *Rhodospirillaceae* ASVs (>500 reads across all samples) categorized by temperature, depth, and ocean basins of the tropical and sub-tropical global ocean. ASVs are in order of their relative abundance. IO = Indian Ocean, MS = Mediterranean Sea, NOA = North Atlantic Ocean, NPO=North Pacific Ocean, SAO = South Atlantic Ocean, SPO = South Pacific Ocean.

### Comparative Genomics

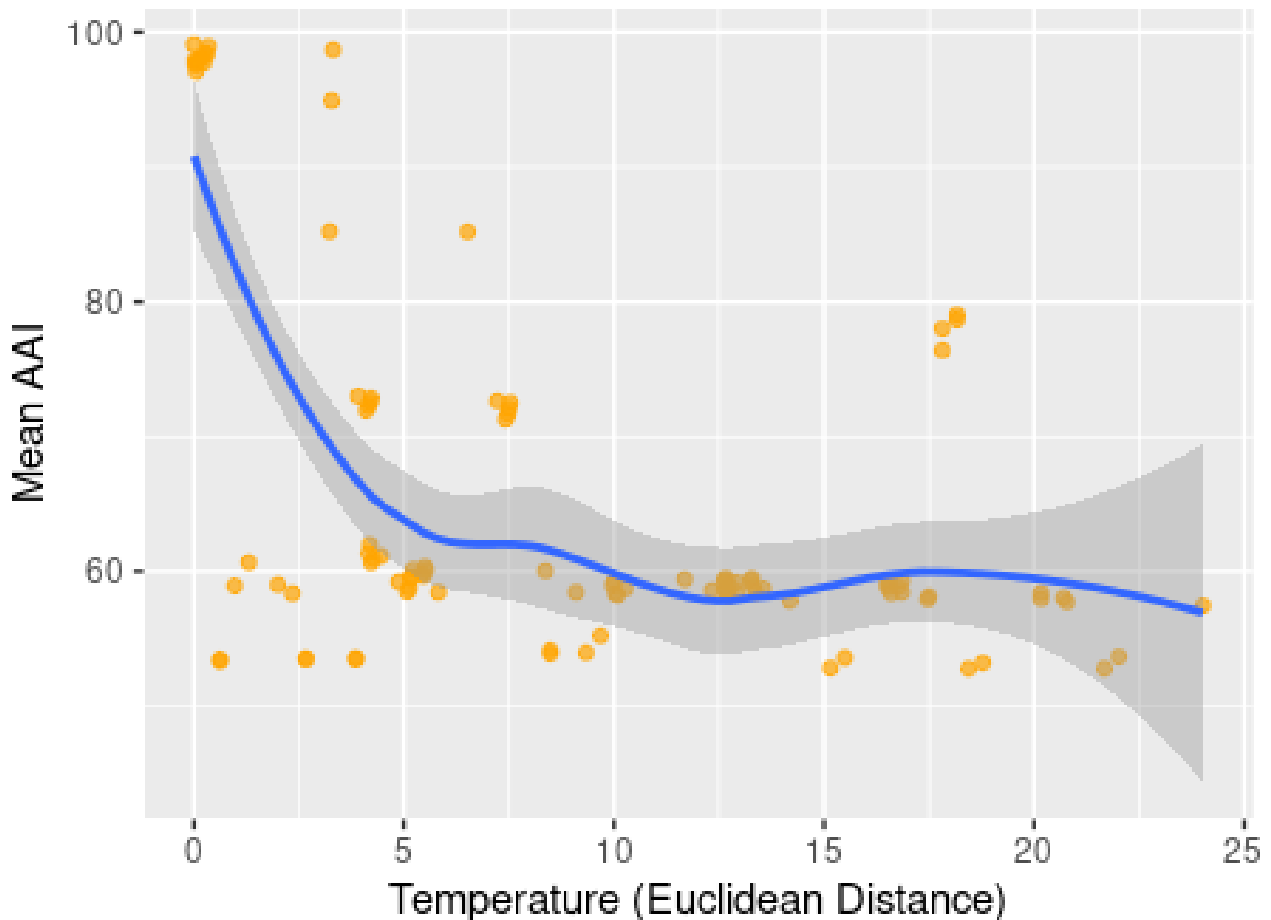
The mean AAI values varied from 46% to 96.8% for the SAGs and from 49.2 to 99.6% for the MAGs (Fig. IV-2). The OF varied from 20% to 87.5% for the SAGs and 18.2% to 80% for the MAGs (Fig. IV-2). Overall, genomes yielding a mean AAI higher than 98% are likely identical. Those genomes with mean AAI >95% can be considered very similar. The OF provides important complementary information to define whether genomes belong to the same species. Genomes with high mean AAI and OF are likely to represent the same species. On the other hand, genomes with low mean AAI and OF probably represent divergent genera within that family.



**Figure IV-2.** Mean amino acid identity (AAI) against the orthologous fraction for each pair of SAGs and MAGs. Orthologous fraction (OF) is defined as the number of orthologous genes divided by the minimum number of genes between a given pair of genomes.

We found an inverse correlation ( $R^2=-0.28$ ,  $p<0.001$ ) between mean AAI and differences in temperature (Fig. IV-3). In other words, *Rhodospirillaceae* genomes tended to be more similar in environments with similar temperatures, indicating that temperature is indeed an important selective force for this bacterial group niche-partitioning pattern. In fact, highly similar genomes, probably representing the same species, were found in environments with the same or very similar temperatures (Fig. IV-3). There were some exceptions of bacterial genomes with AAI smaller than 60% inhabiting environments with little temperature differences, suggesting that other environmental variables are shaping their genomes. Finally, we found a strong negative correlation ( $r=-0.47$ ,  $p<0.001$ ) between genome size and temperature for the *Rhodospirillaceae* MAGs (Fig. IV-4).

## Rhodospirillaceae MAGs



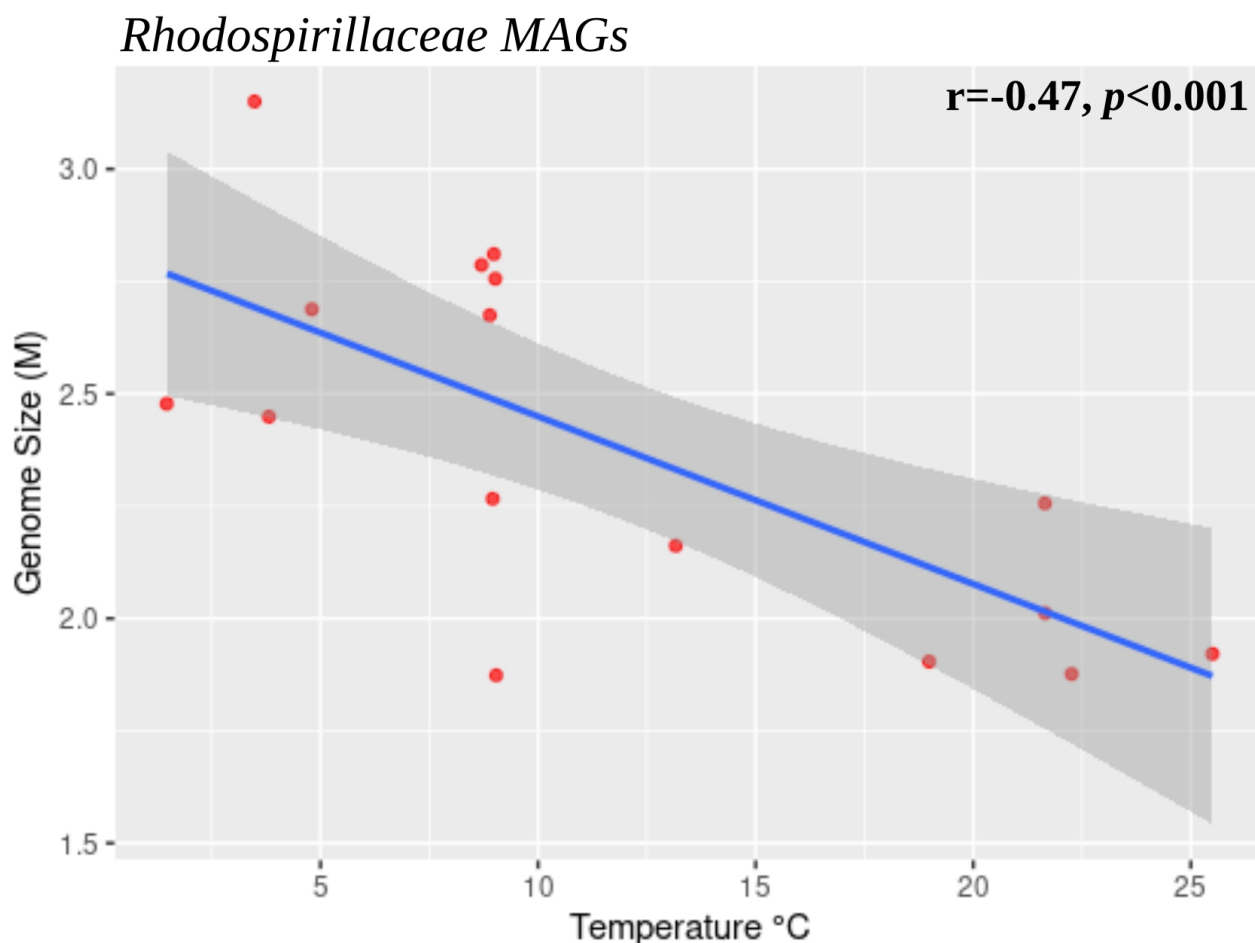
**Figure IV-3.** Gamma regression between mean AAI and temperature (Euclidean distance) for the MAGs obtained from the OceanDNA database (NISHIMURA; YOSHIZAWA, 2022).

## DISCUSSION

Temperature-driven niche partitioning within closely related marine bacteria has been previously reported for several bacterial groups (AULADELL et al., 2022), including ubiquitous and abundant groups such as *Prochlorococcus* (JOHNSON et al., 2006), SAR86 (HOARFROST et al., 2020) and SAR11 (SALTER et al., 2015; VERGIN et al., 2013). Here, we show that *Rhodospirillaceae*, an abundant marine bacteria found across ocean depths, also displays niche segregation based on temperature differences. Such niche dissimilarity tends to increase with genetic distance (nucleotide divergence), which has been detectable among SAR11 clades using the marker 16S rRNA gene (AULADELL et al., 2022). Indeed, changes in the 16S rRNA gene can reflect several changes across complete bacterial genomes (VANINSBERGHE et al., 2020), ultimately leading to niche differentiation (AULADELL et al., 2022). This represents the genomic



basis of selection (also called ‘environmental filtering’), an important ecological process shaping prokaryotic communities in the ocean (JUNGER et al., 2023; LOGARES et al., 2020).



**Figure IV–4.** Negative correlation between Genome Size (M) and temperature (°C) for the MAGs obtained from the OceanDNA database (NISHIMURA; YOSHIZAWA, 2022). The spearman’s rank correlation coefficient is depicted on the plot.

Bacterial lifestyle and adaptation to its ecological niche strongly determine genome size and complexity (RODA-GARCIA et al., 2023). The negative correlation between *Rhodospirillaceae* genome size and temperature has been previously reported for prokaryotes (ROYO-LLONCH et al., 2021). Arctic prokaryotic MAGs have larger genome sizes than those inhabiting lower latitudes, probably because of the high availability of resources and fast growth rates during polar spring/summer (ROYO-LLONCH et al., 2021). Likewise, particle-attached prokaryotes were shown to have larger genome sizes than free-living prokaryotes, regardless of the ocean depth (LEU et al., 2022). These patterns may be explained by the fact that larger genomes present a higher number of ribosomal gene copies, which is in turn associated with smaller minimum generation times (ROLLER; STODDARD; SCHMIDT, 2016; ROYO-LLONCH et al., 2021). Alternatively, the larger genomes of psychrophilic (adapted to cold environments) bacteria could also be related to

the presence of genes underpinning adaptations to low temperatures. For instance, ice-binding proteins are ecologically important enzymes for organisms growing in cold conditions (DORRELL et al., 2023; WINDER et al., 2023; ZHANG et al., 2020). On the other hand, the smaller genome sizes in bacteria inhabiting oligotrophic warmer waters could be associated with a higher mutation rate due to the loss of genes related to DNA repair (BOURGUIGNON et al., 2020). Decreasing bacterial genome size due to environmental filtering has also been referred to in the literature as genomic streamlining (DUPONT et al., 2012; RODA-GARCIA et al., 2023; SWAN et al., 2013).

Understanding the genes involved in life adaptation to thermal niches is particularly important in the context of an increasingly warm ocean. It will allow us to make predictions regarding the genomic basis underpinning the ecological processes shaping the ocean microbiome. Our work, alongside several recent reports, suggests the loss of bacterial genes with ocean warming. Future research should investigate how such genomic reduction could affect genes responsible for global biogeochemical cycles, which are essential for the maintenance of the biosphere.

## REFERENCES

ALNEBERG, Johannes et al. Genomes from uncultivated prokaryotes: a comparison of metagenome-assembled and single-amplified genomes. **Microbiome**, [S. l.], v. 6, n. 1, p. 173, 2018. DOI: 10.1186/s40168-018-0550-0. Disponível em: <https://doi.org/10.1186/s40168-018-0550-0>.

AULADELL, Adrià; BARBERÁN, Albert; LOGARES, Ramiro; GARCÉS, Esther; GASOL, Josep M.; FERRERA, Isabel. Seasonal niche differentiation among closely related marine bacteria. **The ISME Journal**, [S. l.], v. 16, n. 1, p. 178–189, 2022. DOI: 10.1038/s41396-021-01053-2. Disponível em: <https://doi.org/10.1038/s41396-021-01053-2>.

BALDANI, José Ivo et al. The Family Rhodospirillaceae. In: ROSENBERG, Eugene; DELONG, Edward F.; LORY, Stephen; STACKEBRANDT, Erko; THOMPSON, Fabiano (org.). **The Prokaryotes: Alphaproteobacteria and Betaproteobacteria**. Berlin, Heidelberg: Springer Berlin Heidelberg, 2014. p. 533–618. DOI: 10.1007/978-3-642-30197-1\_300. Disponível em: [https://doi.org/10.1007/978-3-642-30197-1\\_300](https://doi.org/10.1007/978-3-642-30197-1_300).

BANKEVICH, Anton et al. SPAdes: A New Genome Assembly Algorithm and Its Applications to Single-Cell Sequencing. **Journal of Computational Biology**, [S. l.], v. 19, n. 5, p. 455–477, 2012. DOI: 10.1089/cmb.2012.0021. Disponível em: <https://doi.org/10.1089/cmb.2012.0021>.

BINDOFF, N. L. et al. Changing Ocean, Marine Ecosystems, and Dependent Communities. In: **IPCC Special Report on the Ocean and Cryosphere in a Changing Climate**. [s.l.: s.n.]. Disponível em: <http://hdl.handle.net/20.500.11850/395234>.

BOLGER, Anthony M.; LOHSE, Marc; USADEL, Bjoern. Trimmomatic: a flexible trimmer for Illumina sequence data. **Bioinformatics**, [S. l.], v. 30, n. 15, p. 2114–2120, 2014. DOI: 10.1093/bioinformatics/btu170. Disponível em: <https://doi.org/10.1093/bioinformatics/btu170>.

BOURGUIGNON, Thomas et al. Increased Mutation Rate Is Linked to Genome Reduction in Prokaryotes. **Current Biology**, [S. l.], v. 30, n. 19, p. 3848–3855.e4, 2020. DOI: <https://doi.org/10.1016/j.cub.2020.07.034>. Disponível em: <https://www.sciencedirect.com/science/article/pii/S0960982220310265>.

CHENG, Lijing et al. Record-Setting Ocean Warmth Continued in 2019. **Advances in Atmospheric Sciences**, [S. l.], v. 37, n. 2, p. 137–142, 2020. DOI: 10.1007/s00376-020-9283-7. Disponível em: <https://doi.org/10.1007/s00376-020-9283-7>.

CRAM, Jacob A.; CHOW, Cheryl-Emiliane T.; SACHDEVA, Rohan; NEEDHAM, David M.; PARADA, Alma E.; STEELE, Joshua A.; FUHRMAN, Jed A. Seasonal and interannual variability of the marine bacterioplankton community throughout the water column over ten years. **The ISME Journal**, [S. l.], v. 9, n. 3, p. 563–580, 2015. DOI: 10.1038/ismej.2014.153. Disponível em: <https://doi.org/10.1038/ismej.2014.153>.

DE LEÓN, L. F.; PODOS, J.; GARDEZI, T.; HERREL, A.; HENDRY, A. P. Darwin's finches and their diet niches: the sympatric coexistence of imperfect generalists. **Journal of Evolutionary Biology**, [S. l.], v. 27, n. 6, p. 1093–1104, 2014. DOI: 10.1111/jeb.12383. Disponível em: <https://doi.org/10.1111/jeb.12383>.

DELMONT, Tom O.; EREN, A. Murat. Linking pangenomes and metagenomes: the Prochlorococcus metapangenome. **PeerJ**, [S. l.], v. 6, p. e4320, 2018. DOI: 10.7717/peerj.4320. Disponível em: <https://doi.org/10.7717/peerj.4320>.

DÍEZ-VIVES, Cristina; NIELSEN, Shaun; SÁNCHEZ, Pablo; PALENZUELA, Oswaldo; FERRERA, Isabel; SEBASTIÁN, Marta; PEDRÓS-ALIÓ, Carlos; GASOL, Josep M.; ACINAS, Silvia G. Delineation of ecologically distinct units of marine Bacteroidetes in the Northwestern Mediterranean Sea. **Molecular Ecology**, [S. l.], v. 28, n. 11, p. 2846–2859, 2019. DOI: 10.1111/mec.15068. Disponível em: <https://doi.org/10.1111/mec.15068>.

DORRELL, Richard G. et al. Convergent evolution and horizontal gene transfer in Arctic Ocean microalgae. **Life Science Alliance**, [S. l.], v. 6, n. 3, p. e202201833, 2023. DOI: 10.26508/lsa.202201833. Disponível em: <http://www.life-science-alliance.org/content/6/3/e202201833.abstract>.

DUARTE, Carlos M. Seafaring in the 21st Century: The Malaspina 2010 Circumnavigation Expedition. **Limnology and Oceanography Bulletin**, [S. l.], v. 24, n. 1, p. 11–14, 2015. DOI: 10.1002/lob.10008. Disponível em: <https://doi.org/10.1002/lob.10008>.

DUPONT, Chris L. et al. Genomic insights to SAR86, an abundant and uncultivated marine bacterial lineage. **The ISME Journal**, [S. l.], v. 6, n. 6, p. 1186–1199, 2012. DOI: 10.1038/ismej.2011.189. Disponível em: <https://doi.org/10.1038/ismej.2011.189>.

FALKOWSKI, Paul G.; FENCHEL, Tom; DELONG, Edward F. The Microbial Engines That Drive Earth's Biogeochemical Cycles. **Science**, [S. l.], v. 320, n. 5879, p. 1034–1039, 2008. DOI: 10.1126/science.1153213. Disponível em: <http://www.sciencemag.org/cgi/doi/10.1126/science.1153213>.

- GIOVANNONI, Stephen J. SAR11 Bacteria: The Most Abundant Plankton in the Oceans. **Annual Review of Marine Science**, [S. l.], v. 9, n. 1, p. 231–255, 2017. DOI: 10.1146/annurev-marine-010814-015934. Disponível em: <https://doi.org/10.1146/annurev-marine-010814-015934>.
- GREGORY, Ann C. et al. Marine DNA Viral Macro- and Microdiversity from Pole to Pole. **Cell**, [S. l.], v. 177, n. 5, p. 1109–1123.e14, 2019. DOI: <https://doi.org/10.1016/j.cell.2019.03.040>. Disponível em: <http://www.sciencedirect.com/science/article/pii/S0092867419303411>.
- HOARFROST, Adrienne; NAYFACH, Stephen; LADAU, Joshua; YOOSEPH, Shibu; ARNOSTI, Carol; DUPONT, Chris L.; POLLARD, Katherine S. Global ecotypes in the ubiquitous marine clade SAR86. **The ISME Journal**, [S. l.], v. 14, n. 1, p. 178–188, 2020. DOI: 10.1038/s41396-019-0516-7. Disponível em: <https://doi.org/10.1038/s41396-019-0516-7>.
- HUTCHINSON, M. Concluding remarks. In: 1957, **Anais [...]**. : Cold Spring Harb Symp Quant Biol, 1957. p. 415–427.
- IBARBALZ, Federico M. et al. Global Trends in Marine Plankton Diversity across Kingdoms of Life. **Cell**, [S. l.], v. 179, n. 5, p. 1084–1097.e21, 2019. DOI: 10.1016/j.cell.2019.10.008. Disponível em: <https://doi.org/10.1016/j.cell.2019.10.008>.
- JOHNSON, Zackary I.; ZINSER, Erik R.; COE, Allison; MCNULTY, Nathan P.; WOODWARD, E. Malcolm S.; CHISHOLM, Sallie W. Niche Partitioning Among *Prochlorococcus* Ecotypes Along Ocean-Scale Environmental Gradients. **Science**, [S. l.], v. 311, n. 5768, p. 1737 LP-1740, 2006. DOI: 10.1126/science.1118052. Disponível em: <http://science.sciencemag.org/content/311/5768/1737.abstract>.
- JUNGER, Pedro C. et al. Global biogeography of the smallest plankton across ocean depths. **bioRxiv**, [S. l.], p. 2023.01.13.523743, 2023. DOI: 10.1101/2023.01.13.523743. Disponível em: <http://biorxiv.org/content/early/2023/01/15/2023.01.13.523743.abstract>.
- LANGMEAD, Ben; SALZBERG, Steven L. Fast gapped-read alignment with Bowtie 2. **Nature Methods**, [S. l.], v. 9, n. 4, p. 357–359, 2012. DOI: 10.1038/nmeth.1923. Disponível em: <https://doi.org/10.1038/nmeth.1923>.
- LEU, Andy O.; EPPLEY, John M.; BURGER, Andrew; DELONG, Edward F. Diverse Genomic Traits Differentiate Sinking-Particle-Associated versus Free-Living Microbes throughout the Oligotrophic Open Ocean Water Column. **mBio**, [S. l.], v. 13, n. 4, p. e01569-22, 2022. DOI: 10.1128/mbio.01569-22. Disponível em: <https://doi.org/10.1128/mbio.01569-22>.
- LEVINE, Jonathan M.; HILLERISLAMBERS, Janneke. The importance of niches for the maintenance of species diversity. **Nature**, [S. l.], v. 461, n. 7261, p. 254–257, 2009. DOI: 10.1038/nature08251. Disponível em: <https://doi.org/10.1038/nature08251>.
- LI, Heng et al. The Sequence Alignment/Map format and SAMtools. **Bioinformatics**, [S. l.], v. 25, n. 16, p. 2078–2079, 2009. DOI: 10.1093/bioinformatics/btp352. Disponível em: <https://doi.org/10.1093/bioinformatics/btp352>.

