Insights into biotic and abiotic modulation of ocean mesopelagic communities

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Abstract

Marine plankton mitigate anthropogenic greenhouse gases, modulate biogeochemical cycles, and provide fishery resources. Plankton is distributed across a stratified ecosystem of sunlit surface waters and a vast, though understudied, mesopelagic 'dark ocean'. In this study, we mapped viruses, prokaryotes, and pico-eukaryotes across 32 globally-distributed cross-depth samples collected during the *Tara* Oceans Expedition, and assessed their ecologies. Based on depth and O_2 measurements, we divided the marine habitat into epipelagic, oxic mesopelagic, and oxygen minimum zone (OMZ) eco-regions. We identified specific communities associated with each marine habitat, and pinpoint environmental drivers of dark ocean communities. Our results indicate that water masses primarily control mesopelagic community composition. Through co-occurrence network inference and analysis, we identified signature communities strongly associated with OMZ eco-regions. Mesopelagic communities appear to be constrained by a combination of factors compared to epipelagic communities. Thus, variations in a given abiotic factor may cause different responses in sunlit and dark ocean communities. This study expands our knowledge about the ecology of planktonic organisms inhabiting the mesopelagic zone.

Keywords: mesopelagic community, metabarcoding, plankton, pan-oceanic expedition, oxygen minimum zone

Introduction

Below the ocean's sunlit layer lies the mesopelagic zone that occupies around 20% of the global ocean volume [1]. The mesopelagic zone is biologically defined as starting where photosynthesis no longer occurs (<1% irradiance around 200m depth), down to its lower boundary where there is no detectable sunlight (around 1000m depth) [2]. This twilight ecosystem cannot rely on photoautotrophy, but sustains its energetic requirements by the combination of heterotrophic, chemoautotrophic, and chemo-mixotrophic metabolisms, together with physicochemical processes. Among the latter, the fraction of upper ocean productivity that escapes epipelagic recycling and sinks by gravity or is delivered by the daily migration of zooplankton constitutes an essential energy source in deep waters and is a vector for attached organisms [3].

The biodiversity and biomass in mesopelagic communities have been underestimated in the past [2, 4], and previous work showed these communities hold an enormous unexploited biological resource [5, 6, 1]. Mesopelagic organisms are considered to be a vast source of fat and protein, potentially becoming the primary source of global bioeconomy [7]. So far, efforts have been made to increase knowledge of mesopelagic mega/macrofauna by studying the abundance and diversity of nekton. These efforts are of great importance, given the rapid increase in the exploitation of this zone by nutraceutical and fisheries industries [6]. However, less attention has been devoted to the mesopelagic community's microscopic fraction, despite the pivotal role of the marine microbiome in biogeochemical cycles. The marine microbiome makes crucial links in the food web between primary production and dark ocean specialized consumers. Previous reports have shown stratification of planktonic communities with depth. In this regard, the mesopelagic zone displays a distinct assemblage of dsDNA viruses [8], giant viruses [9], prokaryotes [10, 11], and eukaryotes [12]. However, unlike the epipelagic layer, mesopelagic plankton diversity does not show the latitudinal diversity gradient trends from pole-to-pole, peaking at lower latitudes [13].

Among the studies conducted in mesopelagic zones, we highlight the efforts to explore regions of extreme conditions, such as oxygen minimum zones (OMZs). These zones are formed by

105 relatively old slowly upwelling waters, often lying below highly productive surface zones [14],
106 and are currently increasing in volume in the oceans [15]. OMZ prokaryotic communities are
107 well documented and predominated by taxa such as Nitrospira, Marinimicrobia, and anammox
108 bacteria from the phylum Planctomycetes, while Thaumarchaeota abundance is frequently
109 lower in these zones [16, 17, 18, 19, 20]. In contrast, knowledge of eukaryotic diversity in OMZs
110 is still rudimentary, but a prevalence of specific taxa such as Ciliophora, Dinoflagellata, MALV,
111 and Acantharia has been reported, together with a higher metabolic activity of these taxa [21,
112 22, 23]. Understanding plankton community structure and dynamics is fundamental to
113 anticipate the impacts of global warming and acidification in these regions.
114 The last decades have seen a significant increase in large-scale oceanic surveys [24, 25, 26].
115 However, most mesopelagic community studies have been limited to geographically or
116 ecologically fragmented regions, or to specific taxonomic groups, mainly because of the
117 inherent difficulties of accessing this zone on a global scale [5]. Hence, these studies have
118 given us a limited picture of community composition. Moreover, the factors influencing
119 community structure, presumably a combination of biotic and abiotic factors [27, 28], have
120 been little explored in the mesopelagic zone.
121 The present study takes advantage of the *Tara* Oceans large-scale survey conducted in
122 different water layers using a systematic sampling protocol, spanning viruses to small
123 eukaryote size fractions, to investigate the mesopelagic biome [29]. We capitalized on genomic
124 data together with extensive contextual data and ocean geography to explore the particularities
125 of mesopelagic communities compared to communities found in the euphotic zone. We also
126 investigated potential water deoxygenation effects on these communities by comparing OMZ
127 communities with those from well-oxygenated waters. This work expands our knowledge of
128 the web of relationships underpinning mesopelagic plankton ecosystems on a broad
129 geographic scale.
130

131 **Materials and Methods**

132 - **Sample collection and pre-processing**

The environmental and biological data were obtained during the *Tara* Oceans expedition (2009- 2012) in 32 oceanographic stations located in the Indian Ocean (IO - 037, 038, 039), Pacific Ocean (PO - 097, 098, 100, 102, 106, 109, 110, 111, 112, 122, 131, 132, 133, 135, 137, 138), South Atlantic Ocean (SAO - 068, 070, 072, 076, 078) and North Atlantic Ocean (NAO - 142, 143, 144, 145, 146, 148, 149, 152) comprising tropical and subtropical regions (Figure 1). Physico-chemical environmental data were obtained along a vertical profile at each station. Temperature, salinity, and oxygen were measured using a CTD-rosette system with a coupled dissolved oxygen sensor. Chlorophyll-a concentrations were measured using high-performance liquid chromatography. Nutrient concentrations were determined using segmented flow analysis. Metadata are available at PANGAEA [30, 31, 32, 33, 34, 35] - (https://doi.pangaea.de/10.1594/PANGAEA.875582).

The vertical distribution of marine particles was investigated with an Underwater Vision Profiler (UVP, [36, 37]) mounted on the CTD-Rosette. The UVP acquires images in a coherent water volume (1 L) delimited by a light sheet issued from red light-emitting diodes. Automatic identification of objects was made using Ecotaxa, based on a learning set of visually identified, manually classified objects and associated features. Images were classified to distinguish mesozooplankton from non-living objects and artifacts (e.g., detritic particles, fibers, and out-of-focus objects).

Water vertical profiles of temperature and salinity generated from the CTD were used to identify the water masses by plotting a temperature x salinity (T/S) diagram using the Ocean Data View V 5.0 (ODV) software package [38].