LOCEY, Kenneth J.; LENNON, Jay T. Scaling laws predict global microbial diversity. **Proceedings of the National Academy of Sciences**, [S. l.], v. 113, n. 21, p. 5970–5975, 2016. DOI: 10.1073/pnas.1521291113. Disponível em: <http://www.pnas.org/lookup/doi/10.1073/pnas.1521291113>.

LOGARES, Ramiro et al. Different processes shape prokaryotic and picoeukaryotic assemblages in the sunlit ocean microbiome. **bioRxiv**, [S. l.], n. Icm, p. 37–49, 2018. Disponível em: <http://biorxiv.org/content/early/2018/07/23/374298.abstract>.

LOGARES, Ramiro et al. Disentangling the mechanisms shaping the surface ocean microbiota. **Microbiome**, [S. l.], v. 8, n. 1, p. 55, 2020. DOI: 10.1186/s40168-020-00827-8. Disponível em: <https://microbiomejournal.biomedcentral.com/articles/10.1186/s40168-020-00827-8>.

MACARTHUR, Robert H. Population Ecology of Some Warblers of Northeastern Coniferous Forests. **Ecology**, [S. l.], v. 39, n. 4, p. 599–619, 1958. DOI: 10.2307/1931600. Disponível em: <https://doi.org/10.2307/1931600>.

MESTRE, Mireia; RUIZ-GONZÁLEZ, Clara; LOGARES, Ramiro; DUARTE, Carlos M.; GASOL, Josep M.; SALA, M. Montserrat. Sinking particles promote vertical connectivity in the ocean microbiome. **Proceedings of the National Academy of Sciences**, [S. l.], v. 115, n. 29, p. E6799 LP-E6807, 2018. DOI: 10.1073/pnas.1802470115. Disponível em: <http://www.pnas.org/content/115/29/E6799.abstract>.

NGUGI, David Kamanda; BLOM, Jochen; STEPANAUSKAS, Ramunas; STINGL, Ulrich. Diversification and niche adaptations of Nitrospina-like bacteria in the polyextreme interfaces of Red Sea brines. **The ISME Journal**, [S. l.], v. 10, n. 6, p. 1383–1399, 2016. DOI: 10.1038/ismej.2015.214. Disponível em: <https://doi.org/10.1038/ismej.2015.214>.

NISHIMURA, Yosuke; YOSHIZAWA, Susumu. The OceanDNA MAG catalog contains over 50,000 prokaryotic genomes originated from various marine environments. **Scientific Data**, [S. l.], v. 9, n. 1, p. 305, 2022. DOI: 10.1038/s41597-022-01392-5. Disponível em: <https://doi.org/10.1038/s41597-022-01392-5>.

PACHIADAKI, Maria G. et al. Charting the Complexity of the Marine Microbiome through Single-Cell Genomics. **Cell**, [S. l.], v. 179, n. 7, p. 1623–1635.e11, 2019. DOI: <https://doi.org/10.1016/j.cell.2019.11.017>. Disponível em: <http://www.sciencedirect.com/science/article/pii/S0092867419312735>.

PESANT, Stéphane et al. Open science resources for the discovery and analysis of Tara Oceans data. **Scientific Data**, [S. l.], v. 2, n. Lmd, p. 150023, 2015. DOI: 10.1038/sdata.2015.23. Disponível em: <http://www.nature.com/articles/sdata201523>.

PYKE, Graham H. Local Geographic Distributions of Bumblebees Near Crested Butte, Colorado: Competition and Community Structure. **Ecology**, [S. l.], v. 63, n. 2, p. 555–573, 1982. DOI: 10.2307/1938970. Disponível em: <https://doi.org/10.2307/1938970>.

REINTJES, Greta et al. On-Site Analysis of Bacterial Communities of the Ultraoligotrophic South Pacific Gyre. **Applied and Environmental Microbiology**, [S. l.], v. 85, n. 14, p. e00184-19, 2019.

DOI: 10.1128/AEM.00184-19. Disponible em: <http://aem.asm.org/content/85/14/e00184-19.abstract>.

REJI, Linta; TOLAR, Bradley B.; SMITH, Jason M.; CHAVEZ, Francisco P.; FRANCIS, Christopher A. Differential co-occurrence relationships shaping ecotype diversification within Thaumarchaeota populations in the coastal ocean water column. **The ISME Journal**, [S. l.], v. 13, n. 5, p. 1144–1158, 2019. DOI: 10.1038/s41396-018-0311-x. Disponible em: <https://doi.org/10.1038/s41396-018-0311-x>.

RODA-GARCIA, Juan J.; HARO-MORENO, Jose M.; RODRIGUEZ-VALERA, Francisco; ALMAGRO-MORENO, Salvador; LÓPEZ-PÉREZ, Mario. Single-amplified genomes reveal most streamlined free-living marine bacteria. **Environmental Microbiology**, [S. l.], 2023. DOI: 10.1111/1462-2920.16348. Disponible em: <https://doi.org/10.1111/1462-2920.16348>.

ROLLER, Benjamin R. K.; STODDARD, Steven F.; SCHMIDT, Thomas M. Exploiting rRNA operon copy number to investigate bacterial reproductive strategies. **Nature Microbiology**, [S. l.], v. 1, n. 11, p. 16160, 2016. DOI: 10.1038/nmicrobiol.2016.160. Disponible em: <https://doi.org/10.1038/nmicrobiol.2016.160>.

ROYO-LLONCH, M.; SÁNCHEZ, P.; GONZÁLEZ, J. M.; PEDRÓS-ALIÓ, C.; ACINAS, S. G. Ecological and functional capabilities of an uncultured *Kordia* sp. **Systematic and Applied Microbiology**, [S. l.], v. 43, n. 1, p. 126045, 2020. DOI: <https://doi.org/10.1016/j.syapm.2019.126045>. Disponible em: <http://www.sciencedirect.com/science/article/pii/S0723202019303406>.

ROYO-LLONCH, Marta et al. Compendium of 530 metagenome-assembled bacterial and archaeal genomes from the polar Arctic Ocean. **Nature Microbiology**, [S. l.], v. 6, n. 12, p. 1561–1574, 2021. DOI: 10.1038/s41564-021-00979-9. Disponible em: <https://doi.org/10.1038/s41564-021-00979-9>.

SALAZAR, Guillem et al. Gene Expression Changes and Community Turnover Differentially Shape the Global Ocean Metatranscriptome. **Cell**, [S. l.], v. 179, n. 5, p. 1068–1083.e21, 2019. DOI: <https://doi.org/10.1016/j.cell.2019.10.014>. Disponible em: <http://www.sciencedirect.com/science/article/pii/S009286741931164X>.

SALAZAR, Guillem; CORNEJO-CASTILLO, Francisco M.; BENÍTEZ-BARRIOS, Verónica; FRAILE-NUEZ, Eugenio; ÁLVAREZ-SALGADO, X. Antón; DUARTE, Carlos M.; GASOL, Josep M.; ACINAS, Silvia G. Global diversity and biogeography of deep-sea pelagic prokaryotes. **The ISME Journal**, [S. l.], v. 10, n. 3, 2016. DOI: 10.1038/ismej.2015.137.

SALAZAR, Guillem; SUNAGAWA, Shinichi. Marine microbial diversity. **Current Biology**, [S. l.], v. 27, n. 11, p. R489–R494, 2017. DOI: <https://doi.org/10.1016/j.cub.2017.01.017>. Disponible em: <http://www.sciencedirect.com/science/article/pii/S0960982217300179>.

SALTER, Ian; GALAND, Pierre E.; FAGERVOLD, Sonja K.; LEBARON, Philippe; OBERNOSTERER, Ingrid; OLIVER, Matthew J.; SUZUKI, Marcelino T.; TRICOIRE, Cyrielle. Seasonal dynamics of active SAR11 ecotypes in the oligotrophic Northwest Mediterranean Sea.

**The ISME Journal**, [S. l.], v. 9, n. 2, p. 347–360, 2015. DOI: 10.1038/ismej.2014.129. Disponível em: <https://doi.org/10.1038/ismej.2014.129>.

SARMENTO, Hugo; MORANA, Cédric; GASOL, Josep M. Bacterioplankton niche partitioning in the use of phytoplankton-derived dissolved organic carbon: quantity is more important than quality. **The ISME Journal**, [S. l.], v. 10, n. 11, p. 2582–2592, 2016. DOI: 10.1038/ismej.2016.66. Disponível em: <https://doi.org/10.1038/ismej.2016.66>.

SCHOENER, Thomas W. The Anolis Lizards of Bimini: Resource Partitioning in a Complex Fauna. **Ecology**, [S. l.], v. 49, n. 4, p. 704–726, 1968. DOI: 10.2307/1935534. Disponível em: <https://doi.org/10.2307/1935534>.

SEEMANN, Torsten. Prokka: rapid prokaryotic genome annotation. **Bioinformatics**, [S. l.], v. 30, n. 14, p. 2068–2069, 2014. DOI: 10.1093/bioinformatics/btu153. Disponível em: <https://doi.org/10.1093/bioinformatics/btu153>.

SHERR, Evelyn B.; SHERR, Barry F. Understanding roles of microbes in marine pelagic food webs: a brief history. In: **Microbial Ecology of the Oceans**. [s.l.: s.n.]. p. 27–44.

SUNAGAWA, Shinichi et al. Structure and function of the global ocean microbiome. **Science**, [S. l.], v. 348, n. 6237, p. 1261359, 2015. DOI: 10.1126/science.1261359. Disponível em: <https://www.science.org/doi/10.1126/science.1261359>.

SWAN, Brandon K. et al. Prevalent genome streamlining and latitudinal divergence of planktonic bacteria in the surface ocean. **Proceedings of the National Academy of Sciences**, [S. l.], v. 110, n. 28, p. 11463–11468, 2013. DOI: 10.1073/pnas.1304246110. Disponível em: <http://www.pnas.org/content/110/28/11463.abstract>.

THOMPSON, Luke R. et al. Red Sea SAR11 and <em>Prochlorococcus</em> Single-Cell Genomes Reflect Globally Distributed Pangenomes. **Applied and Environmental Microbiology**, [S. l.], v. 85, n. 13, p. e00369-19, 2019. DOI: 10.1128/AEM.00369-19. Disponível em: <http://aem.asm.org/content/85/13/e00369-19.abstract>.

VANINSBERGHE, David; AREVALO, Philip; CHIEN, Diana; POLZ, Martin F. How can microbial population genomics inform community ecology? **Philosophical Transactions of the Royal Society B: Biological Sciences**, [S. l.], v. 375, n. 1798, p. 20190253, 2020. DOI: 10.1098/rstb.2019.0253. Disponível em: <https://doi.org/10.1098/rstb.2019.0253>.

VELLEND, Mark. **The Theory of Ecological Communities. Monographs in Population Biology**, 2016. DOI: 10.1016/B978-0-7234-5558-5.00001-4.

VERGIN, Kevin L.; BESZTERI, Bánk; MONIER, Adam; CAMERON THRASH, J.; TEMPERTON, Ben; TREUSCH, Alexander H.; KILPERT, Fabian; WORDEN, Alexandra Z.; GIOVANNONI, Stephen J. High-resolution SAR11 ecotype dynamics at the Bermuda Atlantic Time-series Study site by phylogenetic placement of pyrosequences. **The ISME Journal**, [S. l.], v. 7, n. 7, p. 1322–1332, 2013. DOI: 10.1038/ismej.2013.32. Disponível em: <https://doi.org/10.1038/ismej.2013.32>.

WINDER, Johanna C.; BOULTON, William; SALAMOV, Asaf; EGGERS, Sarah Lena; METFIES, Katja; MOULTON, Vincent; MOCK, Thomas. Genetic and Structural Diversity of Prokaryotic Ice-Binding Proteins from the Central Arctic Ocean. **Genes**, [S. l.], v. 14, n. 2, p. 363, 2023. DOI: 10.3390/genes14020363. Disponível em: <https://www.mdpi.com/2073-4425/14/2/363>.

ZHANG, Jiajie; KOBERT, Kassian; FLOURI, Tomáš; STAMATAKIS, Alexandros. PEAR: a fast and accurate Illumina Paired-End reAd mergeR. **Bioinformatics**, [S. l.], v. 30, n. 5, p. 614–620, 2013. DOI: 10.1093/bioinformatics/btt593. Disponível em: <https://doi.org/10.1093/bioinformatics/btt593>.

ZHANG, Zhenhua et al. Adaptation to Extreme Antarctic Environments Revealed by the Genome of a Sea Ice Green Alga. **Current Biology**, [S. l.], v. 30, n. 17, p. 3330–3341.e7, 2020. DOI: 10.1016/j.cub.2020.06.029. Disponível em: <https://linkinghub.elsevier.com/retrieve/pii/S0960982220308459>.



A dramatic sunset or sunrise over the ocean. The sky is filled with large, dark clouds, some of which are illuminated from below by the sun, creating a vibrant orange and yellow glow. The ocean below is dark and textured with small waves, reflecting the light from the sky. The horizon line is visible in the distance, separating the dark water from the colorful sky.

# CONSIDERAÇÕES FINAIS

## CONSIDERAÇÕES FINAIS

Nesta tese, investiguei como processos ecológicos (seleção, dispersão e deriva ecológica) atuam na estruturação do microbioma (procariontes e eucariontes unicelulares) em três dimensões do oceano: espaço, tempo e profundidade. Também investiguei como fatores ambientais e geográficos exercem pressão de seleção ou influenciam taxas de dispersão nessas comunidades microbianas. De maneira geral, os resultados apontam que a importância relativa dos processos ecológicos atuando na montagem dessas comunidades é dependente da escala espacial, da profundidade e da latitude, podendo variar ao longo do tempo. Mais precisamente, o balanço espaço-temporal desses processos parece estar associado principalmente à heterogeneidade ambiental, a fatores de dispersão (ex.: correntes marítimas) e a barreiras geográficas (ex.: montanhas submersas e massas d'água). Também demonstrei que a importância relativa desses processos ecológicos varia entre procariontes e picoeucariontes. Essa diferença entre domínios biológicos resulta, principalmente, da diferença em tamanho populacional (ou abundância microbiana) e do tamanho do organismo. Esse resultado corrobora com outros estudos que demonstram que o tamanho corporal (ou celular) é um importante traço funcional em comunidades ecológicas.

No capítulo 1 demonstrei, por meio de uma revisão bibliográfica que a ‘Teoria de Ecologia de Comunidades’ de VELLEND, 2010 foi amplamente utilizada na literatura desde a sua publicação em 2010 até 2018, ano de início do desenvolvimento desta tese. Também demonstrei que esse marco conceitual foi mais citado e aplicado em estudos focados em comunidades microbianas. Esta tendência se deve, principalmente, à abordagem matemática proposta STEGEN et al., 2013, que permite a quantificação relativa da seleção, dispersão e deriva ecológica em comunidades microbianas.

No capítulo 2 utilizamos a mencionada abordagem para determinar a importância relativa da seleção, dispersão e deriva ecológica em comunidades picoplanctônicas habitando distintas camadas de profundidade (epi-, meso- e batipelágico) do oceano em escala global e regional. No presente trabalho analisamos uma base de dados com 451 amostras de amplicons cobrindo as zonas epi- (0-200 m), meso- (200-1,000 m) e batipelágicas (1,000-4,000 m) do oceano tropical e subtropical, assim como do Mar Mediterrâneo. Esse é um dos mais amplos conjuntos de dados para investigar esta questão e inclui dados de duas campanhas oceanográficas: a expedição circumglobal Malaspina-2010 e a expedição trans-mediterrânea Hotmix. Descobrimos que diferentes combinações de processos ecológicos (seleção, dispersão e deriva) estruturam comunidades de procariontes e minúsculos eucariontes unicelulares em camadas de profundidade do oceano global e do Mar Mediterrâneo. Assim, os mecanismos que sustentam a montagem do microbioma marinho

aparentam ser dependentes da profundidade. Esses resultados devem ser considerados em um contexto de mudanças ambientais (e.g.: aquecimento do oceano, mudanças nos padrões de correntes e massas de água), as quais podem alterar o equilíbrio entre esses mecanismos, levando potencialmente a novas configurações de microbiomas no oceano global. Resumimos os principais resultados deste trabalho em um modelo conceitual que fornece informações sobre como a heterogeneidade ambiental, a batimetria e massas de água podem afetar os processos ecológicos que atuam na montagem do microbioma nas diferentes camadas de profundidade do oceano. Esse modelo pode ser utilizado para fazer previsões sobre a montagem de comunidades desde a superfície até o fundo (4,000 m de profundidade) do oceano. Além disso, novos estudos poderiam testar se este modelo conceitual é aplicável quando considerada não a composição, mas as funções do microbioma marinho.

Além da abordagem ‘space-for-time’ utilizada em expedições globais (capítulo 2), estudos temporais em observatórios microbianos são essenciais para obter uma visão mais completa da dinâmica dos processos ecológicos nos ecossistemas. Nesse sentido, no capítulo 3 utilizamos dados de dois observatórios marinhos localizados em diferentes latitudes do oceano pra entender como o balanço entre esses processos pode variar com o tempo. De maneira geral, os resultados obtidos nos referidos capítulos são coerentes com aqueles obtidos na escala espacial (Capítulo 2). No capítulo 3, demonstramos que a importância relativa da seleção é sempre maior na estação de clima temperado do que tropical. Por outro lado, fatores estocásticos foram relativamente mais importantes no oceano tropical. Esses resultados corroboram com as redes de coocorrência, com as quais demonstramos que as métricas de redes se correlacionam mais com os fatores ambientais de seleção (temperatura e duração do dia) na estação temperada. Por outro lado, na estação tropical, essas métricas apenas se correlacionam com nutrientes, o que está provavelmente associado a variações interanuais do que sazonais.

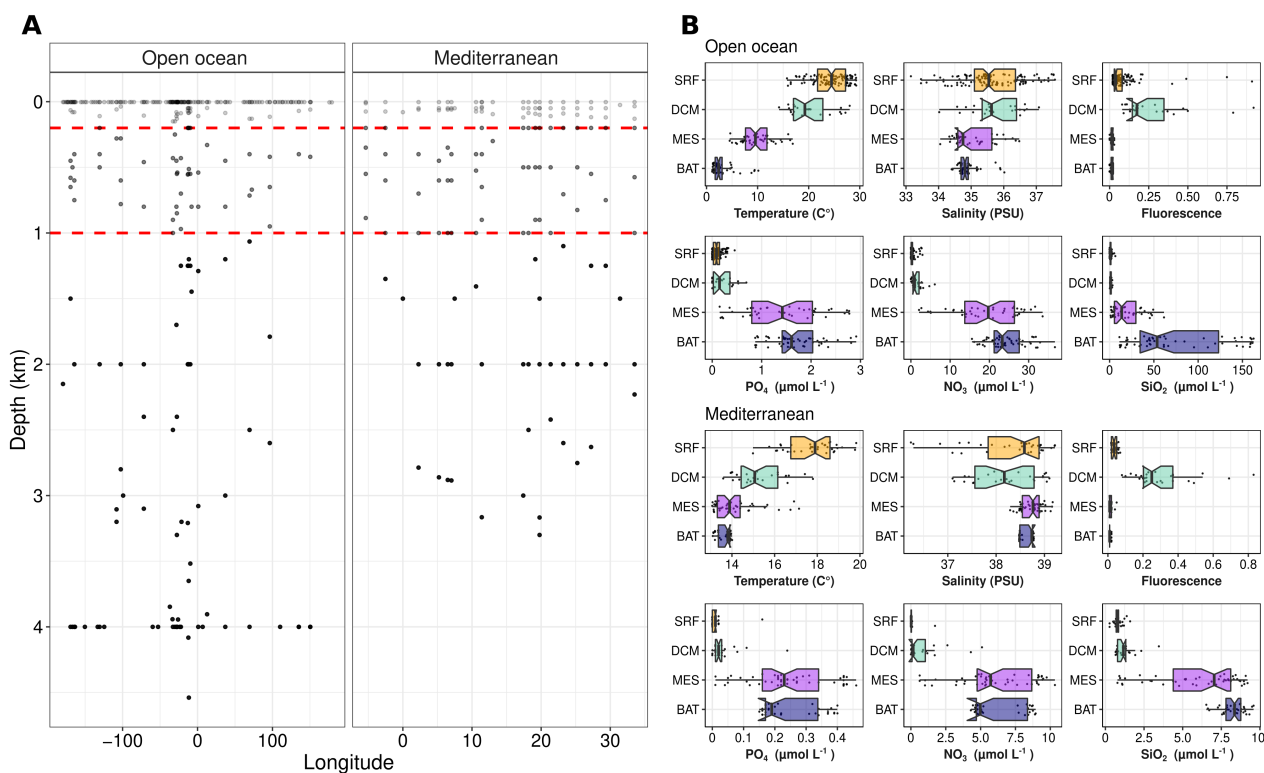
Por fim, no capítulo 4, avaliamos as bases genômicas do efeito de seleção da temperatura sobre *Rhodospirillaceae*, um grupo de bactérias marinhas abundante e que apresenta um padrão de partição de nicho estruturado pela temperatura. Para isso, combinamos metabarcoding com métodos de sequenciamento de genomas ambientais (MAGs e SAGs). Encontramos uma forte correlação negativa entre a temperatura e o tamanho dos genomas de *Rhodospirillaceae*, o que demonstra a ação do processo de seleção na adaptação bacteriana a ambientes com diferentes temperaturas. Compreender a adaptação genética das comunidades microbianas a diferentes nichos de temperatura é particularmente importante no contexto de um oceano cada vez mais quente. Esse passo permitiria fazer previsões sobre as bases genômica dos processos ecológicos que moldam o microbioma oceânico. Esse trabalho, juntamente com estudos recentes, sugere a perda de genes

bacterianos com o aquecimento dos oceanos. Pesquisas futuras devem investigar como essa redução genômica pode afetar os genes responsáveis pelos ciclos biogeoquímicos globais, essenciais para a manutenção da biosfera.