Three different water layers were sampled: surface (SRF, 3-7 m), deep chlorophyll maximum (DCM - depth identified according to the peak of chlorophyll-a fluorescence obtained *in situ*), and mesopelagic (ranging from 200-1000 m) [39]. The planktonic community was sampled by partitioning the seawater by filtering each sampled depth with different filter sizes [34]. Among the mesopelagic zones, 13 of them were identified as deficient in oxygen and classified as

oxygen minimum zone (OMZ, stations IO - 037, 038, 039 / PO - 100, 102, 106, 109, 110, 111, 133, 135, 137, 138). The OMZ were categorized as suboxic: <10 µM O_2/kg seawater and anoxic: (<0.003 µM/kg seawater or undetectable with most sensitive techniques, e.g., STOX sensors) [Units of O_2 concentration: 1 $mL.L^{-1}$=1.43 mg. L^{-1}=44.64 µM] [22].

Our dataset comprises different organismal size-fractions from viruses (two dsDNA-virus families *Podoviridae* and *Myoviridae* - hereafter named as phages and NCDLV giant viruses - hereafter named as giruses) to pico-eukaryotes. Phage libraries were constructed from seawater samples filtered at 0.22 µm, concentrated using iron chloride flocculation, and treated with deoxyribonuclease (DNase). Girus *polB* and prokaryotic 16S rDNA sequences were extracted from plankton metagenomes sequenced from 0.22–1.6 or 0.22-3 µm filters, and the pico-eukaryote dataset was obtained by V9-18S rDNA marker amplification from 0.8-3 or 0.8-5 µm filters. Details of sample preparation and sequencing procedures are described in Alberti et al. [40].

Phage relative abundance was accessed through the search for the marker genes *gp23* (*Myoviridae*) and *polA* (*Podoviridae*) in the protein collection GOV2.0 derived from metagenomic sequencing described in Gregory et al. [8]. The girus abundance profile was obtained from *polB* marker gene gathered from the OM-RGC.v2 catalog [11] as described in Endo et al. [9]. The Prokaryote 16S rDNA marker derived from the metagenome assembly, named 16S Mitag, is described in Sunagawa et al. [10]. Sequences matching “Eukaryota”, “chloroplasts”, and “mitochondria” were removed from the final table. Clustering and annotation of pico-eukaryote V9-18S rDNA amplicons are described in de Vargas et al. [41], and functional annotation of taxonomically assigned V9-18S rDNA metabarcodes was improved afterward; in this case, we conserved in the final data only sequences assigned to the “Eukaryota” domain.

We concatenated SRF and DCM samples for each taxonomic group to obtain an epipelagic dataset (EPI). Counts of OTUs shared in SRF and DCM samples were summed. OTU abundance was normalized by the total counts for each taxonomic group within each sample.

- **Ecological Analysis**

Epipelagic and Mesopelagic Community and Environmental differences

We applied an NMDS analysis based on the Bray-Curtis dissimilarity matrix on relative abundances using the 'metaNMDS' function from the vegan R package [42] to confirm community differences between epipelagic and mesopelagic layers. Homogeneity of the sampled environmental parameters was checked using the 'betadisper' function (Homogeneity of multivariate dispersions in the vegan package). The analysis was conducted using the Euclidean distance matrix of the environmental variables using the depths (epipelagic, mesopelagic) as group factor. A permutation test statistically confirmed the results.

Ecological model selection

We used the Species Abundance (SAD) and Rank Abundance Distribution curves (RAD: log abundances vs. rank order) to fit some of the most popular ecological models assessing how communities are assembled [43, 44]. Niche/deterministic models presume that the community is under selection due to biotic and abiotic interactions, while neutral/stochastic models assume that random processes structure the community such as drift, migration, birth, and death [45, 46]. SAD is essential for describing and understanding community assemblage and its management [44]. Further, by showing logarithmic species abundance against a rank order, RAD is commonly used to investigate a community's structure from observations made at one point in space and time [43, 44]. All the abundance distributions were fitted using 'fitsad' (sads R package - [47]), and 'radfit' (vegan) functions using maximum likelihood estimation. A set of candidate models was selected *a priori* to be tested by SAD: Log-series, Poisson-lognormal, Broken Stick, Power Law, and the neutral ecological model Zero-sum multinomial distribution. The neutral ecological model describes the SAD of a sample taken from a neutral metacommunity under random drift. The models selected to be tested by RAD were niche pre-emption models (geometric series or Motomura model), lognormal, Zipf, and Zipf–Mandelbrot,

and the null model that infers that the individuals are randomly distributed among observed species. For a complete description of each model see Magurran and McGill [48].

The Akaike's Information Criterion (AIC) was used to evaluate the fitted model's quality based on log-likelihood penalized by the number of estimated parameters. AIC estimates the loss of information if the model is assumed for a given dataset. In this manner, models with lower AIC values are selected as better fit conditions [49]. The AIC values produced for each model were compared using the delta AIC (Δ_i). A Δ_i value <2 indicates equally likely models, values 3 <Δ_i <9 indicate less likely models, and Δ_i > 10 for no likely models [49].

Ecological inferences and statistics

Ecological patterns were inferred using environmental variables to constrain the variation observed in biological data for planktonic samples using Canonical Correspondence Analysis (CCA) in the vegan R package. A set of physico-chemical variables for the discrete depths were selected for the ecological inferences, such as nitrate (NO_3^-), oxygen, temperature, salinity, density, and particles using particle flux UVP data. In order to avoid collinearity among factors, the selected variables were checked for variance inflation factor using the vif.cca function and tested for significance by 'anova' implemented in vegan with 999 permutations. Each variable effect significance was tested individually using all the others as covariables (independently from their order in the model) by applying the option 'margin' to the 'anova' function in vegan.

Permutational multivariate analysis of variance (PERMANOVA) was performed with the function 'adonis' in vegan to determine the relationship between mesopelagic community composition and predefined water masses based on 999 permutations.

Organism Eco-region classification

In order to detect organisms specific to epipelagic (EPI), oxic mesopelagic (Oxic MES), and OMZ eco-regions, only data containing both epipelagic and mesopelagic information were

considered: in total, 25 stations for giruses, prokaryotes, and pico-eukaryotes, and 13 for phages. First, we discarded OTUs with fewer than 100 reads to remove biases due to rare species' presence, and then recalculated the relative abundances for each dataset. Next, we ran a Kruskal-Wallis test ('kruskal.test' from stats R package [50]) to detect differential OTU relative abundances between eco-regions, followed by a Bonferroni correction to avoid Type I error. Organisms with a p-value <0.05, indicating a difference within groups, were subject to a post-hoc Dunn test ('dunn.test' from dunn.test R package [51]) to identify preferential Eco-regions for each OTU. From these results, OTUs statistically equally abundant in all Eco-regions or non-significant Kruskal-Wallis tests were assigned to the "ubiquitous" group. In contrast, those with significant p-values were classified as EPI, Oxic MES, or OMZ if only the corresponding Eco-region was elected according to the Dunn test. Organisms with no significant differences between Oxic MES and OMZ were assigned to Core MES.

Co-occurrence Network

For investigation of associations between organisms across Eco-regions, a co-occurrence network was inferred. In this analysis, phage samples were not included due to the lower number of stations sampled. Therefore, samples for giruses, prokaryotes and pico-eukaryotes from stations 038, 039, 068, 070, 072, 076, 078, 098, 100, 102, 109, 110, 111, 112, 122, 132, 133, 137, 138, 142, 145, 146, 148, 149 and 152 were retained. OTUs with a relative abundance $<10^{-4}$ and counting fewer than 5 observations were discarded. Next, each sample was normalized by applying a centered-log-ratio (CLR) transformation. Network inferences were performed using Flashweave version 0.18 developed in Julia version 1.2 [52], using the sensitive and heterogeneous mode.