# **ANEXO I**

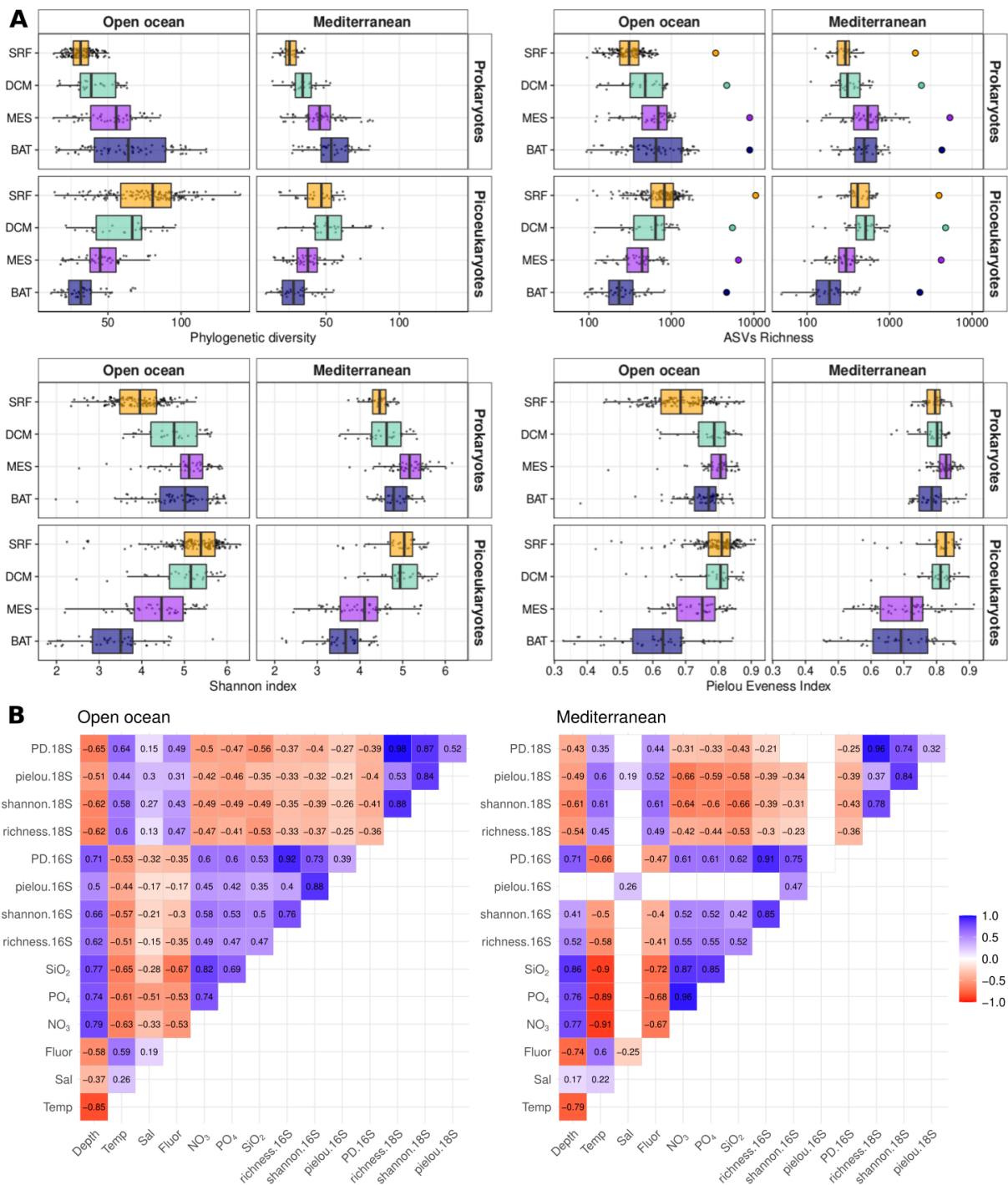
## **MATERIAL SUPLEMENTAR**

**SUPPLEMENTARY MATERIAL – CHAPTER 2: GLOBAL BIOGEOGRAPHY OF THE SMALLEST PLANKTON ACROSS OCEAN DEPTHS**  
**SUPPLEMENTARY FIGURES**

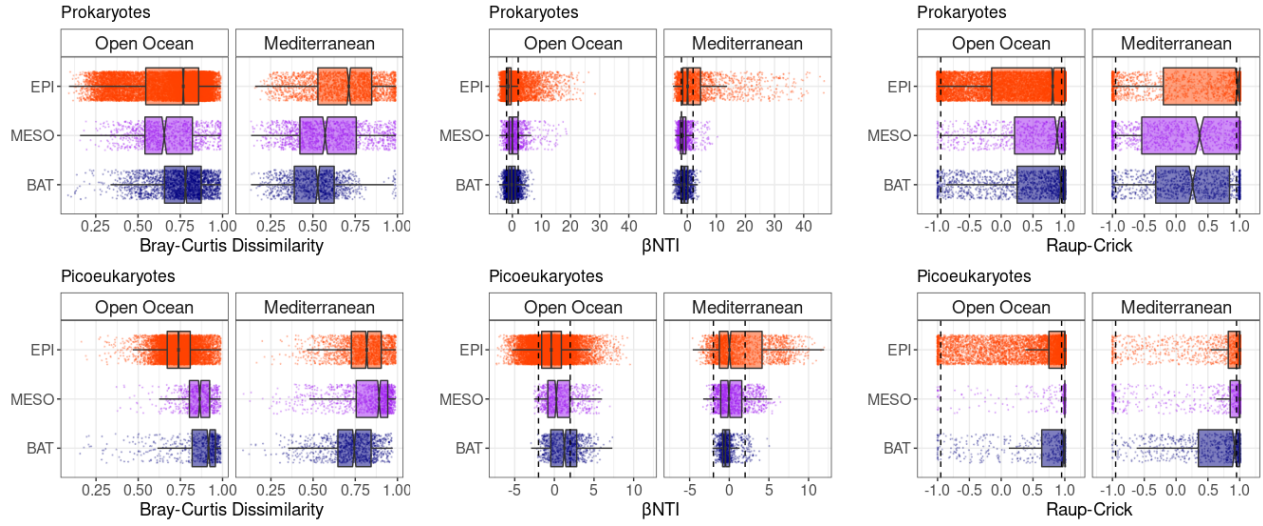


**Figure II–S1. (A)** Distribution of the samples across depth and longitude. The dashed red lines depict the division between zones: epi- (0-200 m), meso- (200-1,000 m) and bathypelagic (>1,000 m) **(B)** Boxplots showing the data variability, by depth zones, of the environmental variables used in this study. Note the difference in scales between the open ocean and the Mediterranean Sea. Means were significantly different (ANOVA, Tukey post-hoc test;  $p < 0.001$ ) between upper (SRF and DCM) and deep (MES and BAT) zones.



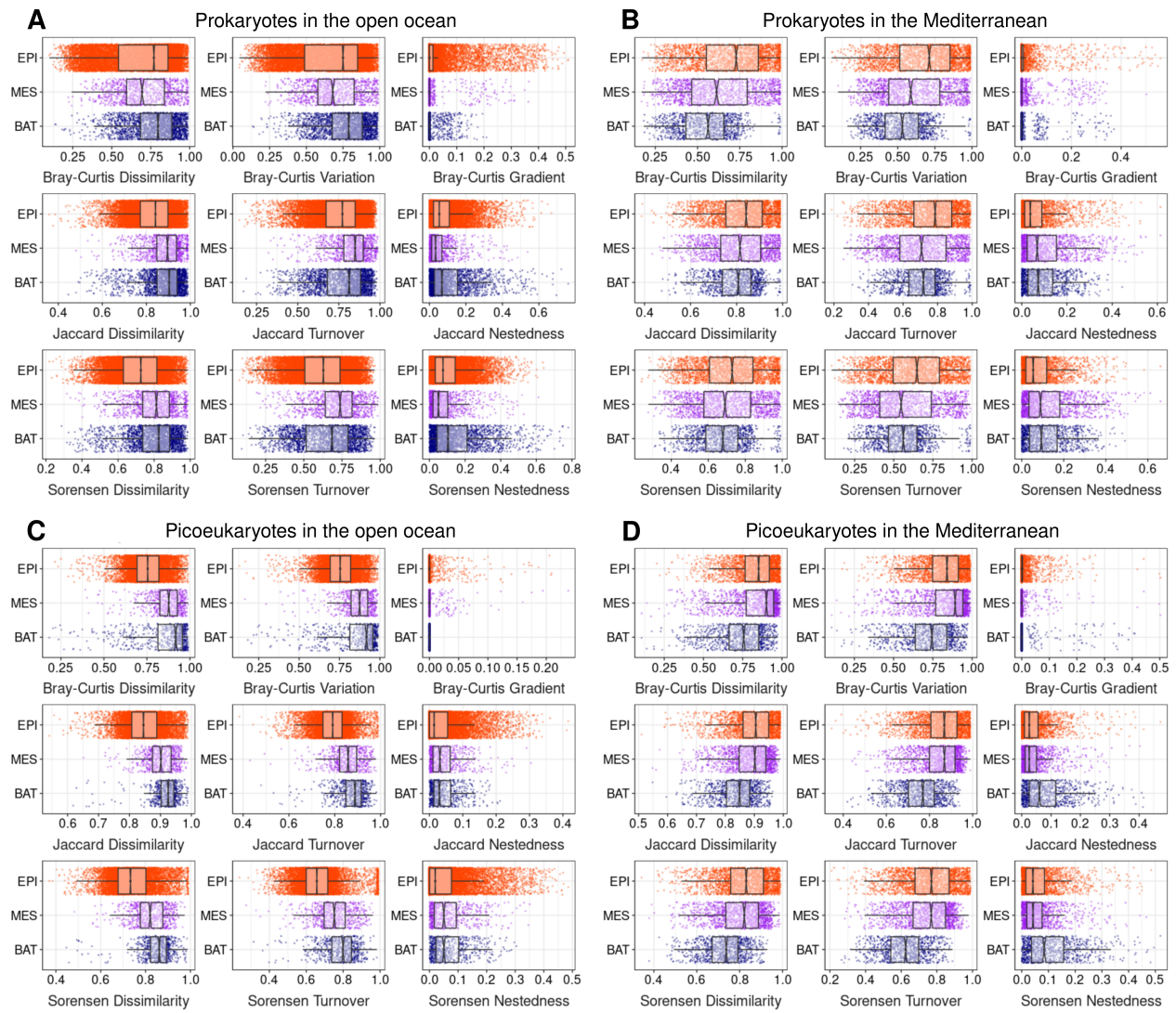


**Figure II-S2. (A)** Picoplankton diversity depicted as phylogenetic diversity, ASVs richness, shannon and Pielou evenness index by depth zones (SRF, surface; DCM, deep chlorophyll maxima; MES, Mesopelagic; BAT, Bathypelagic). The circles in the ASVs richness boxplots stand for gamma diversity adjusted by sampling size. **(B)** Correlation matrix of Pearson (R) correlation values between picoplankton diversity metrics and environmental variables in the open ocean and the Mediterranean Sea. The empty boxes represent non-significant correlations ( $p>0.05$ ). The ‘16S’ tags in the metrics depict prokaryotic communities, while ‘18S’ tags represent picoeukaryotic communities. PD = phylogenetic diversity; Temp = Temperature; Sal = Salinity; Fluor = Fluorescence.

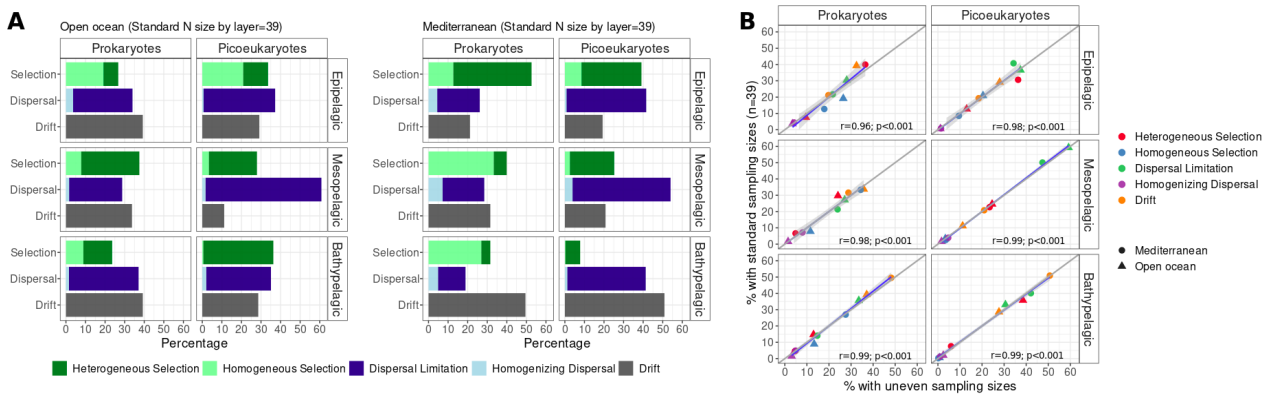


**Figure II-S3. Bray-Curtis,  $\beta$ NTI and RCbray metrics by depth zones for (A) prokaryotes and (B) picoeukaryotes.** See Fig. S4. for  $\beta$ -diversity partitioning plots. Means were significantly different (ANOVA, Tukey post-hoc test;  $p < 0.001$ ) between depth zones for both prokaryotes and picoeukaryotes.

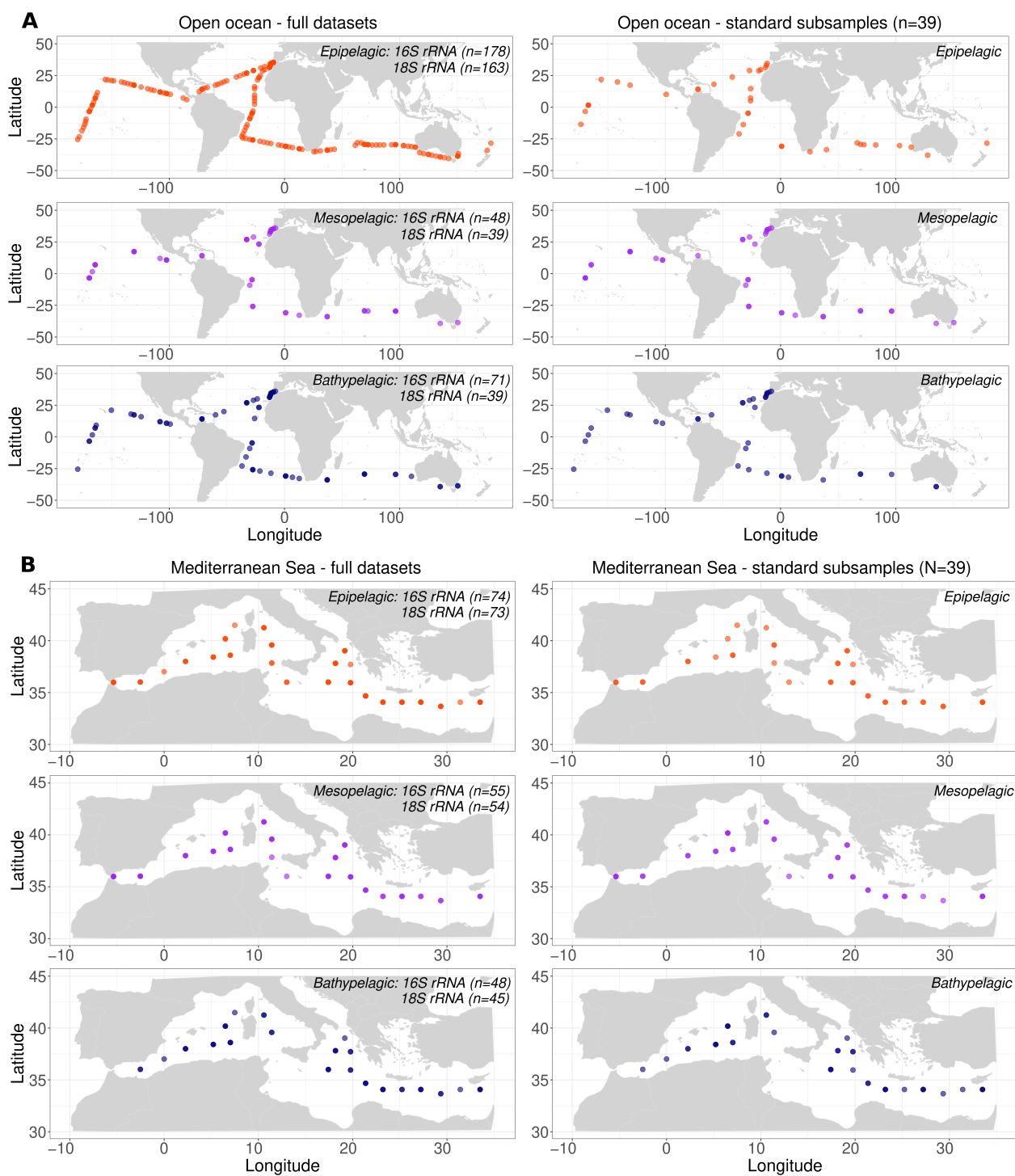




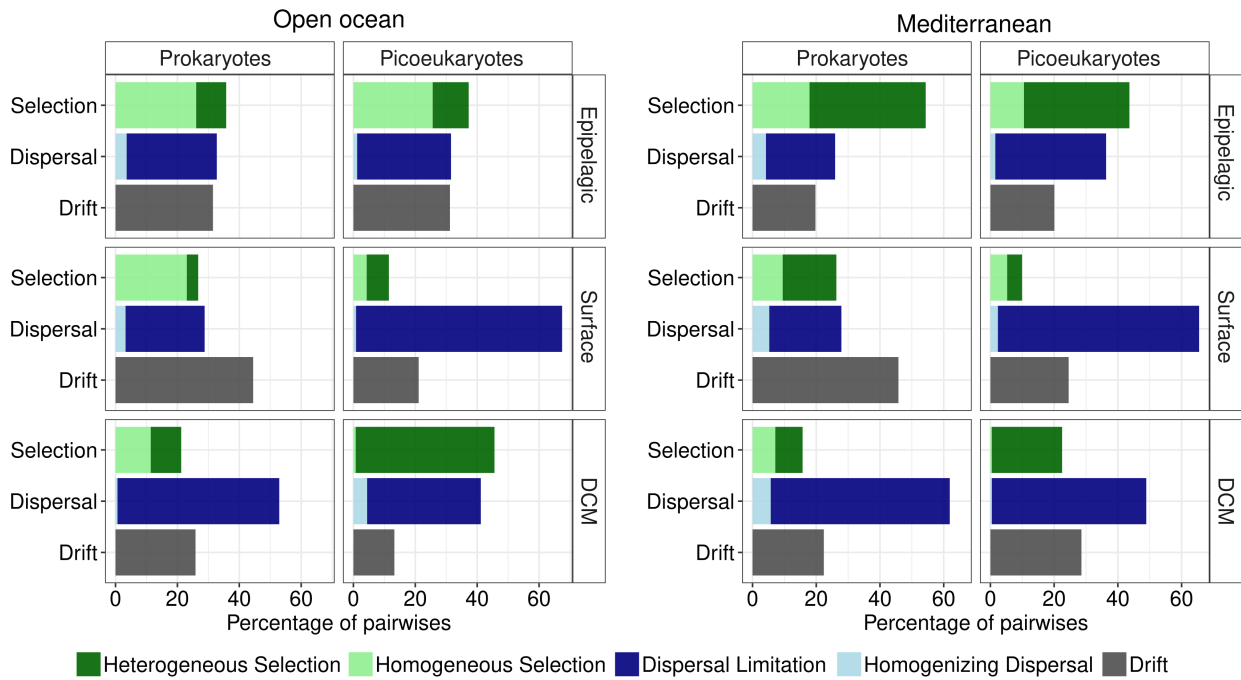
**Figure II-S4. Picoplankton  $\beta$ -diversity partitioning in the different ocean depth zones.** Bray-Curtis dissimilarity (variation and gradient), Jaccard dissimilarity (turnover and nestedness) and Sorensen (turnover and nestedness) for prokaryotes and picoeukaryotes in the open ocean (left panels) and Mediterranean Sea (right panels). Means were significantly different (ANOVA, Tukey post-hoc test;  $p < 0.001$ ) between depth zones for both prokaryotes and picoeukaryotes.



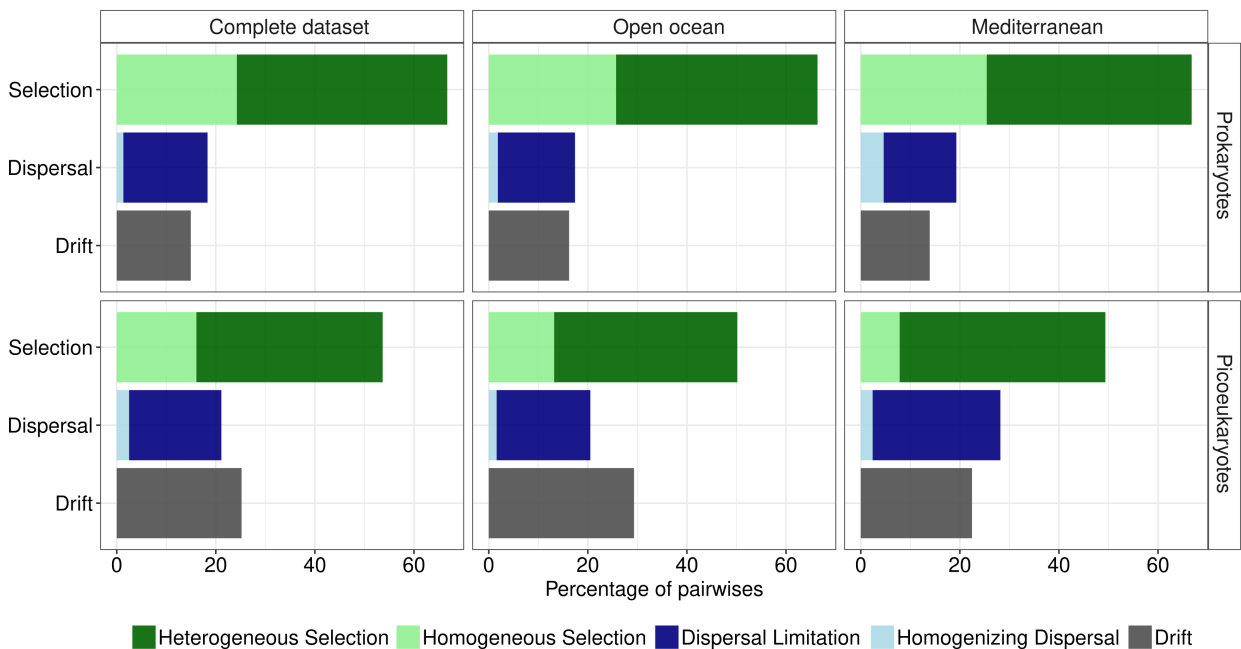
**Figure II-S5. Picoplankton community assembly processes across ocean depth zones using standardized sampling sizes (n=39).** (A) Relative importance of the ecological processes structuring the picoplankton communities at different depth zones of the open ocean and Mediterranean Sea: Epi- (n=39), Meso- (n=39) and Bathypelagic (n=39). (B) Linear regression between results obtained with total (unbalanced) and standardized sampling size dataset. These samples were evenly distributed across space as shown in Fig S6.



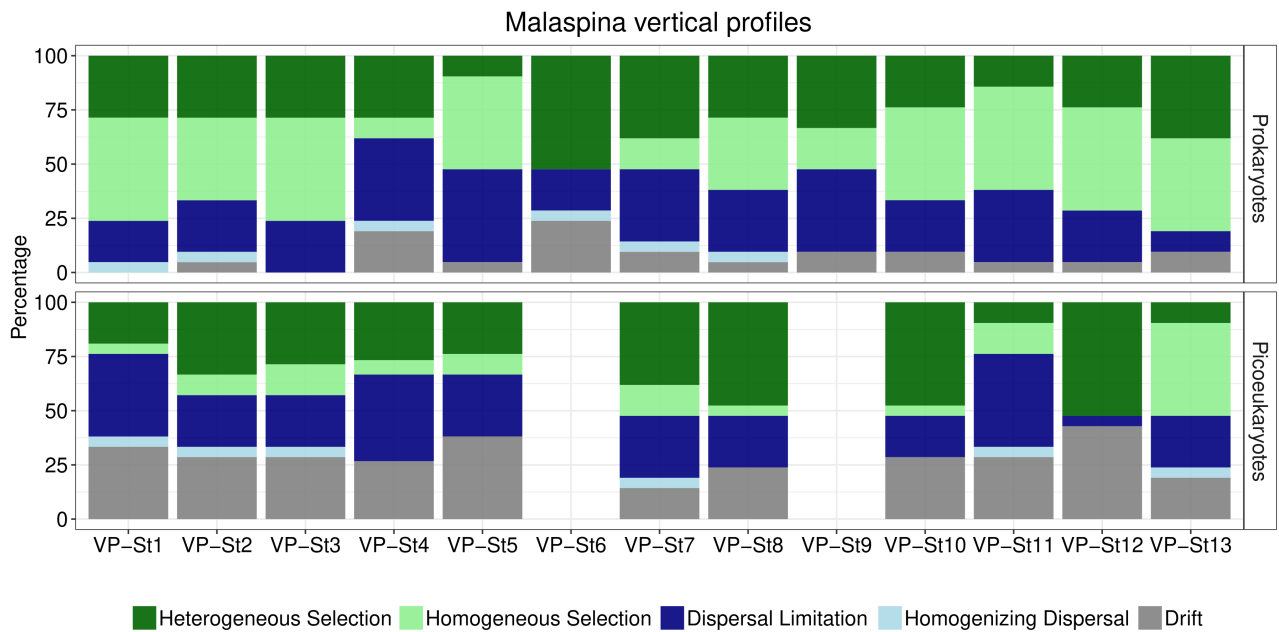
**Figure II-S6.** Geographic distribution of sampling stations in each zone of full datasets and subsets with standardized sampling size (n=39) in the **(A)** open ocean and **(B)** Mediterranean Sea. Samples were evenly distributed across space.



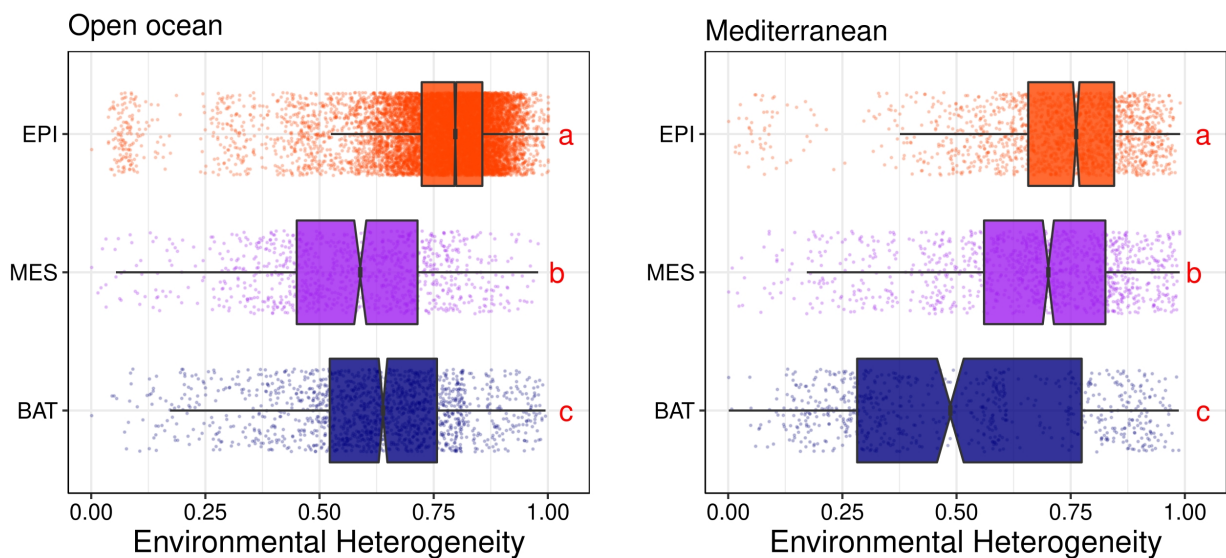
**Figure II–S7. Picoplankton community assembly processes in distinct depth zones of the epipelagic.** Relative importance of the ecological processes structuring the picoplanktonic community in the SRF and DCM zones of the open ocean and Mediterranean Sea.



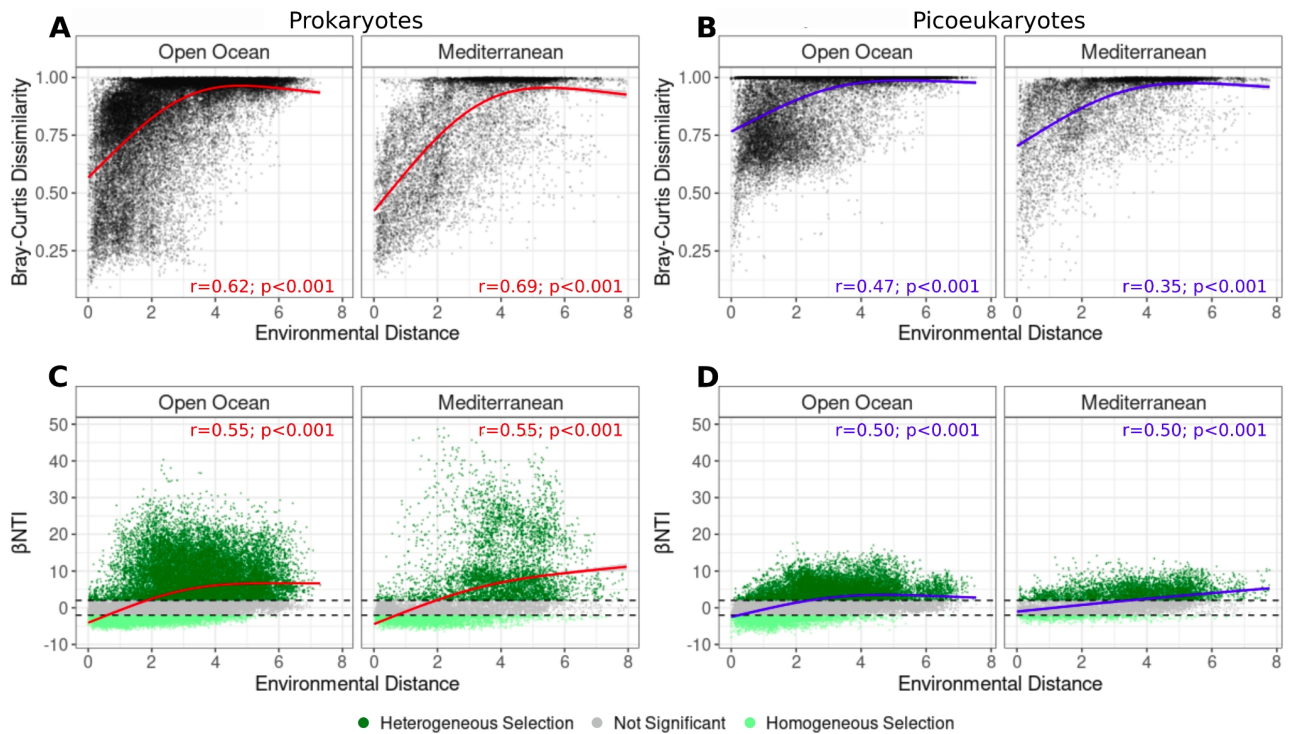
**Figure II–S8. Picoplankton community assembly processes integrating all depth zones.** Relative importance of the ecological processes structuring the picoplanktonic community using the complete dataset as well as separated by the open ocean and the Mediterranean Sea.



**Figure II-S9. Picoplankton community assembly processes in the *Malaspina* vertical profile stations.** Relative importance of the ecological processes structuring the picoplanktonic community integrating all depths (from 3 to 4000 m, i.e. 7 different depths) in each of the 13 vertical-profile (VP) stations (labeled as in Fig. 1A).

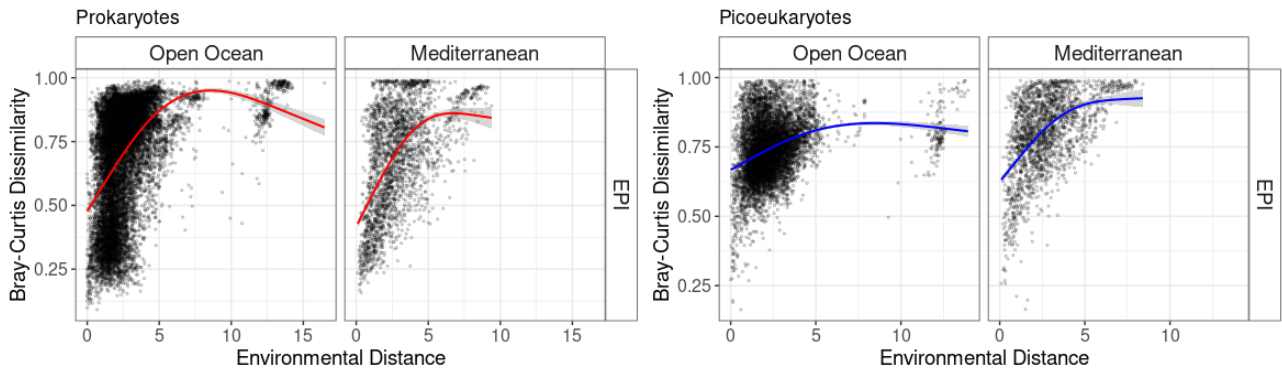


**Figure II-S10.** Environmental heterogeneity computed as the mean environmental dissimilarity between samples considering the main environmental variables (Temperature, Salinity, Fluorescence,  $\text{NO}_3$ ,  $\text{PO}_4$ ,  $\text{SiO}_2$ ) in the open ocean and the Mediterranean Sea. Different red letters represent significantly different means [Kruskal-Wallis, Wilcoxon post-hoc test,  $p < 0.05$ ] between depth zones.

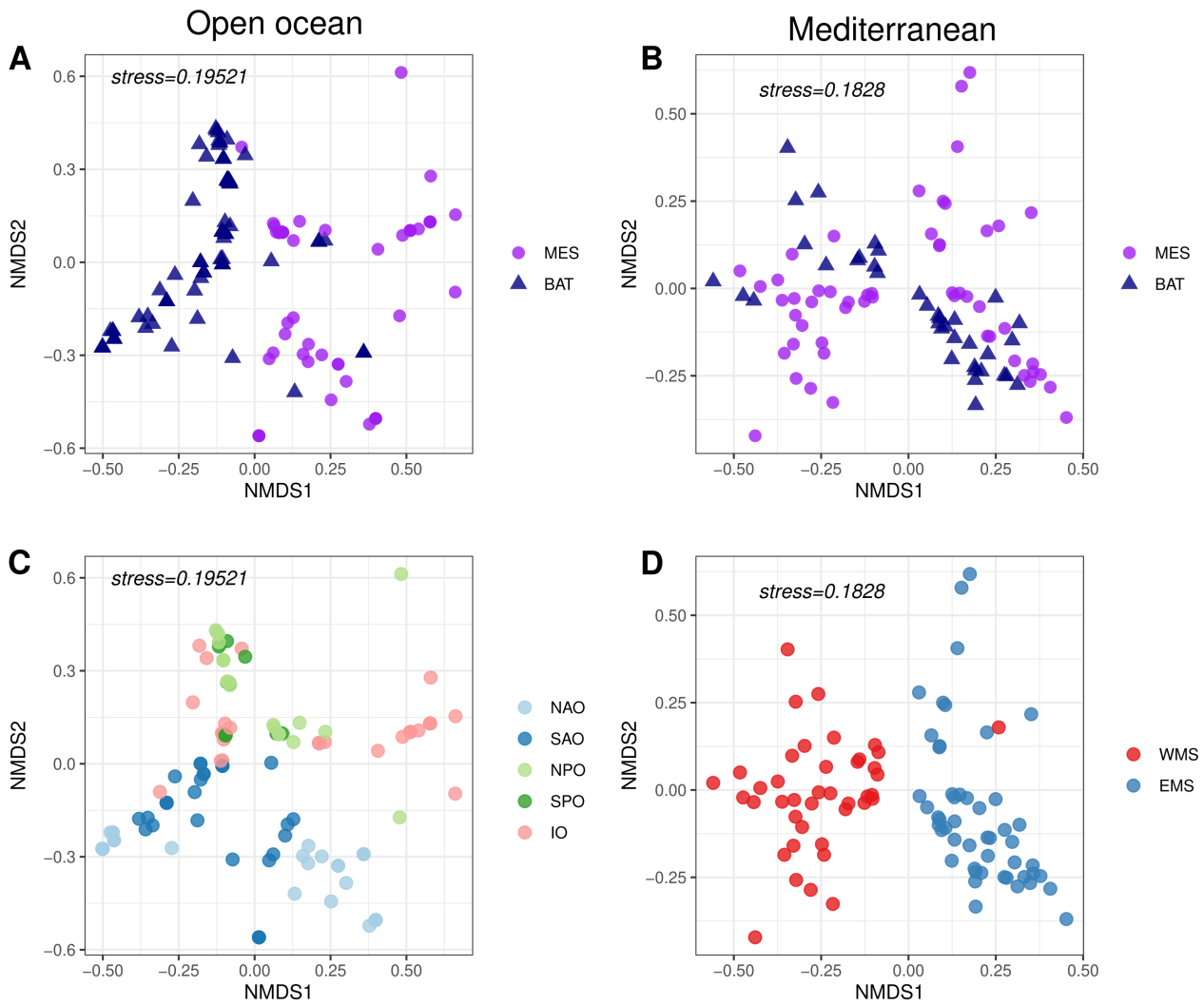


**Figure II-S11. Picoplankton community composition and phylogeny are positively related to environmental heterogeneity.** Difference in taxonomic (Bray-Curtis dissimilarity) and phylogenetic ( $\beta$ NTI) composition for all pairwise picoplankton community comparisons as a function of environmental distance for both prokaryotes (**A, C**) and picoeukaryotes (**B, D**) in the open ocean and Mediterranean Sea. The solid curves illustrate the nonlinear regressions. Spearman's rank correlation coefficients are depicted on the panel. Outliers with high environmental distances ( $>10$ ) corresponding to pairwise comparisons with epipelagic samples from the Costa Rica Dome upwelling system were removed from the open ocean plot (see Fig S13).



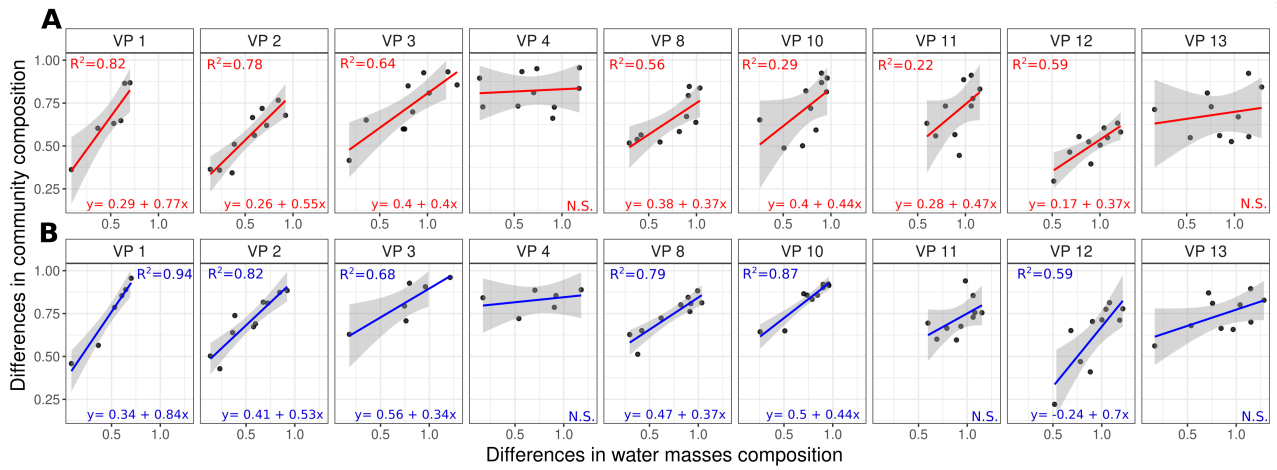


**Figure II-S12. Picoplankton community compositions are positively related to environmental heterogeneity.** Difference in composition (Bray-Curtis dissimilarity) for all pairwise picoplankton community comparisons as a function of environmental distance for both **(A)** prokaryotes and **(B)** picoeukaryotes in the epipelagic of the open ocean and Mediterranean Sea. The points with high environmental distances (>10) correspond to the pairwise comparisons with epipelagic samples from the Costa Rica Dome. The solid curves illustrate the nonlinear regressions. Spearman's rank correlation coefficients are depicted on the panel.

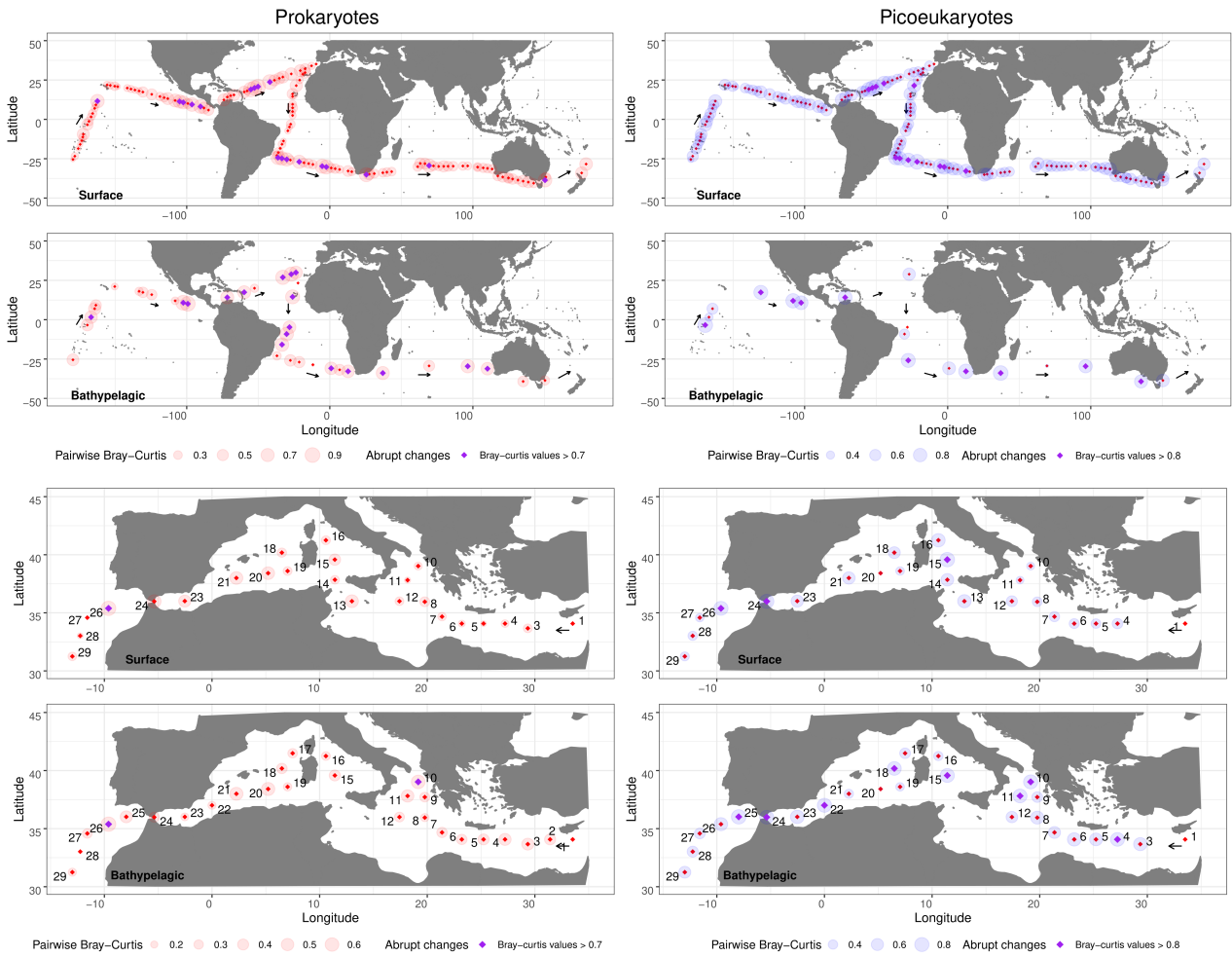


**Figure II-S13. Differences in water mass composition are segregated by depth zones and ocean basins.** Nonmetric multidimensional scaling (NMDS) based on the Euclidean distance of the samples' water mass composition – labeled by zones and basin – in the open ocean (**A, C**) and the Mediterranean Sea (**B, D**). MES = Mesopelagic; BAT = Bathypelagic. NAO = North Atlantic Ocean, SAO = South Atlantic Ocean, NPO = North Pacific Ocean, SPO = South Pacific Ocean, IO = Indian Ocean, WMS = Western Mediterranean Sea, EMS = Eastern Mediterranean Sea.