We analyzed this global co-occurrence network by delineating communities (or modules) using the Clauset-Newman-Moore algorithm [53]. These modules are subsets of OTUs, obtained by maximizing the co-occurrences within the module and minimizing connections between them. Next, we investigated modules enriched in OTUs from specific Eco-regions using Fisher's exact test using the "fisher.test" function from the stats R package.

Results and Discussion

Leveraging the resources produced by the *Tara* Oceans project, we deciphered differences between epipelagic and mesopelagic beta-diversity stratification, with a particular emphasis on the role of environmental variables such as temperature, oxygen, salinity, NO_3^-, chlorophyll-a, and particle flux (see Methods). As previously reported, we observed a stratification by depth of the epipelagic and the mesopelagic communities (i.e., phages, giruses, prokaryotes, and pico-eukaryotes) (Supplementary Figure 1). Consequently, we investigated differences among epipelagic and mesopelagic sampling sites based on Euclidean distance of physicochemical characteristics from each site. We observed a high dissimilarity gradient among sites for both layers (Supplementary Figure 2a, b). Mesopelagic samples were spread in the plot, with most of the points placed distant from the group centroid (located in the center of the cloud of points identified for each group) (Supplementary Figure 2a). In contrast, epipelagic points displayed a large variance due to a few samples positioned apart from the main cluster (Supplementary Figure 2a). These results probably underlie the heterogeneity of environmental conditions encountered in both sampled layers, and this environmental variation may be an important factor that can directly influence community composition.

Community composition variations can also be shaped by four main eco-evolutionary processes: selection, dispersal, drift, and speciation [54]. Mathematical models based on these processes and applied to species abundance (SAD) and rank abundance (RAD) distributions are historically used to infer the ecological or evolutionary mechanisms that structure a given community [55]. Here, we addressed these biological processes to infer the abundance distributions as observed in such natural communities [56, 57] in the most diverse environments (terrestrial and aquatic) [45, 46, 43]. We used both SAD and RAD as evidence of ecological processes related to variations in plankton community composition. When applied at the local scale, we excluded random/neutral evolutive effects because all epipelagic and

mesopelagic communities fit the niche/deterministic ecological models. In fact, in all cases, we found a Δ_i value <2 for both methods evaluated, which allows us to select one best-fitting model without a subjective judgment (Supplementary Material Table 1 and 2) [49]. In these cases, the community assemblages are mediated by a combination of environmental conditions, interspecies interactions (competition, predation, or mutualisms), and species traits (for instance, phototrophy, parasitism). Specifically, 93% and 65% of epipelagic and mesopelagic samples better fit the Lognormal model (Poisson Lognormal SAD [58] and Lognormal RAD [59]), respectively). These results suggest that EPI and MESO plankton populations are affected by the combination of many independent variables, including competitive biotic interactions and abiotic factors [43]. According to this model, the community has a broad and elementary form of organization [57]. Ser-Giacomi et al. [60] stated that the eukaryotic rare-biosphere (non-dominant OTU's) composed of "transient" or occasional taxa in the ocean sunlit layer are mainly governed by dispersal/neutral events. In contrast, and in the case of our findings, the dominant fraction of the assemblage follows idiosyncratic environmental conditions.

Next, to quantify how much of the differences in the assemblages' variance can be explained by environmental conditions, we employed canonical correspondence analysis (CCA) using the environmental variables measured at discrete depths as constraint variables. The results showed that the environment explains only a small fraction of community variance for both layers (32% on average) (Figure 2). The phage assemblage was the exception, for which about 55% of the epipelagic variation and 65% of the mesopelagic variation could be explained by the variables investigated (Figure 2). We tested the variance explained by single explanatory variables individually. In contrast with epipelagic communities mainly governed by temperature and oxygen, as observed here and elsewhere [10, 61, 8, 13, 12, 62], we could not identify a single environmental predictor structuring entire mesopelagic assemblages. However, a few different variables appeared to be significant for each group (Table 1, complete analysis in Supplementary Material Table 3). Notably, our analysis identified oxygen as the main

mesopelagic driver for phage and prokaryote assemblages, confirming previous reports [20, 63, 64].

Even though we observed in the ordination-plots the distinction of OMZ and oxic mesopelagic (Oxic MES) stations for giruses and pico-eukaryote assemblages (Figure 2, diamond and down triangle), we could not disentangle the effect of oxygen from the other variables included in the analyses (Table 1, complete analysis in Supplementary Material Table 3). This result shows that these assemblages are probably affected by a combination of the predictors evaluated, reflecting their need to cope with a broader environmental gradient that maximizes their niche-space partitioning. Previous studies have identified oxygen as one of the main drivers of the eukaryotic community structure in OMZ regions [65, 22, 23]. These studies mainly compared community composition along the oxygen gradient within the water column depth, from the surface downwards. However, depth stratification of plankton communities is evident even in regions with high oxygen concentrations, so distinct parameters co-varying with depth must be taken into account in addition to the oxygen gradients [66].

In addition to the physicochemical parameters, our results show that particle flux derived from UVP measurements was also a significant variable structuring the phage assemblages in both epipelagic and mesopelagic layers (Table 1, complete analysis in Supplementary Material Table 3). This data supports previous reports about the high correlation of this environmental factor with phages, finding possible relevance for the carbon pump's functioning in epipelagic layers [67]. This observation may also reflect the association with virus inputs from overlaying water layers via sinking particles [68].

In situ physico-chemical measurements have revealed the dynamics and fluctuating nature of the ocean, even at short time scale [69]. The heterogeneity in mesopelagic layers given by deep currents, and by the impact of the surface production, together with the low mixing levels, may favor a diversification in the mesopelagic community living in different water masses, leading to species adaptation-acclimation. The *Tara* Oceans expedition route included

347 samples from common or distinct water masses defined by temperature/salinity profiles - T/S,
348 comprising regionally connected or unconnected stations. We identified nine different water
349 masses in the mesopelagic sampled locations (Figure 3). We could confirm significant
350 differences in mesopelagic communities sampled in different water masses based on the
351 PERMANOVA test (Table 2). This result indicates that the oceanic patchiness created by
352 distinct water masses can favor beta-diversity diversification, indicating it to be a critical
353 component for mesopelagic community variation for all the assemblages studied (phages,
354 giruses, prokaryotes, and pico-eukaryotes). Thus, we hypothesize that this result may be
355 explained by two non-exclusive causes related to water masses: (i) past common origin among
356 water masses that have drifted or (ii) constant connectivity by ocean circulation between
357 sampled sites belonging to the same water mass.

358 Following the biotic control suggested by the deterministic model previously identified, we
359 addressed another lingering question, resolving planktonic community signatures of Oxic MES
360 and OMZ regions for epipelagic communities. For this, we classified OTUs based on their
361 relative abundance into three eco-regions: 1) EPI, 2) Oxic MES, and 3) OMZ. OTUs were
362 classified as Core MES when commonly present in Oxic MES and OMZ samples. The taxa
363 that were either equally abundant in all three eco-regions or not statistically confirmed to a
364 single eco-region were classified as ubiquitous (Supplementary Figure 3). Using this approach,
365 we could identify ubiquitous taxa, that are likely to thrive in a wide range of environmental
366 conditions, or that may be detected in mesopelagic samples due to the simple vertical
367 movement of sinking particles. This classification should help avoid putative biases inherent of
368 the metabarcoding methodology.

369 More specifically, we were able to identify Oxic MES and OMZ signatures mainly at the infra-
370 taxonomic level (OTU-species) for all biotic groups investigated (Figure 4, Supplementary
371 Figure 3, 4, 5, 6, 7). This reflected the wide ecological niche occupied by the different species
372 at a higher taxonomic level (i.e. family). At the species level, we observed large taxonomic
373 plasticity of OTUs that occurred equally in both Oxic MES and OMZ samples, called Core MES,

principally for girus assemblages. However, most OTUs are not yet classified at the infra-taxonomic level (data not shown). This observation reflects the knowledge gap about the biodiversity and functional plasticity of species thriving in this ecosystem.

The great majority of phage taxa occurred at similar abundance in all regions (ubiquitous) (Supplementary Figure 3, 4). Surprisingly, this ubiquity is vertically linked at each independent station (Supplementary Figure 4), supporting the seed-bank hypothesis raised by Brum et al. [60], and the correlation to the sinking particles observed here. We observed taxa specific to the mesopelagic layer in second place, mostly related to the OMZ eco-region (Figure 4a, Supplementary Figures 3, 8). This mesopelagic specificity agrees with the sharp increase in marine phage microdiversity following depth, as previously shown by Gregory et al. [8]. Our results emphasize that one cause for phage stratification in the water column might be the adaptation to the mesopelagic environment. Two hypotheses arise here, 1) the environment acts as a strong driver, directly selecting phages independently of their hosts, and 2) there is higher phage-host specificity in the mesopelagic layer, promoting phage selection. Following the first hypothesis, we can posit that the environment can directly impact phage assemblage composition. The direct contact with the environment of free phage particles (released from their hosts) may reduce infectivity, degrade, or remove virus particles, and adversely affect adsorption to the host [70]. This direct environmental effect over marine phages was reported for different ionic gradients [71], daylight conditions, and temperature [72]. However, the enrichment of prokaryotic OTUs specific to mesopelagic regions (Supplementary Figures 3, 8), especially in OMZs, does not exclude the phage-host indirect selection relationship.

We found fewer but abundant mesopelagic-specific girus OTUs in both Oxic and OMZ eco-regions, indicating that giruses can be less diverse in the mesopelagic layer (Figure 4b, Supplementary Figures 5, 3, 8). Also, giruses can encode genes such as transporters for ammonium, magnesium, and phosphate that are important in marine oligotrophic areas [73]. This characteristic can improve the host's fitness in the short-term but ultimately favor girus fecundity and endurance. This property is named NCLDV-mediated host reprogramming [73].

The great majority of mesopelagic girus OTUs were assigned to the Core MES group, indicating that these entities may infect a wide range of hosts adapted to diverse environmental conditions.

We could better distinguish the prokaryotic mesopelagic signatures between Oxic MES and OMZ, confirming the influence of oxygen reported here and in previous studies [18, 19, 20] (Figure 4c, Supplementary Figures 6, 3, 8). Among the planktonic microorganisms, prokaryotes have been, so far, the most investigated group in OMZ regions, especially in the Pacific Ocean [60, 19, 18]. We observed similar occurrence and abundance for the OMZ signature taxa in the Indian Ocean stations (IO - 037, 038, 039) and in stations PO - 100, 137, and 138 from the Pacific Ocean (Figure 4c). These Pacific stations are located in the open ocean (PO - 137 and 138 located in the Equatorial upwelling zone and station PO - 100 in the South Pacific Subtropical Gyre). They present a strong upwelling signature, disclosing an intense decrease in oxygen concentration almost reaching shallow waters. Likewise, the sampling stations in the Indian Ocean are located in well-stratified waters, markedly characterized by the abrupt decrease of oxygen concentration below the thermohaline at 100-120m depth. In all these stations, the oxygen concentration ranges from 0.83 to 3 µmol/kg, characterizing functionally anoxic waters since this oxygen level cannot sustain aerobic metabolisms [74]. The other OMZ stations in the Pacific Ocean (PO - 102, 109, 110, 111) are located in coastal areas. Although they are also under the influence of upwellings presenting an oxygen depletion, the oxygen level does not achieve anoxic conditions, and thus are classified as suboxic waters. This microoxic environment is enough to completely change the microbial metabolism delineating the community composition in those sites. Also, differences in offshore and coastal upwelling formation, for instance, or the influence of river runoffs, transporting anthropogenic nutrient enrichment from the continent to coastal zones [74], could be crucial to support the differences in OMZ communities we observed.

The same combination of OMZ anoxic and suboxic samples was observed for the pico-eukaryotic groups MALV-II and Diplonemida, suggesting these OTUs as the true OMZ

428 eukaryotic signatures (Figure 4d). Some OTUs of these groups exhibited similar occurrences
429 in the anoxic Indian and Pacific Oceans but not in suboxic samples from the Pacific Ocean.
430 However, we observed a lower number of pico-eukaryotic taxa in the OMZ eco-region, the
431 prevailing OTUs being specific to Oxic MES locations in most cases. Although the CCA
432 analysis did not disentangle the oxygen from the other variables to explain the pico-eukaryote
433 assemblage variations, here we can verify that OMZ conditions do act negatively on selection
434 of pico-eukaryotes in marine environments.

435 Another step to better understand mesopelagic community dynamics is to dissect the
436 ecological relationships among species that thrive in this layer. Co-occurrence networks can
437 indicate how the environment may structure the community acting as a filter for resident
438 species [76]. They can also give us glimpses of organisms' ecological interactions based on
439 species connectivity [76, 77]. Combining the girus, prokaryote, and pico-eukaryote data, we
440 inferred a network containing 6,154 nodes and 12,935 edges (Figure 5a, Table 3). Due to the
441 lower number of stations sampled for phages, we excluded this group from the analysis. We
442 found mainly positive relationships (94%), suggesting a predominance of putative biotic
443 interactions (e.g. competition, symbiosis) rather than taxa avoidance or exclusion. This
444 dominance of positive relations was also reported for epipelagic plankton communities [27,78].
445 The global network had a modularity value greater than 0.4 (Table 3), indicating that the
446 network has a modular structure [74]. Using a module detection algorithm, we were able to
447 identify 36 distinct modules in the global network. Three of them were mainly composed of
448 OTUs significantly enriched in mesopelagic OTUs (Oxic MES enriched module 1 and OMZ
449 enriched modules 4 and 17; Figure 5). Together, these three modules cover almost the total
450 richness found in the mesopelagic zone (Figure 5b), and present similar values for the average
451 degree, clustering coefficient, and average path length (Table 3). These parameters indicate
452 a network complexity [76], hinting at distinct ecological niches within the mesopelagic at the
453 level of investigated organismal fractions.