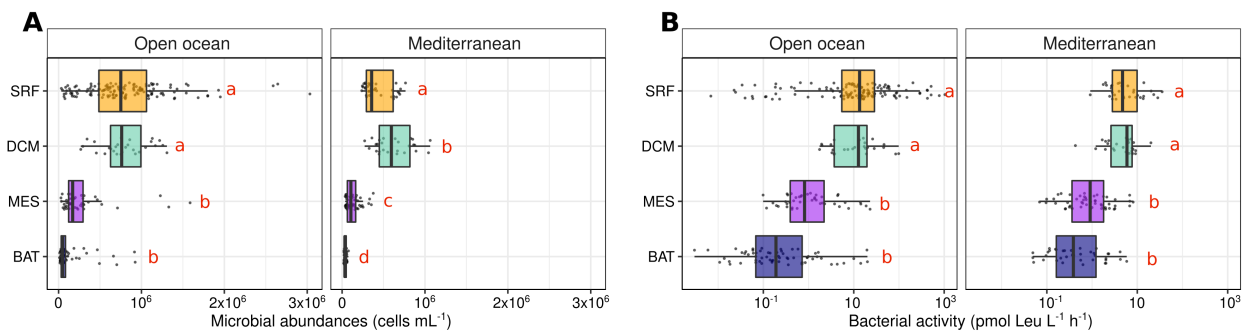




**Figure II-S14. Picoplankton community composition and potential dispersal are vertically linked to differences in water mass composition.** Difference in community composition (Bray-Curtis dissimilarity) as a function of water mass composition dissimilarity (Euclidean distances) for prokaryotes (in red) (A) and picoeukaryotes (in blue) (B) in Malaspina vertical profiles. Note that only meso- and bathypelagic samples were used in this analysis. The equation, the explanatory power of the linear regression models (adjusted  $R^2$ ), and the significance of the smooth terms ( $p < 0.001$ ) are shown on the plots.



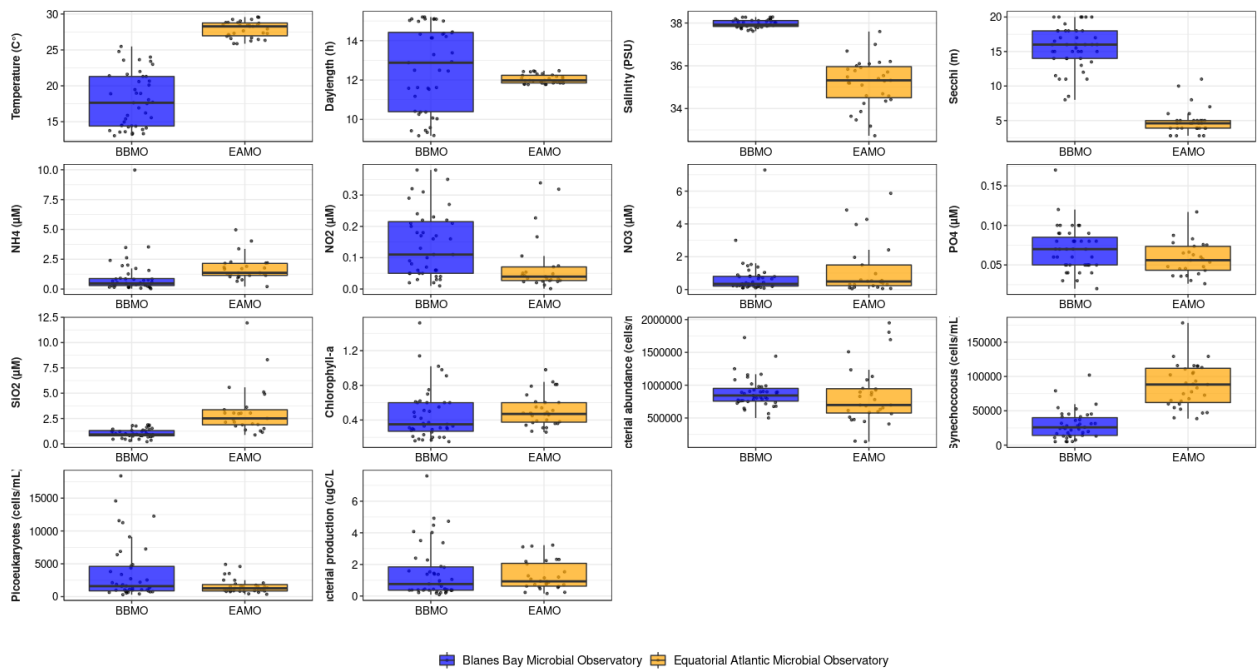
**Figure II-S15. Sequential change in community composition across space (sequential  $\beta$ -diversity).** Communities were sampled along the *Malaspina* and *Hotmix* expeditions (black arrows), and the composition of each community was compared against its immediate predecessor. The size of each bubble represents the Bray-Curtis dissimilarity between a given community and the community sampled previously.



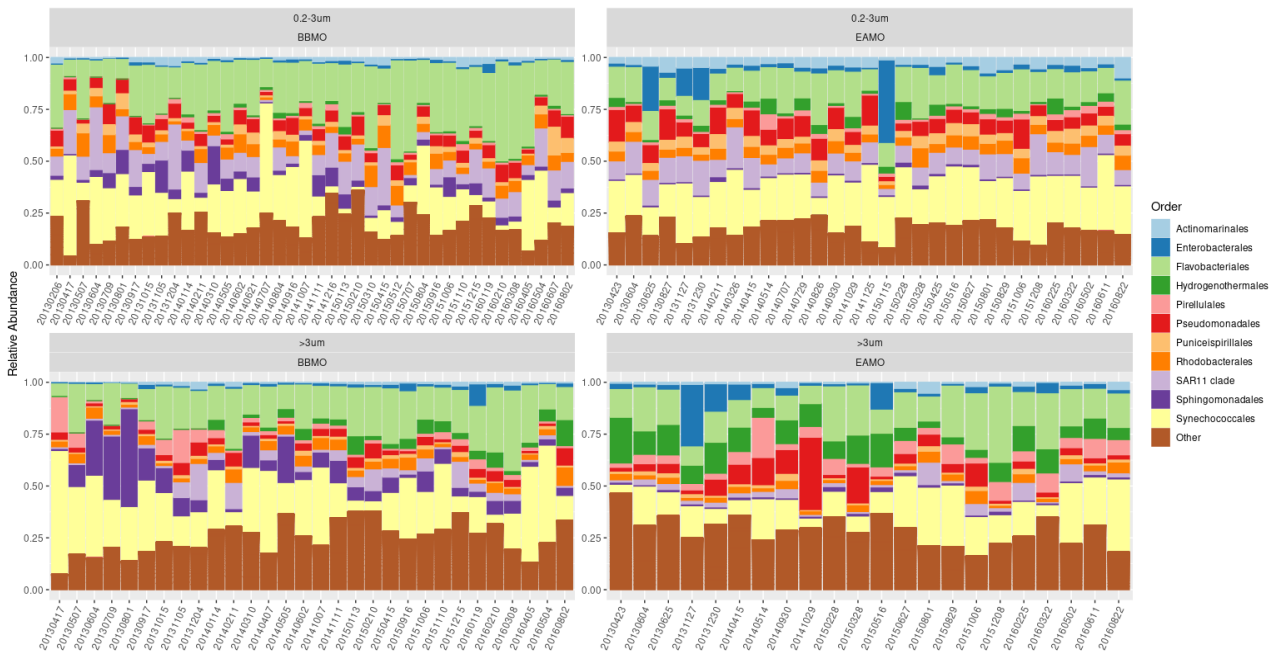
**Figure II-S16. Microbial abundances and bacterial activity sharply decrease in deep waters. (A)** Microbial abundances (prokaryotes + picoeukaryotes) as measured by flow cytometry; **(B)** bacterial activity as measured by leucine incorporation rates in each zone (SRF, surface; DCM, deep chlorophyll maxima; MES, Mesopelagic; BAT, Bathypelagic) of the open ocean and the Mediterranean Sea. Different red letters represent significantly different means [ANOVA, Tukey post-hoc test,  $p < 0.05$ ] between depth zones.

# SUPPLEMENTARY MATERIAL – CHAPTER 3: ECOLOGICAL PROCESSES SHAPING MICROBIAL ASSOCIATIONS OVER TIME IN CONTRASTING LATITUDES OF THE OCEAN

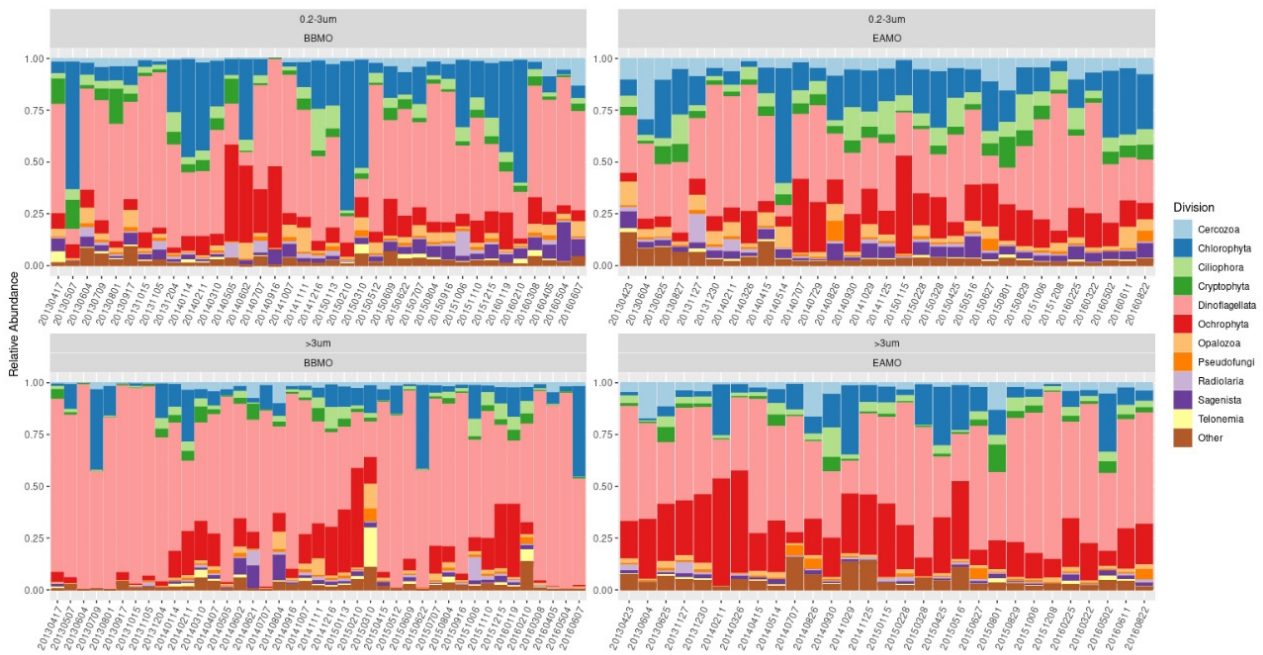
## SUPPLEMENTARY FIGURES



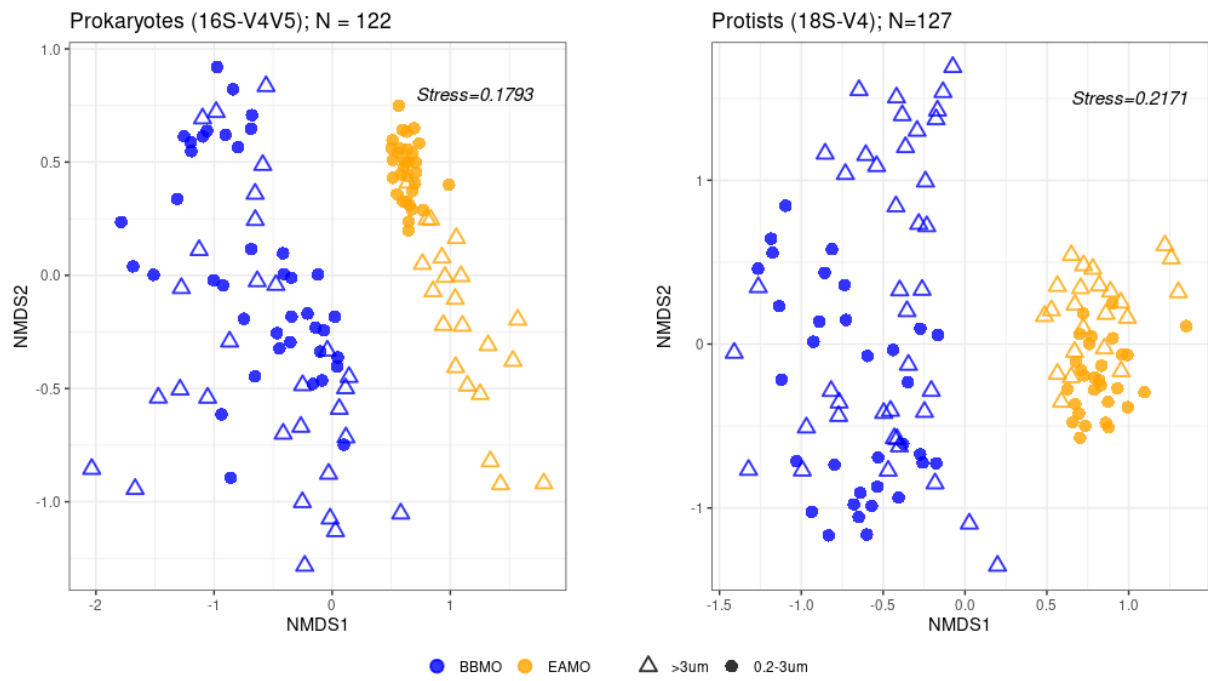
**Figure III–S1.** Box-plots comparing (BBMO vs. EAMO) the mean values of all environmental and biological variables considered in this study.



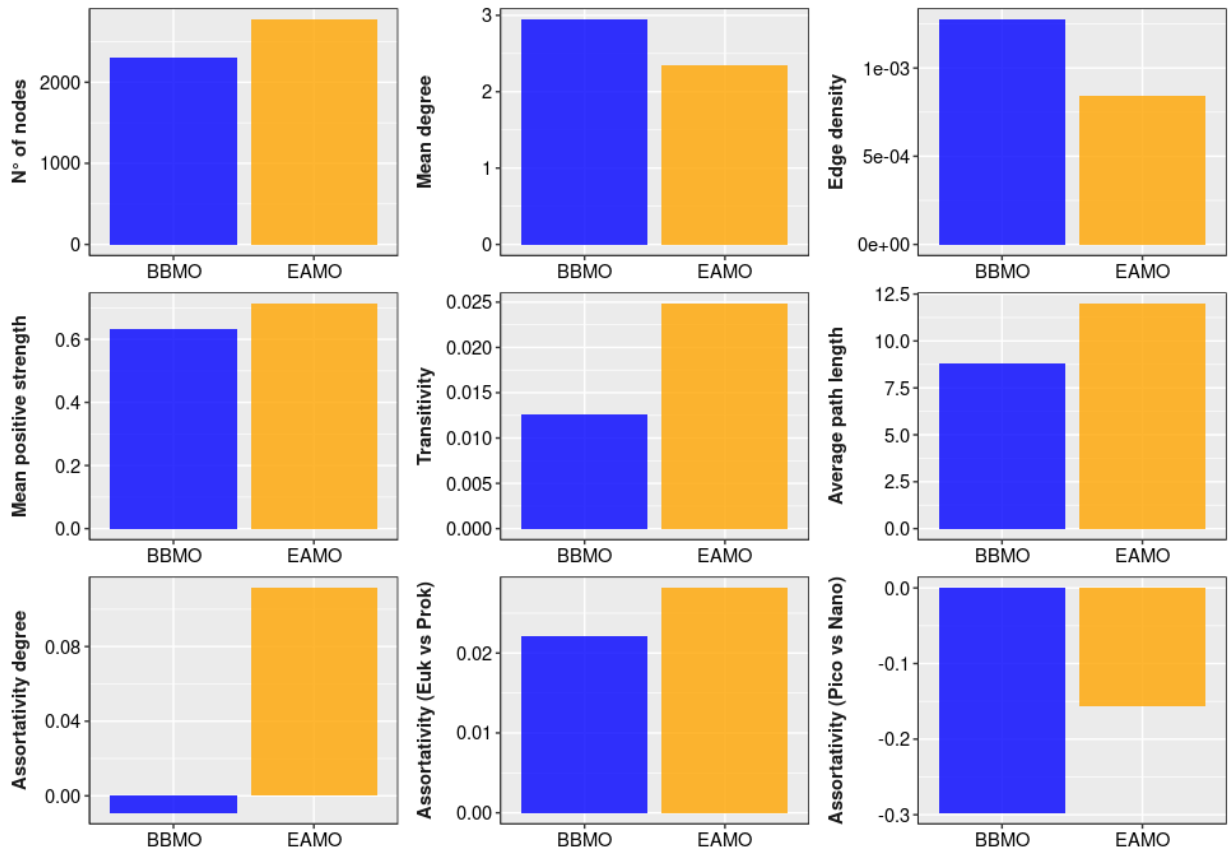
**Figure III-S2.** Bar plots showing the prokaryotic taxonomic composition at the order level in each sample separated by size-fraction of the Blanes Bay Microbial Observatory (BBMO) and the Equatorial Atlantic Microbial Observatory (EAMO). Only the most abundant taxonomic groups are represented, and the remaining prokaryotic taxonomic groups are pooled together as “other”. Abundances are expressed as percentages of the total number of sequences in each sample.



**Figure III-S3.** Bar plots showing the protist taxonomic composition at the order level in each sample separated by size-fraction of the Blanes Bay Microbial Observatory (BBMO) and the Equatorial Atlantic Microbial Observatory (EAMO). Only the most abundant taxonomic groups are represented, and the remaining protist taxonomic groups are pooled together as “other”. Abundances are expressed as percentages of the total number of sequences in each sample.

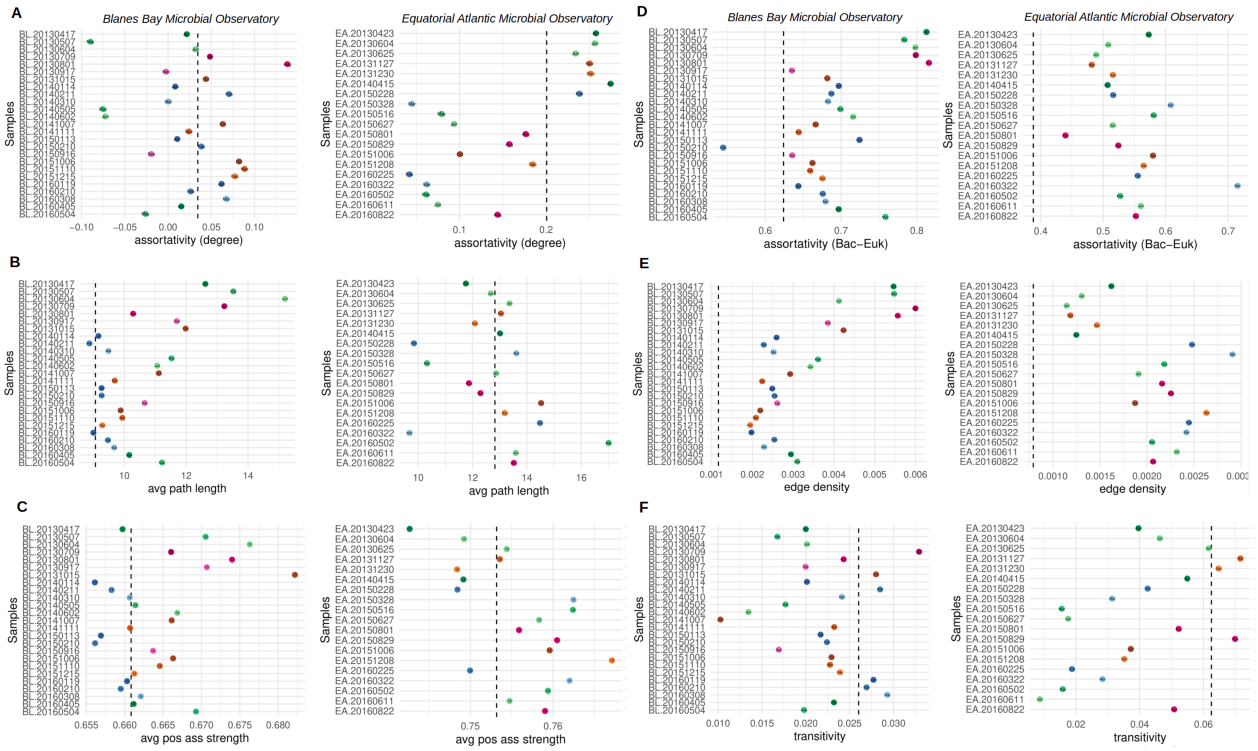


**Figure III-S4.** Differences in microbial communities compositions. Nonmetric multidimensional scaling (NMDS) based on the Bray-Curtis dissimilarities among prokaryotic and eukaryotic samples – labeled by observatory and size-fraction. BBMO – Blanes Bay Microbial Observatory; EAMO – Equatorial Atlantic Microbial Observatory.



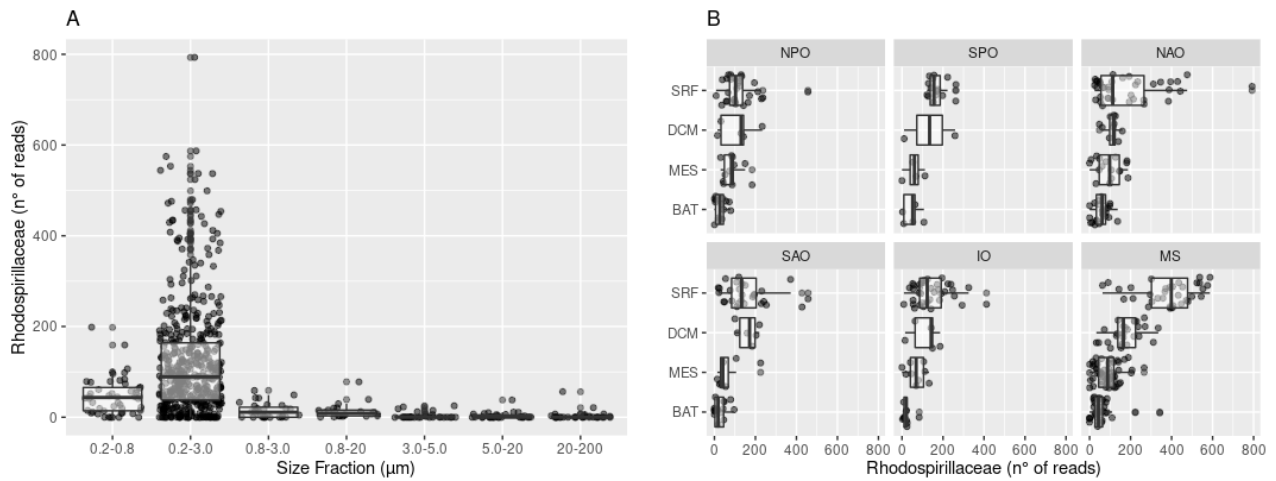
**Figure III–S5.** Bar plots showing the topological metrics from the static networks from the Blanes Bay Microbial Observatory (BBMO) and the Equatorial Atlantic Microbial Observatory (EAMO).