In more detail, the OMZ signature modules were composed of a few connected nodes (323 and 175 nodes for modules 4 and 17, respectively), potentially indicating two distinct OMZ community niches. The Oxic MES module 1 counted more nodes composing the network associations (731 nodes), and both modules presented a variation in taxonomic composition and proportions. OMZ module 4 contained mainly prokaryotic (23%) and girus (55%) OTUs (Figure 5c, d). Among the prokaryotes, we detected taxa previously determined as OMZ signatures (Nitrospinae, Marinimicrobia SAR 406, and Planctomycetes). Module 17 is mainly comprised of pico-eukaryotes (82%), notably MALV-II (14%) and Diplonemida (17%) previously indicated as OMZ signatures. Module 1 is taxonomically more diverse but is mainly comprised of giruses and pico-eukaryotes. These groups accounted for 36% and 55%, respectively, of OTUs in this module (Figure 5c, d). Giruses contributed to 598 associations (edges) in mesopelagic module 1, of which 177 occurred between giruses and pico-eukaryotes. Giruses from the *Mimiviridae* family are the most numerous taxa in all three mesopelagic modules. *Mimiviridae* is a very abundant family in the ocean, present in various size ranges from piconanoplankton (0.8-5 µm) up to mesoplankton (180- 2,000 µm) [9, 79]. This observation supports our findings that giruses are a prosperous group in mesopelagic waters, undertaking different strategies to endure in such environmental conditions. In all three modules, we observed the presence of Foraminifera, of which some species can use nitrate over oxygen as an electron acceptor, favoring their survival in OMZ regions [80].

Sugihara [57] affirms that a hierarchical niche structure can explain the lognormal abundance pattern in communities, and this assertion is valid for small assemblages and for large ensembles. Consistently, the observation of three distinct community modules supports the evidence of the lognormal ecological model empirically defined locally for each assemblage. Our results converge and suggest that the mesopelagic presents at least three well-defined ecological niches (Oxic MES, OMZ-4 and OMZ-17), with established conditions and resources (abiotic and biotic) that allow the survival of a given species in these environments. Differences between OMZ and Oxic MES networks suggest a potential loss of connections and interactions

among mesopelagic community members, directly affecting ecosystem stability due to habitat change.

Conclusions

In this study, we explored mesopelagic pico-plankton ecological structuring, and concluded that this component of oceanic plankton is heterogeneous regarding its environmental parameters. The ecological parameters drive mesopelagic community assemblages, as they fit niche ecological models. Although we could not identify a single driver of community composition (such as temperature for sunlit ocean) for all organisms, we could pinpoint the relevance of oxygen for phages and prokaryotic fractions and the relation of the former with particle flux. Also, we show that water masses defined by their T/S profiles can explain the differences in the observed pico-plankton structure, pointing to the role of a set of environmental parameters rather than single drivers for community composition.

By establishing Eco-regions (Epipelagic, Oxic MES, and OMZ), we were able to discriminate specific OTUs for all fractions studied. While we recovered known markers for Oxic MES and OMZ regions at high taxonomic levels, we also found that most of these OTU signatures are observed at low taxonomic levels, which sometimes cannot be resolved using current databases. Crossing these specific OTUs with co-occurrence networks, we identified three niches with biotic and abiotic conditions that characterize mesopelagic waters.

The limiting access to data is usually the bottleneck for knowledge about mesopelagic dynamics. Our study benefits from a more significant number of organism samples and distinct oceanic provinces than previous ones, allowing us to combine data to derive an expanded vision of mesopelagic composition. Our results emphasize the need for better understanding of mesopelagic life, in particular by improving our knowledge about oxic and oxygen-depleted mesopelagic-dwelling communities, especially as climate change can be expected to expand marine OMZs shortly.

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543 **Conflict of Interest**
544
545 The authors declare that they have no conflict of interest.

546

547 **References**
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791

792 **Figure legends**

793 **Figure 1**: Geographical locations of *Tara* Oceans epipelagic and mesopelagic sampling sites
794 included in this study. Symbols: ▲ refers to epipelagic, ▼ Oxic MES and ♦ OMZ eco-regions.
795 Symbol colors represent organism groups evaluated in the present study.

796 **Figure 2**: Ordination plot of epipelagic (left) and mesopelagic (right) communities based on
797 OTU composition based on canonical correspondence analysis (CCA). Percentages in
798 parentheses are the amount of variation constrained, in titles represent the total in each
799 analysis, and in the axis the correspondent value for each dimension. Arrows represent
800 environmental quantitative explanatory variables with arrowheads indicating their direction of
801 increase. Shapes represent sampling sites. Shape formats represent eco-regions, epi:
802 epipelagic, Oxic MES: oxic mesopelagic, OMZ: oxygen minimum zone mesopelagic. IO: Indian
803 Ocean, NAO: North Atlantic Ocean, NPO: North Pacific Ocean, SAO: South Atlantic Ocean,
804 SPO: South Pacific Ocean.

805 **Figure 3**: Temperature and salinity plot indicating water mass designation for all mesopelagic
806 samples. Formats represent the different oceanic basins (■ - North Atlantic Ocean, ● - South
807 Atlantic Ocean, ▲- Pacific Ocean, ★- Indian Ocean). Colors indicate the oxygen concentration
808 at the sampling depth. LSW - Labrador Sea Water; AAIW - Antarctic Intermediate Water;
809 tNPIW transitional North Pacific Intermediate Water; SAMW - Subantarctic Mode Water;
810 SPSTMW - South Pacific Subtropical Mode Water; modAAIW - modified Antarctic Intermediate
811 Water; PGW - Persian Gulf Water mass; RSW - Red Sea Water mass; NASTMW - North
812 Atlantic Subtropical Mode Water.

Figure 4: Relative abundance of OTUs assigned to mesopelagic eco-regions. A) Phages, B) Giruses, C) Prokaryotes and D) pico-Eukaryotes.

Figure 5: Co-occurrence network in epipelagic and mesopelagic communities. A) Global network, with connected modules for OMZ (purple and orange) and MES (green) highlighted. B) Relative taxa abundance in each module in each station and depth. C) Relative number of OTUs classified in taxonomic groups. D) Network representation of modules.

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Supplementary Figure 3: Relative abundance of OTUs classified into different eco-regions by ocean layers.

Supplementary Figure 4: Normalized relative abundance of phages and their preferred eco-region.

Supplementary Figure 5: Normalized relative abundance of giruses and their preferred eco-region.

Supplementary Figure 6: Normalized relative abundance of prokaryotes and their preferred eco-region.

Supplementary Figure 7: Normalized relative abundance of pico-eukaryotes and their preferred eco-region.

Supplementary Figure 8: Relative abundance of OTUs from taxonomic groups for epipelagic and mesopelagic (Oxic MES and OMZ) samples enriched in each eco-region (UBI: ubiquitous, EPI: epipelagic, Core MES: core mesopelagic, Oxic MES: oxic mesopelagic, OMZ: oxygen minimum zone. A) Phages B) Giruses C) Prokaryotes D) pico-eukaryotes.