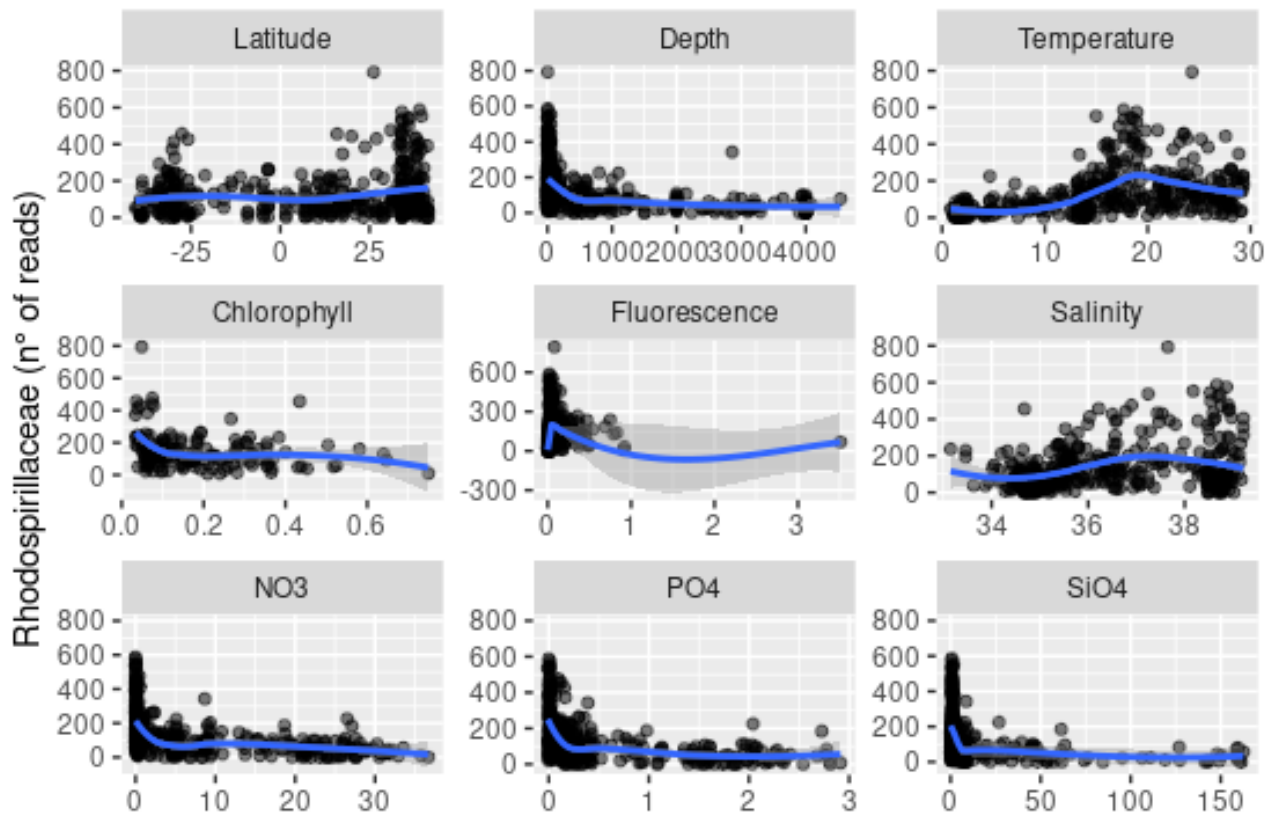


**Figure III-S6.** Main topological metrics from the temporal network across samples of the Blanes Bay Microbial Observatory – BBMO and the Equatorial Atlantic Microbial Observatory – EAMO.

**SUPPLEMENTARY MATERIAL – CHAPTER 4: NICHE-PARTITIONING OF RHODOSPIRILLACEAE AN UBIQUITOUS, ABUNDANT AND PREVIOUSLY OVERLOOKED MARINE BACTERIA**



**Figure IV–S1.** *Rhodospirillaceae* distribution in different (A) size-fractions and (B) depth layers of the tropical and sub-tropical ocean basins. SRF = Surface, DCM = Deep Chlorophyll Maxima, MES = Mesopelagic, BAT = Bathypelagic. NPO = North Pacific Ocean, SPO = South Pacific Ocean, NAO = North Atlantic Ocean, SAO = South Atlantic Ocean, IO = Indian Ocean, MS = Mediterranean Sea.



**Figure IV-S2.** Rhodospirillaceae n° of reads against the main environmental variables measured in this study.

# **ANEXO II**

## **STATUS DOS MANUSCRITOS**

**CAPÍTULO 2:**

**Global biogeography of the smallest plankton across ocean depths**

Pedro C. Junger<sup>1,2\*</sup>, Hugo Sarmiento<sup>1</sup>, Caterina. R. Giner<sup>3</sup>, Mireia Mestre<sup>4,5,6</sup>, Marta Sebastián<sup>3</sup>, Xosé Anxelu G. Morán<sup>7</sup>, Javier Arístegui<sup>8</sup>, Susana Agustí<sup>9</sup>, Carlos M. Duarte<sup>9</sup>, Silvia G. Acinas<sup>3</sup>, Ramon Massana<sup>3</sup>, Josep M. Gasol<sup>3</sup>, Ramiro Logares<sup>3\*</sup>

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**Pre-print:** Junger, P. C., Sarmiento, H., Giner, C. R., Mestre, M., Sebastian, M., Moran, X. A. G., Arístegui, J., Agustí, S., Duarte, C. M., Acinas, S. G., Massana, R., Gasol, J. M., Logares, R. Global biogeography of the smallest plankton across ocean depths. *BioRxiv* 2023. 01.13.523743. <https://doi.org/10.1101/2023.01.13.523743>

Manuscript submitted to *Science Advances* (February 2023)

### **CAPÍTULO 3:**

#### **Ecological processes shaping microbial associations over time in contrasting latitudes of the ocean**

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Manuscript in preparation to be submitted to *Molecular Ecology*

## **CAPÍTULO 4:**

### **Niche-partitioning of *Rhodospirillaceae*, an ubiquitous, abundant and previously overlooked group of marine bacteria**

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# **ANEXO III**

## **ARTIGOS SUBMETIDOS**





# **ANEXO IV**

## **ARTIGOS PUBLICADOS**

RESEARCH

Open Access

# Disentangling the mechanisms shaping the surface ocean microbiota



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## Abstract

**Background:** The ocean microbiota modulates global biogeochemical cycles and changes in its configuration may have large-scale consequences. Yet, the underlying ecological mechanisms structuring it are unclear. Here, we investigate how fundamental ecological mechanisms (*selection*, *dispersal* and *ecological drift*) shape the smallest members of the tropical and subtropical surface-ocean microbiota: prokaryotes and minute eukaryotes (picoeukaryotes). Furthermore, we investigate the agents exerting abiotic selection on this assemblage as well as the spatial patterns emerging from the action of ecological mechanisms. To explore this, we analysed the composition of surface-ocean prokaryotic and picoeukaryotic communities using DNA-sequence data (16S- and 18S-rRNA genes) collected during the circumglobal expeditions *Malaspina-2010* and *TARA-Oceans*.

**Results:** We found that the two main components of the tropical and subtropical surface-ocean microbiota, prokaryotes and picoeukaryotes, appear to be structured by different ecological mechanisms. Picoeukaryotic communities were predominantly structured by dispersal-limitation, while prokaryotic counterparts appeared to be shaped by the combined action of dispersal-limitation, selection and drift. Temperature-driven selection appeared as a major factor, out of a few selected factors, influencing species co-occurrence networks in prokaryotes but not in picoeukaryotes, indicating that association patterns may contribute to understand ocean microbiota structure and response to selection. Other measured abiotic variables seemed to have limited selective effects on community structure in the tropical and subtropical ocean. Picoeukaryotes displayed a higher spatial differentiation between communities and a higher distance decay when compared to prokaryotes, consistent with a scenario of higher dispersal limitation in the former after considering environmental heterogeneity. Lastly, random dynamics or *drift* seemed to have a more important role in structuring prokaryotic communities than picoeukaryotic counterparts.

(Continued on next page)

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# Global diversity and distribution of aerobic anoxygenic phototrophs in the tropical and subtropical oceans

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## Summary

The aerobic anoxygenic phototrophic (AAP) bacteria are common in most marine environments but their global diversity and biogeography remain poorly characterized. Here, we analyzed AAP communities across 113 globally-distributed surface ocean stations sampled during the Malaspina Expedition in the tropical and subtropical ocean. By means of amplicon sequencing of the *puM* gene, a genetic marker for this functional group, we show that AAP communities along the surface ocean were mainly

composed of members of the Halieaceae (Gammaproteobacteria), which were adapted to a large range of environmental conditions, and of different clades of the Alphaproteobacteria, which seemed to dominate under particular circumstances, such as in the oligotrophic gyres. AAP taxa were spatially structured within each of the studied oceans, with communities from adjacent stations sharing more taxonomic similarities. AAP communities were composed of a large pool of rare members and several habitat specialists. When compared to the surface ocean prokaryotic and picoeukaryotic communities, it appears that AAP communities display an idiosyncratic global biogeographical pattern, dominated by selection processes and less influenced by dispersal limitation. Our study contributes to the understanding of how AAP communities are distributed in the horizontal dimension and the mechanisms underlying their distribution across the global surface ocean.

## Introduction

The discovery of marine aerobic photoheterotrophs (i.e., aerobic anoxygenic phototrophic (AAP) bacteria and proteorhodopsin-containing bacteria) (Béjà *et al.*, 2000; Kolber *et al.*, 2000) challenged the classic view of bacterioplankton being composed of photoautotrophic microorganisms as primary producers and of chemoheterotrophs as consumers. Since then, many studies have investigated their abundance, diversity and distribution in the ocean, and ultimately tried to understand their role in the marine ecosystem (DeLong and Béjà, 2010; Kirchman and Hanson, 2013; Koblížek, 2015; Pinhassi *et al.*, 2016). AAP bacteria are photoheterotrophs that use dissolved organic matter but harvest solar energy using bacteriochlorophyll *a* (Bchl<sub>a</sub>) to supplement their metabolism. In the marine environment, these organisms can typically constitute up to 10% of total prokaryotes (Schwalbach and Fuhrman, 2005; Sieracki *et al.*, 2006; Jiao *et al.*, 2007; Hojerová *et al.*, 2011), and are an active part of the community because they consist of large cells that display higher growth rates and receive higher grazing pressure than most bacteria (Sieracki *et al.*, 2006;

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## ECOLOGY

## Environmental vulnerability of the global ocean epipelagic plankton community interactome

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Marine plankton form complex communities of interacting organisms at the base of the food web, which sustain oceanic biogeochemical cycles and help regulate climate. Although global surveys are starting to reveal ecological drivers underlying planktonic community structure and predicted climate change responses, it is unclear how community-scale species interactions will be affected by climate change. Here, we leveraged *Tara Oceans* sampling to infer a global ocean cross-domain plankton co-occurrence network—the community interactome—and used niche modeling to assess its vulnerabilities to environmental change. Globally, this revealed a plankton interactome self-organized latitudinally into marine biomes (Tropics, Westerlies, Polar) and more connected poleward. Integrated niche modeling revealed biome-specific community interactome responses to environmental change and forecasted the most affected lineages for each community. These results provide baseline approaches to assess community structure and organismal interactions under climate scenarios while identifying plausible plankton bioindicators for ocean monitoring of climate change.

## INTRODUCTION

Marine plankton and associated processes are at the core of global biogeochemical cycles, shaping ecosystem structure and influencing climate regulation (1). While global biodiversity maps for viruses, prokaryotes, and microbial eukaryotes are beginning to emerge (2–4), identifying and understanding the complex network of interactions between these organisms and their environment is in its infancy (5). These interactions are critical to establish the ecosystem trophic links that underpin biogeochemical cycles and feedbacks that drive climate regulation and response (6, 7). While abiotic factors, such as temperature, can explain a large fraction of microbial

community composition in the global ocean (8), biotic interactions can differentially shape ecosystem diversity (9) and can even influence the adaptation to new environments (10). Thus, determining how plankton ecological interactions are structured and affected by environmental change remains a notable challenge.

Large-scale holistic marine ecosystem sampling facilitates conceptualization of plankton community interactomes as co-occurrence networks that are useful to model the complex community structure of ecological associations (11, 12). These networks have enabled the detection of communities assembled through niche overlap across biomes (13) and also the prediction of putative interactions such as parasitism or symbioses (14). Likewise, plankton co-occurrence networks have been instrumental in detecting interrelated changes in community structure from surface to depth (15), as well as in identifying specific communities of key lineages (e.g., *Synechococcus*, its phages, and Collodaria) associated with global open ocean processes such as carbon export (16). Community interactomes are also useful to identify central, highly connected lineages that may play significant ecological roles and confer stability to the community (17). These central lineages can correspond to keystone taxa that are good indicators of community shifts (18). Understanding the mechanisms affecting these central taxa may help us to predict responses of microbiome structure and functioning to perturbations (19).

While community interactomes inferred from global-scale samplings summarize well the complexity and potential interactions within microbial assemblages (12), they usually do not reflect dynamic processes shaping the observed system, as measured by longitudinal high-frequency sampling (20). Thus, alternative strategies need to be developed to capture ecosystem dynamics and responses from spatial samplings. Plankton species display various ecological and evolutionary responses to global environmental change (21, 22). Within marine ecosystems, the interplay between species ecological niche and climate change can induce abrupt community shifts,

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## Effects of seasonality, trophic state and landscape properties on CO<sub>2</sub> saturation in low-latitude lakes and reservoirs



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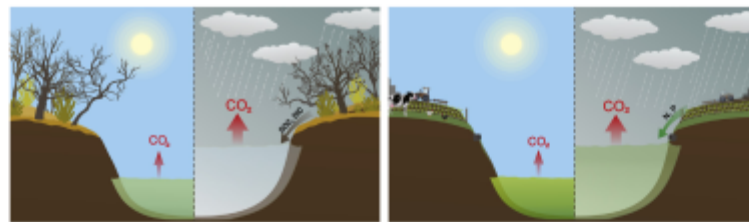
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### HIGHLIGHTS

- CO<sub>2</sub> supersaturation is prevalent in eutrophic low-latitude fresh waters
- The pCO<sub>2</sub> was significantly higher in these lakes than at higher latitudes
- Rainy season resulted in high pCO<sub>2</sub> in low-latitude freshwater systems
- pCO<sub>2</sub> increased as eutrophication decreased with higher water volume
- Land-use types directly affected trophic state but not water pCO<sub>2</sub>

### GRAPHICAL ABSTRACT



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### ABSTRACT

The role of tropical lakes and reservoirs in the global carbon cycle has received increasing attention in the past decade, but our understanding of its variability is still limited. The metabolism of tropical systems may differ profoundly from temperate systems due to the higher temperatures and wider variations in precipitation. Here, we investigated the spatial and temporal patterns of the variability in the partial pressure of carbon dioxide (pCO<sub>2</sub>) and its drivers in a set of 102 low-latitude lakes and reservoirs that encompass wide gradients of precipitation, productivity and landscape properties (lake area, perimeter-to-area ratio, catchment size, catchment area-to-lake area ratio, and types of catchment land use). We used multiple regressions and structural equation modeling (SEM) to determine the direct and indirect effects of the main in-lake variables and landscape properties on the water pCO<sub>2</sub> variance. We found that these systems were mostly supersaturated with CO<sub>2</sub> (92% spatially and 72% seasonally) regardless of their trophic status and landscape properties. The pCO<sub>2</sub> values (9–40,020 μatm) were within the range found in tropical ecosystems, and higher ( $p < 0.005$ ) than pCO<sub>2</sub> values recorded from high-latitude ecosystems. Water volume had a negative effect on the trophic state ( $r = -0.63$ ), which mediated a positive indirect effect on pCO<sub>2</sub> ( $r = 0.4$ ), representing an important negative feedback in the context of climate change-driven reduction in precipitation. Our results demonstrated that precipitation drives the pCO<sub>2</sub> seasonal

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OPEN

# Limnological effects of a large Amazonian run-of-river dam on the main river and drowned tributary valleys

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Run-of-river dams are often considered to have lower environmental impacts than storage dams due to their smaller reservoirs and low potential for flow alteration. However, this has been questioned for projects recently built on large rivers around the world. Two of the world's largest run-of-river dams—Santo Antônio and Jirau—were recently constructed on the Madeira River, a major tributary to the Amazon River in Brazil. Here we evaluate the effects of the creation of the Santo Antônio dam on the water chemistry and thermal structure of the Madeira River mainstem and back-flooded valleys of tributaries within the reservoir inundated area. In contrast to the mainstem river, some back-flooded tributaries periodically developed thermal stratification, which is associated with higher water residence times. Additionally, biochemical oxygen demand, partial pressure of CO<sub>2</sub>, and organic carbon all increased in the tributary valleys inundated by the reservoir, possibly due to increased input of allochthonous organic matter and its subsequent mineralization upon back-flooding—a common feature of newly flooded impoundments. The mainstem did not show detectable dam-related changes in water chemistry and thermal structure. Although the majority of the reservoir area maintained riverine conditions, the lateral valleys formed upon back-flooding—corresponding to ~30% of the Santo Antônio reservoir area—developed lake-like conditions akin to a typical reservoir of a storage dam.

Run-of-river hydropower plants have smaller reservoirs with limited water storage potential, unlike storage dams, which generally form large reservoirs with lacustrine conditions and variable water volumes. Inflowing water typically passes through a run-of-river reservoir quickly, and the electricity generation is a function of the flow of the river at a given time. Run-of-river dams are often considered to have lower environmental impacts due to their smaller reservoirs and lower potential for flow alteration<sup>1,2</sup>, although this has been questioned for projects recently built on large rivers around the world<sup>3,4</sup>. Considering that most new dam construction is of the run-of-river design<sup>5</sup>, there is an urgent need to more fully document the effects of this dam design on river systems.

Two of the world's largest run-of-river dams—Santo Antônio and Jirau—have recently been constructed on the Madeira River, the largest tributary to the Amazon River in terms of water and sediment discharge<sup>6,7</sup>. The Jirau dam is immediately upstream of the reservoir created by the Santo Antônio dam, and the combined installed








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## Not all viruses in nature are human enemies: a perspective on aquatic virus ecology in Brazil

Nem todos os vírus na natureza são inimigos humanos: uma perspectiva sobre a ecologia dos vírus aquáticos no Brasil

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**Abstract:** Viruses cause various diseases in humans through vector-borne (e.g., Zika and dengue fever), airborne (e.g., measles) and water-borne (e.g., hepatitis) transmission, as well as direct physical contact (e.g., AIDS and herpes). Recently, the new coronavirus (SARS-CoV-2) pandemic has triggered the greatest global health crisis in a century. However, not all viruses in nature are human enemies. A vast body of literature indicates that viral infection is vital for ecosystem functioning by affecting nutrient cycling, controlling species growth and enhancing biodiversity. Here we provide a perspective on the ecological role of viruses in nature, with special focus on Brazilian aquatic ecosystems.

**Keywords:** viral ecology; viroplankton; microorganisms; aquatic ecosystems; tropics.

**Resumo:** Os vírus causam doenças em humanos por meio de vetores (e.g., Zika e dengue), pelo ar (e.g., sarampo), pela água (e.g., hepatite) e por contato físico direto (e.g., AIDS e herpes). Recentemente, a pandemia do novo coronavírus (SARS-CoV-2) ocasionou a maior crise sanitária do século. No entanto, nem todos os vírus na natureza são inimigos humanos. Diversos estudos têm mostrado que a infecção viral é fundamental para o funcionamento de ecossistemas, afetando o ciclo de nutrientes, controlando o crescimento de algumas espécies e aumentando a biodiversidade. Esta mini-revisão apresenta uma perspectiva do papel ecológico dos vírus na natureza, com foco em ambientes aquáticos Brasileiros.

**Palavras-chave:** ecologia viral; viroplâncton; microrganismos; ecossistemas aquáticos; trópicos.



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OPEN

DATA DESCRIPTOR

# A georeferenced rRNA amplicon database of aquatic microbiomes from South America

Sebastian Metz *et al.*<sup>‡</sup>

The biogeography of bacterial communities is a key topic in Microbial Ecology. Regarding continental water, most studies are carried out in the northern hemisphere, leaving a gap on microorganism's diversity patterns on a global scale. South America harbours approximately one third of the world's total freshwater resources, and is one of these understudied regions. To fill this gap, we compiled 16S rRNA amplicon sequencing data of microbial communities across South America continental water ecosystems, presenting the first database  $\mu$ SudAqua[db]. The database contains over 866 georeferenced samples from 9 different ecoregions with contextual environmental information. For its integration and validation we constructed a curated database ( $\mu$ SudAqua[db.sp]) using samples sequenced by Illumina MiSeq platform with commonly used prokaryote universal primers. This comprised ~60% of the total georeferenced samples of the  $\mu$ SudAqua[db]. This compilation was carried out in the scope of the  $\mu$ SudAqua collaborative network and represents one of the most complete databases of continental water microbial communities from South America.

## Background & Summary

Microorganisms are the main drivers of biogeochemical cycles in freshwater ecosystems<sup>1–4</sup>. Due to their high abundances and activities and to their collective metabolic and phylogenetic diversity, prokaryotes support aquatic food webs and regulate the magnitude and recycling rates of major elements<sup>5</sup>. Thus, understanding the microbial diversity patterns is a fundamental topic in modern Microbial Ecology and a key step for advancing our knowledge on bacterial-mediated processes across continental water ecosystems.

Despite the extensive application of amplicon sequencing by high-throughput technologies (HTS), there are still important gaps in the study of aquatic microbial diversity<sup>6–8</sup>. For example, a rough mapping of the world-wide distribution of amplicon sequencing studies (Fig. 1), clearly shows that most of them are from the northern hemisphere, particularly from Europe and the United States – Canada, while the Southern Hemisphere has a contrasting underrepresentation<sup>9,10</sup>. This is especially true in South America and Africa, where sequencing studies are still scarce and generated from isolated efforts.

The Southern Hemisphere covers a comparatively high share of the surface and volume of the continental and marine ecosystems in the world. In particular, South America is considered the “continent of water”, harboring 6 out of the 10 largest rivers in the world in terms of water discharge, draining about 30% of the continental freshwater that reaches the ocean<sup>11</sup>. This water flows through five huge hydrological river basins: the Amazonas (6,000,000 km<sup>2</sup>), Del Plata-Paraná/Paraguay (2,600,000 km<sup>2</sup>), Orinoco (990,000 km<sup>2</sup>), Araguaia-Tocantins (757,000 km<sup>2</sup>), and São Francisco (634,000 km<sup>2</sup>)<sup>12,13</sup>. In addition, a great number and diversity of lentic water bodies are also prominent features that tend to occur in lake districts and wetlands as a result of the main climatic and geomorphological processes acting on regional scales<sup>14</sup>.