Figures:

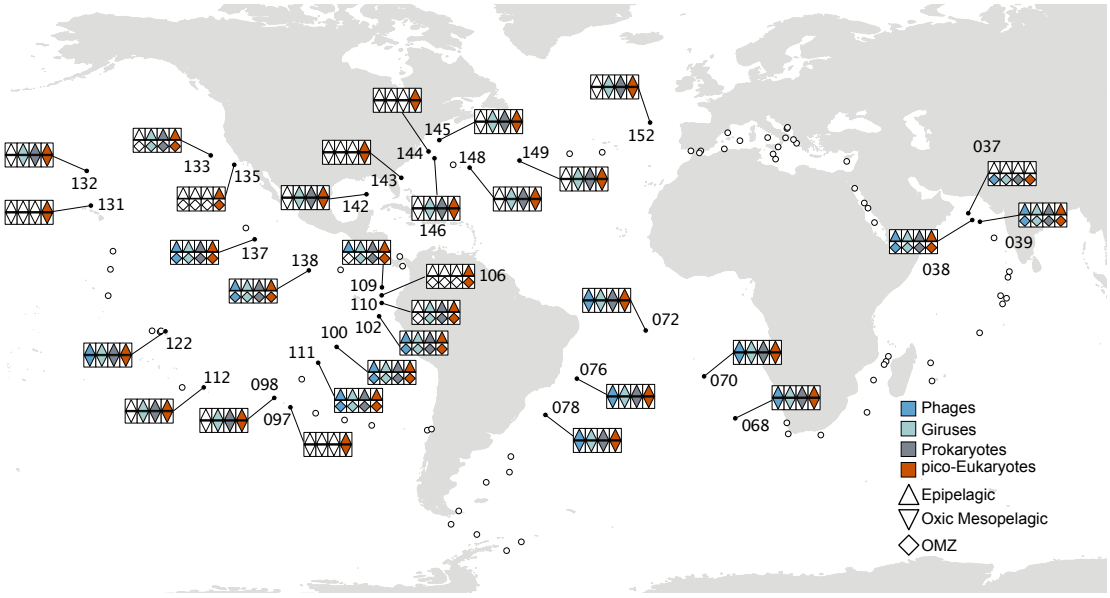


Figure 1: Geographical locations of Tara Oceans epipelagic and mesopelagic sampling sites included in this study. Symbols up-triangle refers to epipelagic, down-triangles Oxic MES and diamond OMZ eco-regions. Symbols colors represent organism groups evaluated in the present study

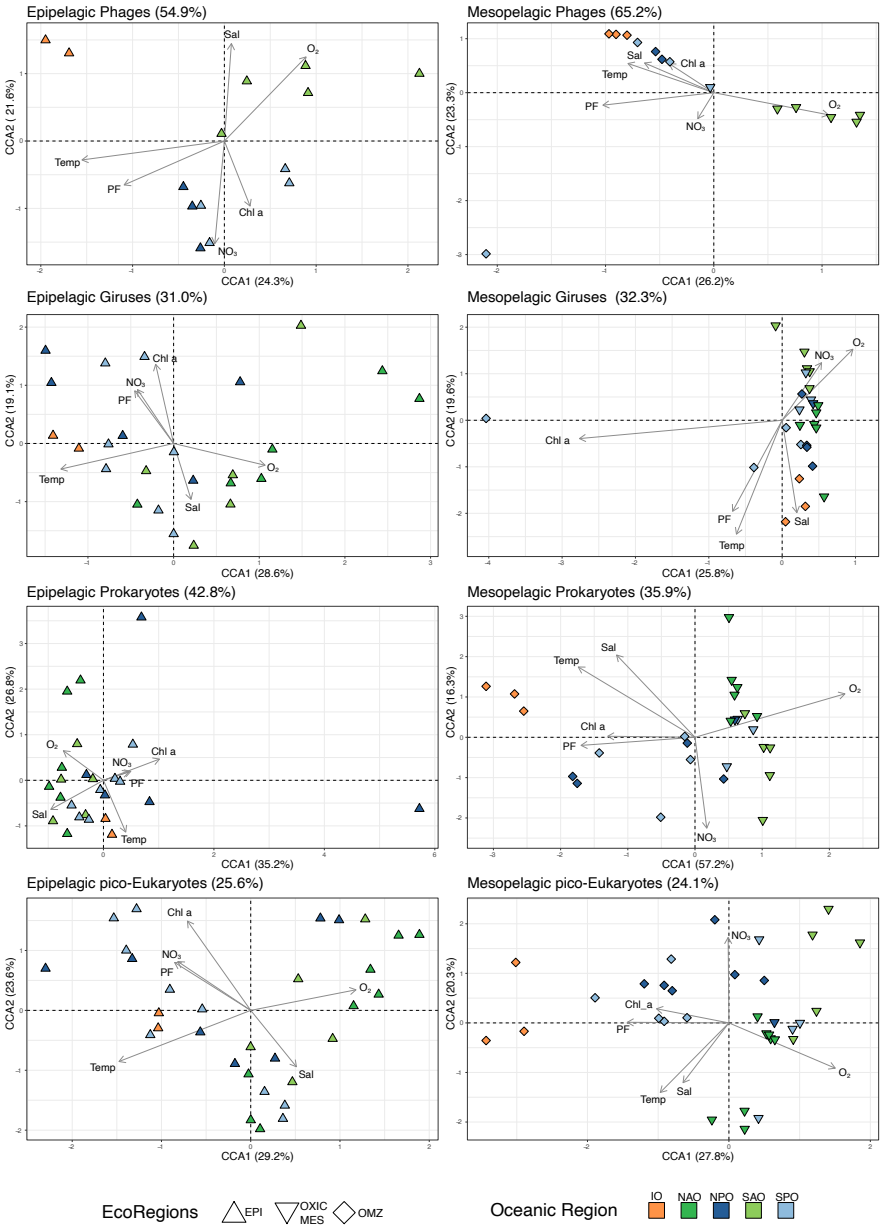


Figure 2: Ordination plot of epipelagic (left) and mesopelagic (right) communities based on OTU's composition based on canonical correspondence analysis (CCA). Percentages in parenthesis are the amount of variation constrained, in titles represents the total in each analysis, and in the axis the correspondent value for each dimension. Arrows represent environmental quantitative explanatory variables with arrowheads indicating their direction of increase. Shapes represent sampling sites. Shape formats represent eco-regions, epi: epipelagic, Oxic_mes: oxic mesopelagic, OMZ: OMZ mesopelagic. IO: Indian Ocean, NAO: North Atlantic Ocean, NPO: North Pacific Ocean, SAO: South Atlantic Ocean, SPO: South Pacific Ocean

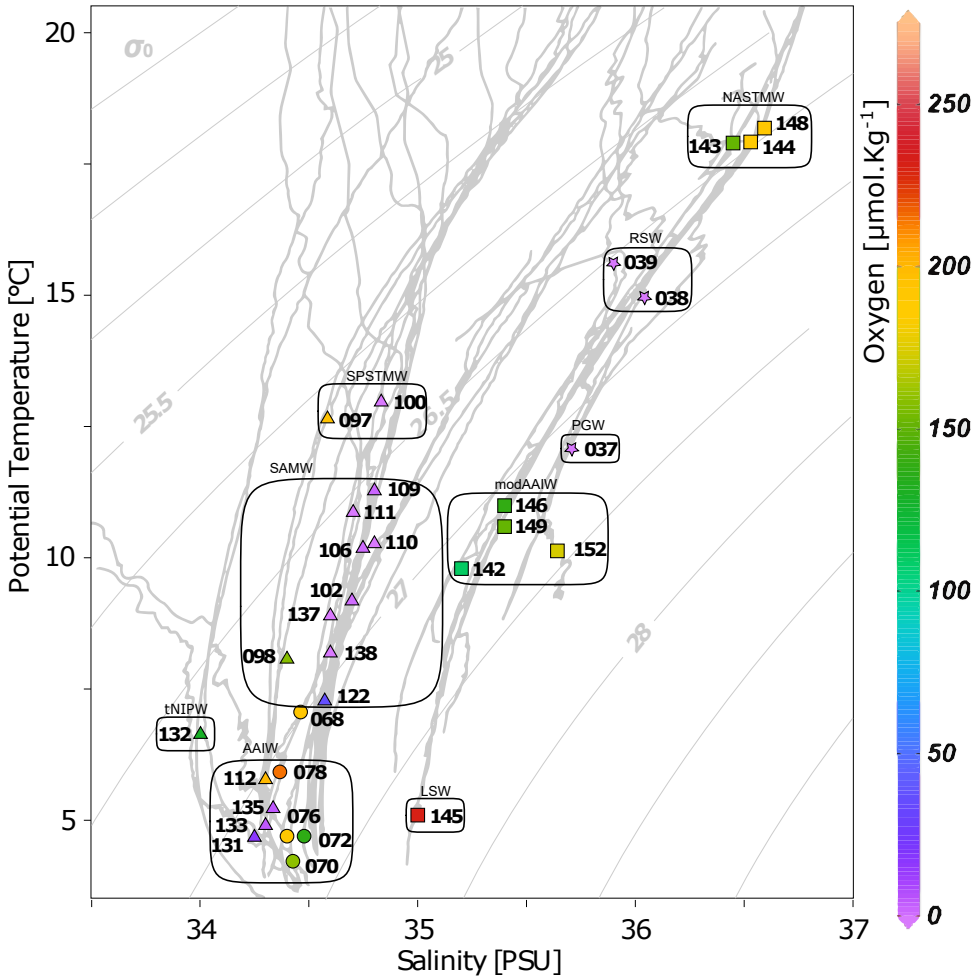


Figure 3: Temperature and salinity plot indicating water masses designation for all mesopelagic samples. Formats represent the different oceanic basins (□- North Atlantic Ocean, ◯ - South Atlantic Ocean, △ - Pacific Ocean, ★ - Indian Ocean). Colours indicate the oxygen concentration at the sampling depth. LSW - Labrador Sea Water; AAIW - Antarctic Intermediate Water; tNPIW ? transitional North Pacific Intermediate Water; SAMW - Subantarctic Mode Water; SPSTMW - South Pacific Subtropical Mode Water; modAAIW - modified Antarctic Intermediate Water; PGW - Persian Gulf Water mass; RSW - Red Sea Water mass; NASTMW - North Atlantic Subtropical Mode Water.

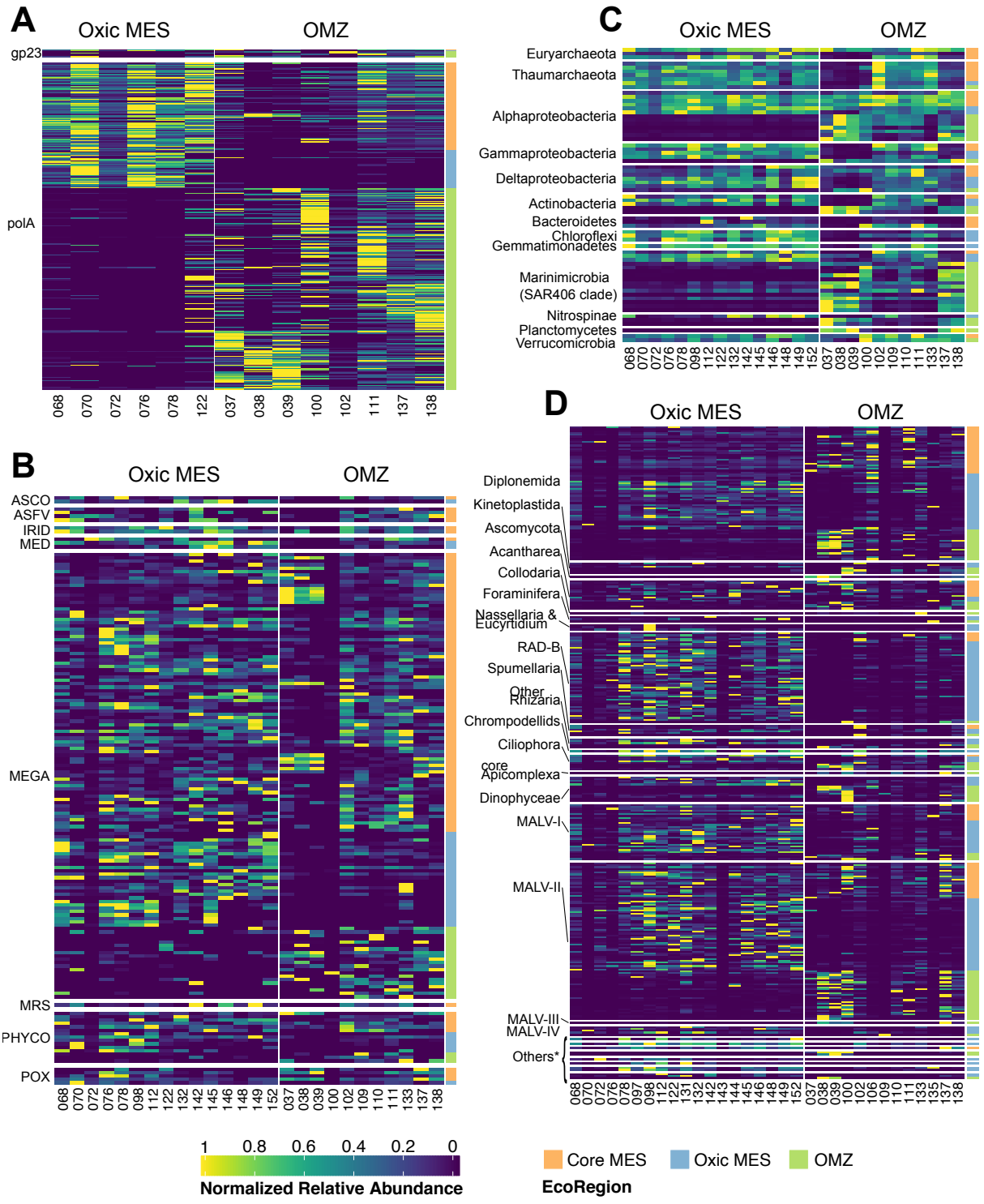


Figure 4: Relative abundance of OTUs assigned to Mesopelagic Eco-regions. A) Phages, B) Giruses, C) Prokaryotes and D) Eukaryotes