Furthermore, the South American continent comprises a large ecological heterogeneity<sup>15,16</sup>. South America covers about 15% of the global land area (17,870,218 km<sup>2</sup>) and spans a broad latitudinal range, extending from 12° 28'N (Punta Gallinas, Colombia) to 55° 59'S (Cabo de Hornos, Chile). According to the biogeographic regionalization by Cabrera & Willink<sup>15</sup>, South America belongs to the Neotropical region, except the southernmost area, which is assigned to the Antarctic region. Owing to the wide latitudinal coverage, a large variety of climates occur, with much of the continental mass located within the intertropical belt, large regions of Chile, Argentina, and Uruguay laying in the Southern Temperate Zone, and the southern tip of the continent extending

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# **ANEXO V**

MANUSCRITO ADICIONAL  
(VERSÃO PRELIMINAR)

## A LATITUDINAL GRADIENT HYPOTHESIS FOR ECOLOGICAL PROCESSES SHAPING MARINE PROTIST COMMUNITIES

### ABSTRACT

Marine protists are diverse and essential components of ocean food-webs. Investigating protist diversity patterns and its drivers is essential, since changes in their community structure can cause important biogeochemical and ecological alterations in the ocean. Recent studies have demonstrated that protists display diversity patterns by latitude and depth in regional and global scales. There have also been several time-series studies showing protist seasonal patterns in coastal microbial observatories located in different latitudes of the global ocean. However, a great majority of these long-term studies were conducted separately and focused on temperate observatories. Here, we compiled a four years (April 2013-2016) 18SV4-rRNA-gene amplicon dataset integrating four time-series located in contrasting latitudes (Polar 78°N, Temperate 42-48°N and Tropical 5°S) to investigate the seasonal drivers of protists in the ocean. We tested a latitudinal gradient hypothesis in which the relative importance of environmental selection structuring protist communities increases with latitude. Conversely, the role of stochastic factors would decrease with latitude, as a result of low amplitude of environmental variation towards the tropics. Our results partially corroborated our hypothesis, demonstrating that selection explained relatively more the community turnover in the temperate and polar observatory than in the tropical one, where stochasticity was the highest. Our work represents a significant contribution to understanding the ecological processes seasonally shaping marine protist assemblages in contrasting latitudes of the ocean.

**Keywords:** polar, temperate, tropical, eukaryotes, microbial time-series, 18SV4 rRNA amplicons, phenology

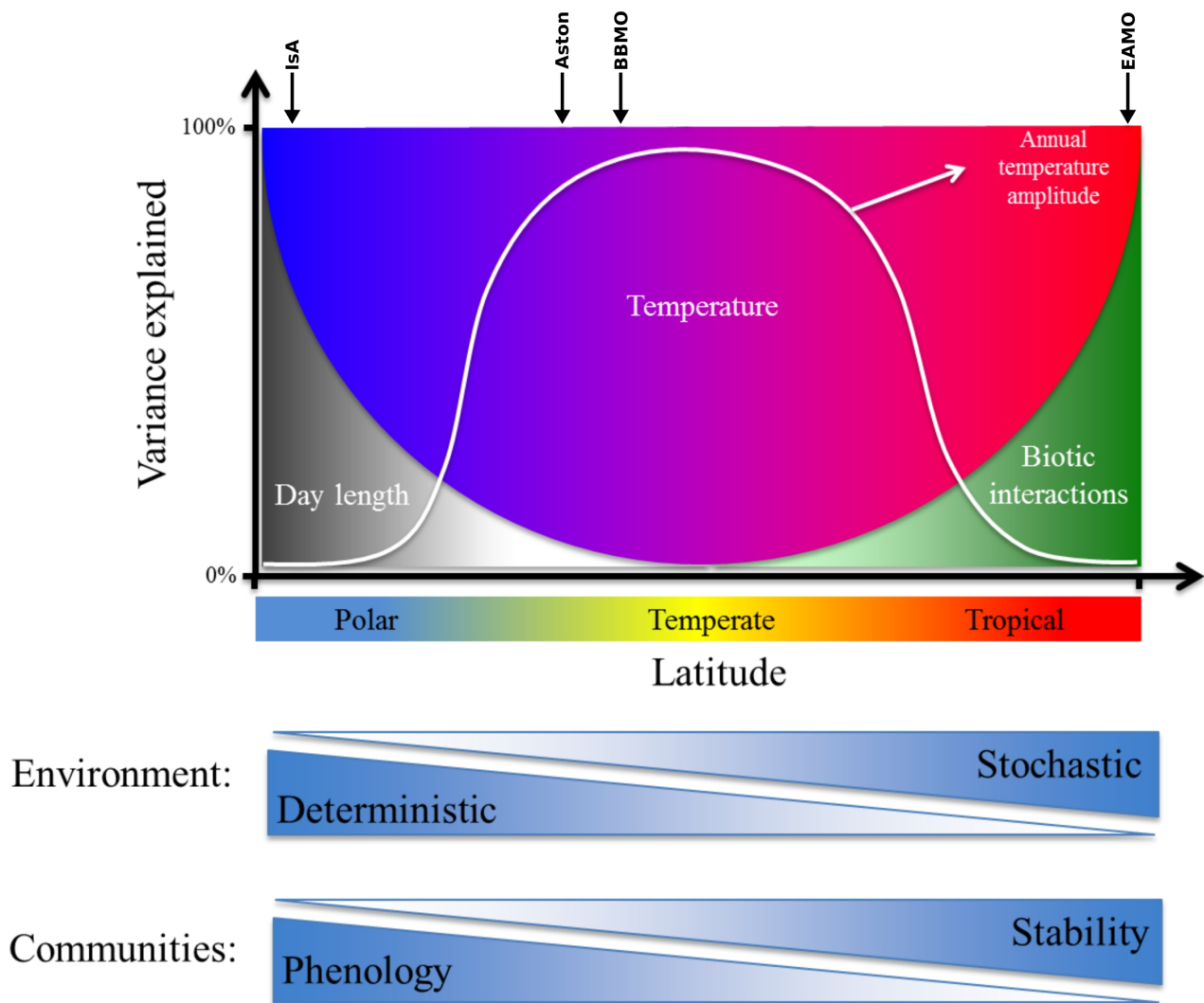
## INTRODUCTION

Marine eukaryotic plankton presents an incredibly high diversity (DE VARGAS et al., 2015) and is an essential component of food webs in the global ocean (SHERR; SHERR, 2008). Understanding the processes shaping marine protist communities is important to predict the consequences of the effect of environmental changes on these communities (DONEY et al., 2012). Global oceanographic expeditions have lately revealed large-scale spatial patterns of protist diversity in the ocean (GINER et al., 2020; IBARBALZ et al., 2019; LOGARES et al., 2020; SOMMERIA-KLEIN et al., 2021). Some time-series studies have revealed the temporal variability of protist assemblages in a range of marine observatories (BROWN et al., 2018; CARACCILO et al., 2022; GINER et al., 2019). However, these temporal studies were mostly focused on mid-latitudes, although a great part of the ocean is covering tropical and sub-tropical waters (BEHRENFELD et al., 2006). Here, we compiled a 18S-rRNA-gene (V4 region) dataset with four coastal marine time-series covering tropical, temperate and polar latitudes of the ocean at a global scale.

A recent theory in community ecology describes the structure of ecological communities as a result of the balance between four main processes: selection, dispersal, ecological drift and diversification (VELLEND, 2016). Selection can be interpreted as determinism, while dispersal limitation and ecological drift as stochastic processes (ZHOU; NING, 2017). (STEGEN et al., 2013) have proposed a methodological approach combining null models that allows the estimation of the relative abundance of selection, dispersal and ecological drift. This approach has been applied to reveal the main ecological processes shaping microbial eukaryotic communities of a range of ecosystems (LOGARES et al., 2018; VASS et al., 2020; ZHAO et al., 2017), including the ocean (JIE et al., 2022; JUNGER et al., 2023; LOGARES et al., 2020; WU et al., 2018) (JUNGER et al., 2023; LOGARES et al., 2020). Tiny eukaryotic communities were found to be mainly structured by dispersal limitation in the tropical and sub-tropical surface ocean (LOGARES et al., 2020). Moreover, a recent report has shown that the role of dispersal limitation and ecological drift, relative to that of selection, increase with depth due to decreasing microbial populations and the presence of dispersal barriers in the deep ocean (JUNGER et al., 2023). The role of dispersal limitation also seems to increase with eukaryotic cell size (SOMMERIA-KLEIN et al., 2021). However, these space-for-time studies lack a temporal dimension, which is essential to fully understand the ecological processes underpinning protist biogeography in the ocean (MOREIRA; LÓPEZ-GARCÍA, 2019). Furthermore, we still do not understand whether the relative role of deterministic and stochastic factors assembling protist communities change with latitude.

Here we aimed to test a latitudinal gradient hypothesis (Fig 1) of the ecological processes

shaping protist communities in marine observatories located in contrasting latitudes. We determined the relative importance of ecological processes (selection vs. stochasticity) shaping protists (0.2-20  $\mu\text{m}$  and 0.45-10  $\mu\text{m}$ ) in each site. To do so, we sequenced 18S rRNA gene amplicons from DNA samples monthly collected during four years in four microbial observatories: one tropical site located in the Western Equatorial Atlantic (6°S), two temperate sites located in the Northwestern Mediterranean Sea (42°N) and the western English Channel (49°N) as well as one polar site located in the Arctic Ocean (78°N).



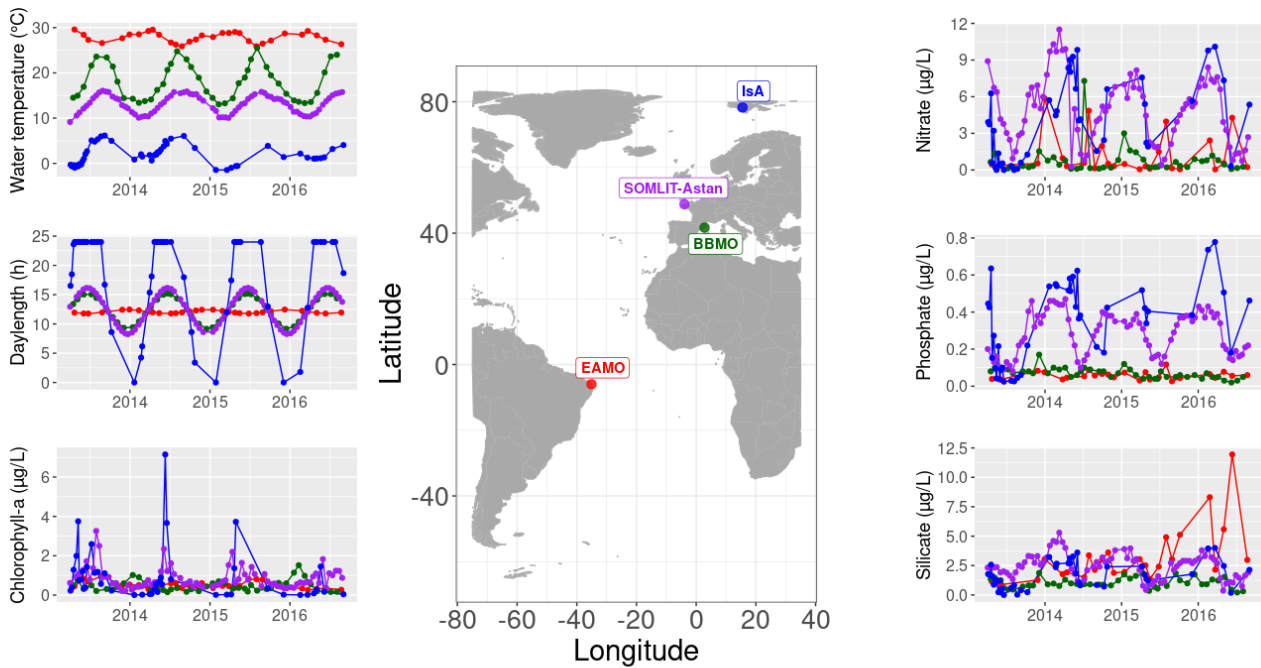
**Figure 1.** Conceptual scheme of the different processes and variables explaining variance in marine protist communities across a wide latitudinal gradient. At low latitudes (tropical regions), community structure should be relatively more driven by stochastic factors in a scenario of strong community temporal stability. At intermediate latitudes (temperate regions) high annual temperature amplitude would be the main factor driving protist communities. At high latitudes (polar regions), deterministic factors (selection driven mainly by light and temperature) leads to less protist community stability (marked succession) over time. The arrows at the top indicate the latitudinal

position of the time-series stations used in this study: IsA – Isfjorden Adventfjorden Time Series (78°N), Astan – SOMLIT-Astan, Roscoff (49°N), BBMO – Blanes Bay Microbial Observatory (42°N), EAMO – Equatorial Atlantic Microbial Observatory (6°S).

## **MATERIAL AND METHODS**

### ***Study sites and sampling procedures***

Seawater samples (1-15 m depth) for 18S ribosomal RNA (rRNA) amplicons, together with physiochemical parameters, were collected from April 2013 to August 2016 at four marine time-series covering a wide latitudinal gradient (Fig. 2 and Table 1). The Equatorial Atlantic Microbial Observatory – EAMO (-5.99°, -35.08°) is a tropical oligotrophic site located in the western coast of the South Atlantic Ocean. The Blanes Bay Microbial Observatory – BBMO (41.66°, 2.80°) is a temperate oligotrophic coastal site located in the Northwestern Mediterranean Sea (GASOL et al., 2016). SOMLIT-Astan station (48.78°, -3.94°) is a temperate coastal site located in the western English Channel, 3.5 km off Roscoff (Brittany, France), that is part of the French SOMLIT monitoring network (Service d’Observation en Milieu Littoral; <http://somlit.epoc.u-bordeaux1.fr/>) (CARACCILO et al., 2022). The Isfjorden Adventfjorden Time-Series – IsA (78.25°, 15.53°) is a polar site located in the Arctic Ocean, at the mouth of Adventfjorden in the inner part of Isfjorden, close to Longyearbyen on the western coast of Spitsbergen, Svalbard (VADER et al., 2015). Day length (hours of light) was calculated for each site based on coordinates and sampling dates using the *geosphere* R package. Technical details about the sampling procedure as well as the protocols to obtain the physiochemical parameters at each site can be found in the Supplementary Material.



**Figure 2.** Geographic location of the four coastal marine microbial observatories used in this study: Equatorial Atlantic Microbial Observatory (EAMO), Blanes Bay Microbial Observatory (BBMO), Service d'Observation en Milieu Littoral – Roscoff (SOMLIT-Astan), Isfjorden Adventfjorden Time Series (IsA). Temporal variability in temperature, day-length, chlorophyll-a as well as inorganic nutrients in these observatories from April 2013 to August 2016.

**Table 1:** Georeference, climate zones, sampling and sequencing information of each microbial time-series used in this study.

Time-series						Primers	
station	Latitude	Longitude	Ocean basin	Climate	Fractions	reference	ENA project
IsA Times Series	78°15.6'N	15°31.8' E	Arctic Ocean	Polar	0.45-10 µm	PIREDDA et al., 2017	XXXXXXX
SOMLIT-Astan	48°44'55" N	3°57'40" W	Eastern North Atlantic Ocean	Temperate	0.2-3 µm; 3-20 µm	STOECK et al., 2010	PRJEB48571
BBMO	41°40'10" N	2°48'01" E	Mediterranean Sea	Temperate	0.2-3 µm; 3-20 µm	STOECK et al., 2010	PRJEB48035
EAMO	05°59'24" S	35°4'48" W	South Atlantic Ocean	Tropical	0.2-3 µm; 3-20 µm	STOECK et al., 2010	PRJEB48035

### **DNA extraction, sequencing and bioinformatic processing**

Prior to extraction, SOMLIT-Astan samples were first incubated 45 min at 37°C with 100 µl lysozyme (20 mg/ml), and 1 h at 56°C with 20 µl proteinase K (20 mg/ml) and 100 µl SDS 20% (CARACCILO et al., 2022). For BBMO, EAMO and SOMLIT-Astan samples, DNA extraction was conducted using a phenol-chloroform protocol (MASSANA et al., 1997), as previously

described in Chapter 3. For the IsA time-series, DNA extraction was carried out using the DNeasy Plant Mini kit (Qiagen, USA) following the manufacturers' protocol, but including a bead-beating step to guarantee efficient lysis (technical details are provided in (VADER et al., 2015). DNA extracts were quantified with a Qubit 1.0 (Thermo Fisher Scientific) and preserved at  $-80^{\circ}\text{C}$ . For BBMO, EAMO and SOMLIT-Astan, PCR amplification was performed using the primers TAREukFWD1 (5'-CCAGCA(G/C)C(CT)GCGG-TAATTCC-3') and TAREukREV3 (5'-ACTTTCGTTCTTGAT(CT)(A/G)A-3') of the 18S rRNA gene hypervariable V4 region ( $\approx 380$  bp) (STOECK et al., 2010). For IsA, PCR amplification was conducted using the modified primers forward (5'-CCAGCASCYGCGGTAATTCC-3') and reverse (5'-ACTTTCGTTCTTGATYRATGA-3'), as detailed in (PIREDDA et al., 2017). Samples were sequenced in an Illumina MiSeq platform and raw reads were processed using DADA2 (CALLAHAN et al., 2016) to determine amplicon sequence variants (ASVs). For BBMO, EAMO and IsA, the forward reads were trimmed at 220 bp, while reverse reads at 190 bp. For SOMLIT-Astan, the forward reads were trimmed at 210 bp and the reverse reads at 200 bp. Then, the maximum number of expected errors (maxEE) was set to 5 for both the forward and reverse reads. Finally, error rates were estimated using DADA2 for the 18S gene to delineate the ASVs.

ASVs taxonomy was assigned with DADA2 using the naïve Bayesian classifier method (QIONG et al., 2007) alongside the Protist Ribosomal Reference database (PR<sup>2</sup>, version 4.14, (GUILLOU et al., 2013)). Holozoan (Metazoa and Fungi), Streptophyta, and nucleomorphs were removed from the 18S ASVs table. Rare ASVs with total abundance below 50 reads and prevalence below 15% were removed from each ASV table to reduce PCR and sequencing depth biases. The DNA sequences used in this study are deposited in the European Nucleotide Archive (<http://www.ebi.ac.uk/ena>) under the accession numbers available in Table 1.

### ***Phylogenetic trees***

Phylogenetic trees were built for the 18S rRNA gene-datasets. First, we used Mothur to align raw ASV sequences against an aligned PR<sup>2</sup> template (SCHLOSS et al., 2009). Poorly aligned regions or sequences were then removed using trimAl (parameters: -gt 0.3 -st 0.001) (CAPELLA-GUTIÉRREZ; SILLA-MARTÍNEZ; GABALDÓN, 2009). Aligned sequences were visually curated with seaview v4 (GOUY; GUINDON; GASCUEL, 2010) and sequences with  $\geq 40\%$  of gaps were removed. Finally, phylogenetic trees were inferred from the aligned quality-filtered sequences using FastTree v2.1.9 (PRICE; DEHAL; ARKIN, 2009).



### ***Computation of ecological processes***

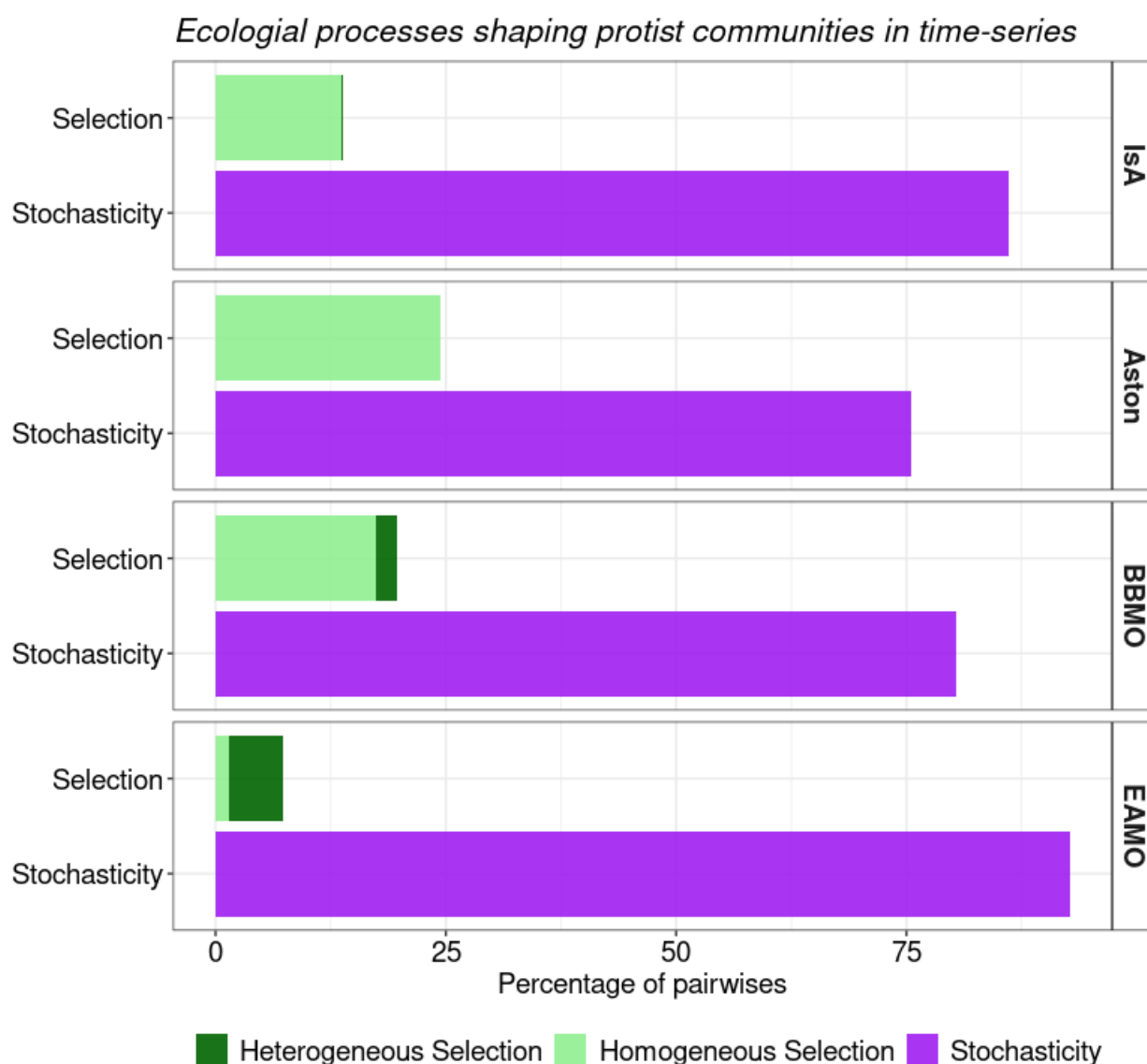
The relative importance of determinism (selection) and stochasticity were here estimated using a null model approach (STEGEN et al., 2013) that has been often applied to single-cell eukaryotic communities (JUNGER et al., 2023; LOGARES et al., 2020; VASS et al., 2020). First, we quantified the phylogenetic turnover using the abundance-weighted  $\beta$ -mean nearest taxon distance ( $\beta$ MNTD) metric (STEGEN et al., 2013), which computes the mean phylogenetic distances between each ASV and its closest relative in each pair of communities (pairwise comparisons). Second, we run null models with 999 randomizations to simulate the community turnover by chance ( $\beta$ MNTD<sub>null</sub>) (STEGEN et al., 2013). Finally, the  $\beta$ -Nearest Taxon Index ( $\beta$ NTI) was calculated from the differences between the observed  $\beta$ MNTD and the mean  $\beta$ MNTD<sub>null</sub> values. Overall,  $|\beta$ NTI| > 2 indicates that taxa are phylogenetically more related or less related than expected by chance, pointing to a strong influence of selection on community assembly (STEGEN et al., 2013).  $\beta$ NTI values higher than +2 indicate the action of heterogeneous selection, while  $\beta$ NTI values lower than -2 points out to the action of homogeneous selection (STEGEN et al., 2013). The  $\beta$ -diversity of communities that were not explained by selection ( $|\beta$ NTI|  $\leq$  2) were considered to be ruled by stochasticity.

### **RESULTS AND DISCUSSION**

We found differences in the relative importance of selection and stochasticity between observatories (Fig. 3). The role of selection was smaller in the tropical (~7.3%) than in the temperate (~20-25%) and polar sites (~14%). Conversely, stochasticity was higher in the tropics (93%) than in the polar (~86%) and temperate sites (~75-80%). These results are coherent to those found in the tropical and sub-tropical surface ocean, where the turnover of picoeukaryotes was explained more by stochastic factors, such as dispersal limitation (~68%) and drift (~21%), than environmental selection (~11%) (LOGARES et al., 2020).

Our findings partially corroborated our latitudinal hypothesis (Fig. 1), demonstrating that selection explained relatively more the community turnover in the temperate and polar observatories than in the tropical one, where stochasticity was the highest. We also found that selection was larger in SOMLIT-Aston than in BBMO, which suggests an increment in the role of selection with increasing environmental variability associated to higher latitudes (Fig. 2). Nevertheless, our results pointed relatively less selection in the polar station than in the temperate stations (Aston and BBMO), which contradicts our hypothesis of increasing selection with latitude (Fig. 1). This result partially contradicts previous studies showing strong seasonality in microbial eukaryotes driven by extreme temporal variability in light, nutrients and, to a less extent,

temperature (KILIAS et al., 2014; MARQUARDT et al., 2016; TREFAULT et al., 2021; WIETZ et al., 2021). The high stochasticity in the Arctic Fjord could be explained by terrestrial runoffs on plankton communities, which has been increasingly important in the Arctic as a consequence of increasing precipitation and glacial melt caused by climate change (ARENDRT et al., 2016; DELPECH et al., 2021). Alternatively, inter-annual changes in factors not measured here, such as hydrographic conditions, may be a source of stochasticity in the temporal assembly of microbial eukaryotic communities (KUBISZYN et al., 2014; THALER; LOVEJOY, 2015). However, it is important to notice that the results from the polar observatory may be biased due to methodological differences in comparison to the other observatories studied here. For instance, the size range (0.45-10  $\mu\text{m}$ ) in IsA was not exactly the same as the other observatories (all others are 0.22-3  $\mu\text{m}$ ). Additionally, slightly different 18S-V4 primers were used in IsA as compared to the other observatories studied here (Table 1). More precisely, the reverse primer used for the 18S-V4 region (TAReuk- Rev3; (STOECK et al., 2010)) in the other observatories may discriminate against Haptophytes, which has been corrected in the primer sets used in the polar station (Table 1), as described by (PIREDDA et al., 2017). Despite these technical issues, the current work suggests that there is a latitudinal difference in the balance of ecological processes shaping the temporal turnover of small eukaryotes in the ocean. However, further studies using standard protocols to compare polar to low-latitude long-term time-series are needed to validate our observations and confirm the latitudinal gradient hypothesis proposed here (Fig. 1). This framework would represent a significant contribution to predict global changes since the surface ocean is rapidly warming and the Arctic Ocean is very likely becoming similar to temperate systems such as the ones represented by SOMLIT- Astan and BBMO.



**Figure 3.** Relative importance of the processes (Selection vs Stochasticity) structuring small protist communities in contrasting time-series. IsA – Isfjorden Adventfjorden Time Series (78°N), Aston – SOMLIT-Astan, Roscoff (49°N), BBMO – Blanes Bay Microbial Observatory (42°N), EAMO – Equatorial Atlantic Microbial Observatory (6°S).

## REFERENCES

ARENDRT, Kristine Engel; AGERSTED, Mette Dalgaard; SEJR, Mikael Kristian; JUUL-PEDERSEN, Thomas. Glacial meltwater influences on plankton community structure and the importance of top-down control (of primary production) in a NE Greenland fjord. **Estuarine, Coastal and Shelf Science**, [S. l.], v. 183, p. 123–135, 2016. DOI: 10.1016/j.ecss.2016.08.026. Disponível em: <https://www.sciencedirect.com/science/article/pii/S0272771416302803>.

BEHRENFELD, Michael J. et al. Climate-driven trends in contemporary ocean productivity. **Nature**, [S. l.], v. 444, n. 7120, p. 752–755, 2006. DOI: 10.1038/nature05317. Disponível em: <https://doi.org/10.1038/nature05317>.

BROWN, Mark V et al. Systematic, continental scale temporal monitoring of marine pelagic microbiota by the Australian Marine Microbial Biodiversity Initiative. **Scientific Data**, [S. l.], v. 5, n. 1, p. 180130, 2018. DOI: 10.1038/sdata.2018.130. Disponível em: <https://doi.org/10.1038/sdata.2018.130>.

CALLAHAN, Benjamin J.; MCMURDIE, Paul J.; ROSEN, Michael J.; HAN, Andrew W.; JOHNSON, Amy Jo A.; HOLMES, Susan P. DADA2: High-resolution sample inference from Illumina amplicon data. **Nature Methods**, [S. l.], v. 13, p. 581, 2016. Disponível em: <https://doi.org/10.1038/nmeth.3869>.

CAPELLA-GUTIÉRREZ, Salvador; SILLA-MARTÍNEZ, José M.; GABALDÓN, Toni. trimAl: a tool for automated alignment trimming in large-scale phylogenetic analyses. **Bioinformatics**, [S. l.], v. 25, n. 15, p. 1972–1973, 2009. DOI: 10.1093/bioinformatics/btp348. Disponível em: <https://doi.org/10.1093/bioinformatics/btp348>.

CARACCILO, Mariarita et al. Seasonal dynamics of marine protist communities in tidally mixed coastal waters. **Molecular Ecology**, [S. l.], v. n/a, n. n/a, 2022. DOI: <https://doi.org/10.1111/mec.16539>. Disponível em: <https://doi.org/10.1111/mec.16539>.

DE VARGAS, Colomán et al. Eukaryotic plankton diversity in the sunlit ocean. **Science**, [S. l.], v. 348, n. 6237, p. 1261605–1261605, 2015. DOI: 10.1126/science.1261605. Disponível em: <http://www.sciencemag.org/cgi/doi/10.1126/science.1261605>.

DELPECH, Lisa-Marie; VONNAHME, Tobias R.; MCGOVERN, Maeve; GRADINGER, Rolf; PRÆBEL, Kim; POSTE, Amanda E. Terrestrial Inputs Shape Coastal Bacterial and Archaeal Communities in a High Arctic Fjord (Isfjorden, Svalbard). **Frontiers in Microbiology**, [S. l.], v. 12, 2021. DOI: 10.3389/fmicb.2021.614634. Disponível em: <https://www.frontiersin.org/articles/10.3389/fmicb.2021.614634>.

DONEY, Scott C. et al. Climate Change Impacts on Marine Ecosystems. **Annual Review of Marine Science**, [S. l.], v. 4, n. 1, p. 11–37, 2012. DOI: 10.1146/annurev-marine-041911-111611. Disponível em: <https://www.annualreviews.org/doi/10.1146/annurev-marine-041911-111611>.

GASOL, Josep M. et al. Seasonal patterns in phytoplankton photosynthetic parameters and primary production at a coastal NW Mediterranean site. **Scientia Marina**, [S. l.], v. 80, n. S1, p. 63–77, 2016. DOI: 10.3989/scimar.04480.06E. Disponível em: <https://scientiamarina.revistas.csic.es/index.php/scientiamarina/article/view/1658>.

GINER, Caterina R.; BALAGUÉ, Vanessa; KRABBERØD, Anders K.; FERRERA, Isabel; REÑÉ, Albert; GARCÉS, Esther; GASOL, Josep M.; LOGARES, Ramiro; MASSANA, Ramon. Quantifying long-term recurrence in planktonic microbial eukaryotes. **Molecular Ecology**, [S. l.], v. 28, n. 5, p. 923–935, 2019. DOI: 10.1111/mec.14929. Disponível em: <https://doi.org/10.1111/mec.14929>.

GINER, Caterina R.; PERNICE, Massimo C.; BALAGUÉ, Vanessa; DUARTE, Carlos M.; GASOL, Josep M.; LOGARES, Ramiro; MASSANA, Ramon. Marked changes in diversity and relative activity of picoeukaryotes with depth in the world ocean. **The ISME Journal**, [S. l.], v. 14, n. 2, p. 437–449, 2020. DOI: 10.1038/s41396-019-0506-9. Disponível em: <https://doi.org/10.1038/s41396-019-0506-9>.

GOUY, Manolo; GUINDON, Stéphane; GASCUEL, Olivier. SeaView Version 4: A Multiplatform Graphical User Interface for Sequence Alignment and Phylogenetic Tree Building. **Molecular Biology and Evolution**, [S. l.], v. 27, n. 2, p. 221–224, 2010. DOI: 10.1093/molbev/msp259. Disponível em: <https://doi.org/10.1093/molbev/msp259>.

GUILLOU, Laure et al. The Protist Ribosomal Reference database (PR2): a catalog of unicellular eukaryote Small Sub-Unit rRNA sequences with curated taxonomy. **Nucleic Acids Research**, [S. l.], v. 41, n. D1, p. D597–D604, 2013. DOI: 10.1093/nar/gks1160. Disponível em: <https://doi.org/10.1093/nar/gks1160>.

IBARBALZ, Federico M. et al. Global Trends in Marine Plankton Diversity across Kingdoms of Life. **Cell**, [S. l.], v. 179, n. 5, p. 1084–1097.e21, 2019. DOI: 10.1016/j.cell.2019.10.008. Disponível em: <https://doi.org/10.1016/j.cell.2019.10.008>.

JIE, Kong; LEI, Wang; CAI, Lin; FANGFANG, Kuang; XIWU, Zhou; A., Laws Edward; PING, Sun; HAO, Huang; BANGQIN, Huang. Contrasting Community Assembly Mechanisms Underlie Similar Biogeographic Patterns of Surface Microbiota in the Tropical North Pacific Ocean. **Microbiology Spectrum**, [S. l.], v. 10, n. 1, p. e00798-21, 2022. DOI: 10.1128/spectrum.00798-21. Disponível em: <https://doi.org/10.1128/spectrum.00798-21>.

JUNGER, Pedro C. et al. Global biogeography of the smallest plankton across ocean depths. **bioRxiv**, [S. l.], p. 2023.01.13.523743, 2023. DOI: 10.1101/2023.01.13.523743. Disponível em: <http://biorxiv.org/content/early/2023/01/15/2023.01.13.523743.abstract>.

KILIAS, Estelle; KATTNER, Gerhard; WOLF, Christian; FRICKENHAUS, Stephan; METFIES, Katja. A molecular survey of protist diversity through the central Arctic Ocean. **Polar Biology**, [S. l.], v. 37, n. 9, p. 1271–1287, 2014. DOI: 10.1007/s00300-014-1519-5. Disponível em: <https://doi.org/10.1007/s00300-014-1519-5>.

KUBISZYN, A. M.; PIWOSZ, K.; WIKTOR, J. M.; WIKTOR, J. M. The effect of inter-annual Atlantic water inflow variability on the planktonic protist community structure in the West Spitsbergen waters during the summer. **Journal of Plankton Research**, [S. l.], v. 36, n. 5, p. 1190–1203, 2014. DOI: 10.1093/plankt/fbu044. Disponível em: <https://doi.org/10.1093/plankt/fbu044>.

LOGARES, Ramiro et al. Disentangling the mechanisms shaping the surface ocean microbiota. **Microbiome**, [S. l.], v. 8, n. 1, p. 55, 2020. DOI: 10.1186/s40168-020-00827-8. Disponível em: <https://microbiomejournal.biomedcentral.com/articles/10.1186/s40168-020-00827-8>.

LOGARES, Ramiro; TESSON, Sylvie V. M.; CANBÄCK, Björn; PONTARP, Mikael; HEDLUND, Katarina; RENGEFORS, Karin. Contrasting prevalence of selection and drift in the community structuring of bacteria and microbial eukaryotes. **Environmental Microbiology**, [S. l.], v. 20, n. 6,

p. 2231–2240, 2018. DOI: 10.1111/1462-2920.14265. Disponible em:  
<https://onlinelibrary.wiley.com/doi/10.1111/1462-2920.14265>.

MARQUARDT, Miriam; VADER, Anna; STÜBNER, Eike I.; REIGSTAD, Marit; GABRIELSEN, Tove M. Strong Seasonality of Marine Microbial Eukaryotes in a High-Arctic Fjord (Isfjorden, in West Spitsbergen, Norway). **Applied and Environmental Microbiology**, [S. l.], v. 82, n. 6, p. 1868–1880, 2016. DOI: 10.1128/AEM.03208-15. Disponible em:  
<https://doi.org/10.1128/AEM.03208-15>.

MASSANA, R.; MURRAY, A. E.; PRESTON, C. M.; DELONG, E. F. Vertical distribution and phylogenetic characterization of marine planktonic Archaea in the Santa Barbara Channel. **Applied and Environmental Microbiology**, [S. l.], v. 63, n. 1, p. 50–56, 1997. DOI: 10.1128/aem.63.1.50-56.1997. Disponible em: <https://journals.asm.org/doi/10.1128/aem.63.1.50-56.1997>.

MOREIRA, David; LÓPEZ-GARCÍA, Purificación. Time series are critical to understand microbial plankton diversity and ecology. **Molecular Ecology**, [S. l.], v. 28, n. 5, p. 920–922, 2019. DOI: 10.1111/mec.15015. Disponible em: <https://doi.org/10.1111/mec.15015>.

PIREDDA, R.; TOMASINO, M. P.; D'ERCHIA, A. M.; MANZARI, C.; PESOLE, G.; MONTRESOR, M.; KOOISTRA, W. H. C. F.; SARNO, D.; ZINGONE, A. Diversity and temporal patterns of planktonic protist assemblages at a Mediterranean Long Term Ecological Research site. **FEMS Microbiology Ecology**, [S. l.], v. 93, n. 1, p. fiw200, 2017. DOI: 10.1093/femsec/fiw200. Disponible em: <https://doi.org/10.1093/femsec/fiw200>.

PRICE, Morgan N.; DEHAL, Paramvir S.; ARKIN, Adam P. FastTree: Computing Large Minimum Evolution Trees with Profiles instead of a Distance Matrix. **Molecular Biology and Evolution**, [S. l.], v. 26, n. 7, p. 1641–1650, 2009. DOI: 10.1093/molbev/msp077. Disponible em: <https://doi.org/10.1093/molbev/msp077>.

QIONG, Wang; M., Garrity George; M., Tiedje James; R., Cole James. Naïve Bayesian Classifier for Rapid Assignment of rRNA Sequences into the New Bacterial Taxonomy. **Applied and Environmental Microbiology**, [S. l.], v. 73, n. 16, p. 5261–5267, 2007. DOI: 10.1128/AEM.00062-07. Disponible em: <https://doi.org/10.1128/AEM.00062-07>.

SCHLOSS, Patrick D. et al. Introducing mothur: Open-Source, Platform-Independent, Community-Supported Software for Describing and Comparing Microbial Communities. **Applied and Environmental Microbiology**, [S. l.], v. 75, n. 23, p. 7537–7541, 2009. DOI: 10.1128/AEM.01541-09. Disponible em: <https://doi.org/10.1128/AEM.01541-09>.

SHERR, Evelyn B.; SHERR, Barry F. Understanding roles of microbes in marine pelagic food webs: a brief history. In: **Microbial Ecology of the Oceans**. [s.l.: s.n.]. p. 27–44.

SOMMERIA-KLEIN, Guilhem; WATTEAUX, Romain; IBARBALZ, Federico M.; KARLUSICH, Juan José Pierella; IUDICONE, Daniele; BOWLER, Chris; MORLON, Hélène. Global drivers of eukaryotic plankton biogeography in the sunlit ocean. **Science**, [S. l.], v. 374, n. 6567, p. 594–599, 2021. DOI: 10.1126/science.abb3717. Disponible em: <http://biorxiv.org/content/early/2020/12/24/2020.09.08.287524.abstract>.

STEGEN, James C.; LIN, Xueju; FREDRICKSON, Jim K.; CHEN, Xingyuan; KENNEDY, David W.; MURRAY, Christopher J.; ROCKHOLD, Mark L.; KONOPKA, Allan. Quantifying community assembly processes and identifying features that impose them. **The ISME Journal**, [S. l.], v. 7, n. 11, p. 2069–2079, 2013. DOI: 10.1038/ismej.2013.93. Disponível em: <http://dx.doi.org/10.1038/ismej.2013.93>.

STOECK, Thorsten; BASS, David; NEBEL, Markus; CHRISTEN, Richard; JONES, Meredith D. M.; BREINER, Hans-Werner; RICHARDS, Thomas A. Multiple marker parallel tag environmental DNA sequencing reveals a highly complex eukaryotic community in marine anoxic water. **Molecular Ecology**, [S. l.], v. 19, n. s1, p. 21–31, 2010. DOI: 10.1111/j.1365-294X.2009.04480.x. Disponível em: <https://doi.org/10.1111/j.1365-294X.2009.04480.x>.

THALER, Mary; LOVEJOY, Connie. Biogeography of Heterotrophic Flagellate Populations Indicates the Presence of Generalist and Specialist Taxa in the Arctic Ocean. **Applied and Environmental Microbiology**, [S. l.], v. 81, n. 6, p. 2137–2148, 2015. DOI: 10.1128/AEM.02737-14. Disponível em: <https://doi.org/10.1128/AEM.02737-14>.

TREFAULT, Nicole; DE LA IGLESIA, Rodrigo; MORENO-PINO, Mario; LOPES DOS SANTOS, Adriana; GÉRIKAS RIBEIRO, Catherine; PARADA-POZO, Génesis; CRISTI, Antonia; MARIE, Dominique; VAULOT, Daniel. Annual phytoplankton dynamics in coastal waters from Fildes Bay, Western Antarctic Peninsula. **Scientific Reports**, [S. l.], v. 11, n. 1, p. 1368, 2021. DOI: 10.1038/s41598-020-80568-8. Disponível em: <https://doi.org/10.1038/s41598-020-80568-8>.

VADER, Anna; MARQUARDT, Miriam; MESHAM, Archana R.; GABRIELSEN, Tove M. Key Arctic phototrophs are widespread in the polar night. **Polar Biology**, [S. l.], v. 38, n. 1, p. 13–21, 2015. DOI: 10.1007/s00300-014-1570-2. Disponível em: <https://doi.org/10.1007/s00300-014-1570-2>.

VASS, Máté; SZÉKELY, Anna J.; LINDSTRÖM, Eva S.; LANGENHEDER, Silke. Using null models to compare bacterial and microeukaryotic metacommunity assembly under shifting environmental conditions. **Scientific Reports**, [S. l.], v. 10, n. 1, p. 2455, 2020. DOI: 10.1038/s41598-020-59182-1. Disponível em: <https://doi.org/10.1038/s41598-020-59182-1>.

VELLEND, Mark. **The Theory of Ecological Communities. Monographs in Population Biology**, 2016. DOI: 10.1016/B978-0-7234-5558-5.00001-4.

WIETZ, Matthias; BIENHOLD, Christina; METFIES, Katja; TORRES-VALDÉS, Sinhué; VON APPEN, Wilken-Jon; SALTER, Ian; BOETIUS, Antje. The polar night shift: seasonal dynamics and drivers of Arctic Ocean microbiomes revealed by autonomous sampling. **ISME Communications**, [S. l.], v. 1, n. 1, p. 76, 2021. DOI: 10.1038/s43705-021-00074-4. Disponível em: <https://doi.org/10.1038/s43705-021-00074-4>.

WU, Wenxue; LU, Hsiao-Pei; SASTRI, Akash; YEH, Yi-Chun; GONG, Gwo-Ching; CHOU, Wen-Chen; HSIEH, Chih-Hao. Contrasting the relative importance of species sorting and dispersal limitation in shaping marine bacterial versus protist communities. **ISME JOURNAL**, MACMILLAN BUILDING, 4 CRINAN ST, LONDON N1 9XW, ENGLAND, v. 12, n. 2, p. 485–494, 2018. DOI: 10.1038/ismej.2017.183.

ZHAO, Dayong; CAO, Xinyi; HUANG, Rui; ZENG, Jin; SHEN, Feng; XU, Huimin; WANG, Sichen; HE, Xiaowei; YU, Zhongbo. The heterogeneity of composition and assembly processes of the microbial community between different nutrient loading lake zones in Taihu Lake. **Applied Microbiology and Biotechnology**, [S. l.], v. 101, n. 14, p. 5913–5923, 2017. DOI: 10.1007/s00253-017-8327-0. Disponível em: <https://doi.org/10.1007/s00253-017-8327-0>.

ZHOU, Jizhong; NING, Daliang. Stochastic Community Assembly: Does It Matter in Microbial Ecology? **Microbiology and Molecular Biology Reviews**, [S. l.], v. 81, n. 4, p. e00002-17, 2017. DOI: 10.1128/MMBR.00002-17. Disponível em: <http://www.ncbi.nlm.nih.gov/pubmed/29021219>. Acesso em: 20 nov. 2017.