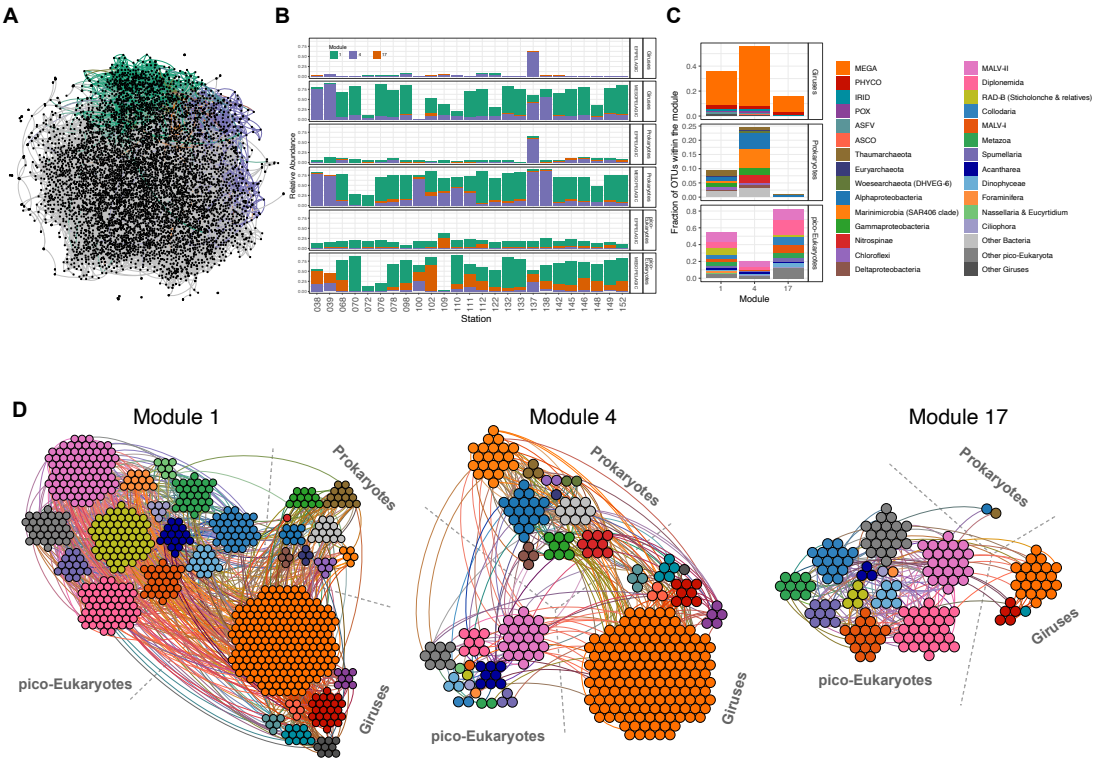


Figure 5: Co-Ocurrence Network in Epipelagic and Mesopelagic communities. A) Total Network, with connected modules for OMZ (purple and orange) and MES (green) highlighted. B) Relative taxa abundance in each Module in each station and depth. C) Relative number of OTUs classified in taxonomic groups. D) Network representation of modules

Tables

Table 1: ANOVA p_value for each environmental factor used as explanatory variable. Global correspond to the p-value for the whole set of variables, the following columns to p-values for each of the environmental variables considering the others as covariable.

assemblage	Depth	Global	Temp. °C	Salinity	O_2 [μmol/kg]	$[NO3]^-$ [μmol/l]	Chl-a [mg/m^3]	Particle flux
Phages	EPI	0.001	0.057	0.144	0.043	0.031	0.420	0.017
Pahges	MES	0.001	0.059	0.004	0.027	0.124	0.110	0.002
Giruses	EPI	0.001	0.023	0.074	0.051	0.152	0.053	0.221
Giruses	MES	0.002	0.032	0.211	0.145	0.134	0.008	0.211
Prokaryotes	EPI	0.035	0.048	0.141	0.002	0.006	0.044	0.568
Prokaryotes	MES	0.006	0.501	0.304	0.039	0.444	0.966	0.486
pico-Eukaryotes	EPI	0.025	0.027	0.166	0.412	0.292	0.216	0.659
pico-Eukaryotes	MES	0.001	0.606	0.191	0.243	0.468	0.271	0.477

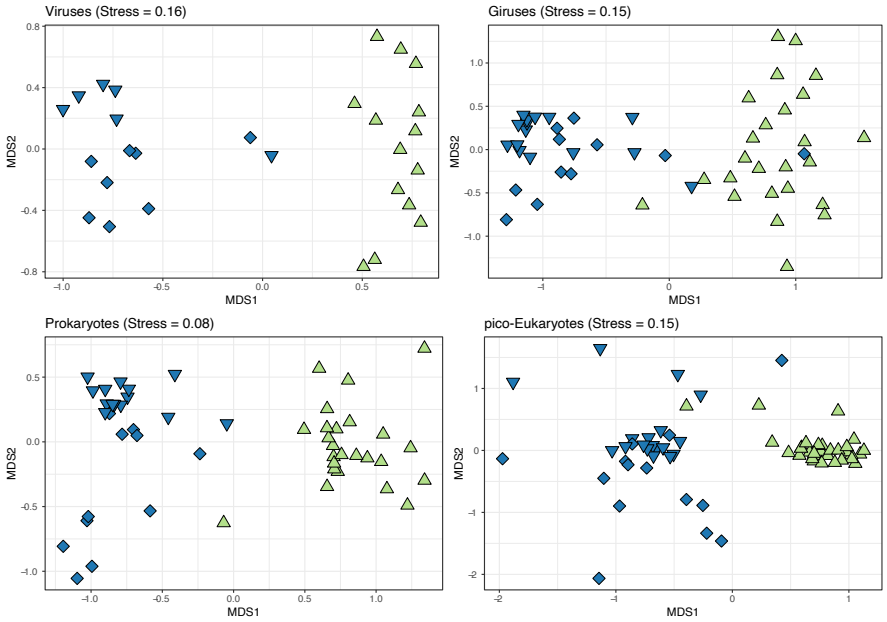
Table 2: Proportion of the variation in community composition that explained by water masses using the Permutation multivariated analysis of variance (PERMANOVA)

assemblage	Df	Sum Of Squares	Mean Squares	F. Model	R^2	Pr(¿F)
Phages	4	1.87	0.47	2.32	0.51	0.002
Giruses	8	3.19	0.40	1.81	0.46	0.001
Prokaryotes	8	1.30	0.16	3.29	0.60	0.001
pico-Eukaryotes	8	2.60	0.32	1.62	0.36	0.001

Table 3: Network topological features derived from global analysis including giruses, prokaryotes and pico-eukaryotes samples in epipelagic and mesopelagic depths

Name	Global	Mod 1	Mod 4	Mod 17
Nodes	6154	731	323	175
Positive Edges	12193	1236	480	223
Negative Edges	742	70	49	9
Avg. degree	4.20	3.57	3.28	2.65
Clustering	0.03	0.03	0.09	0.05
Density	0.00	0.00	0.01	0.02
Average.path.length	7.28	6.01	6.27	6.30
Betweenness	0.01	0.05	0.10	0.22
Degree Centralization	0.00	0.01	0.02	0.04
Modularity	0.47	0.60	0.67	0.66

Supplementary Figures:



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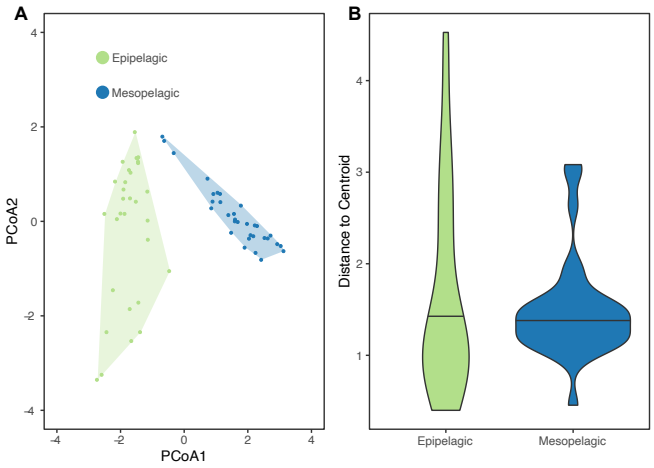

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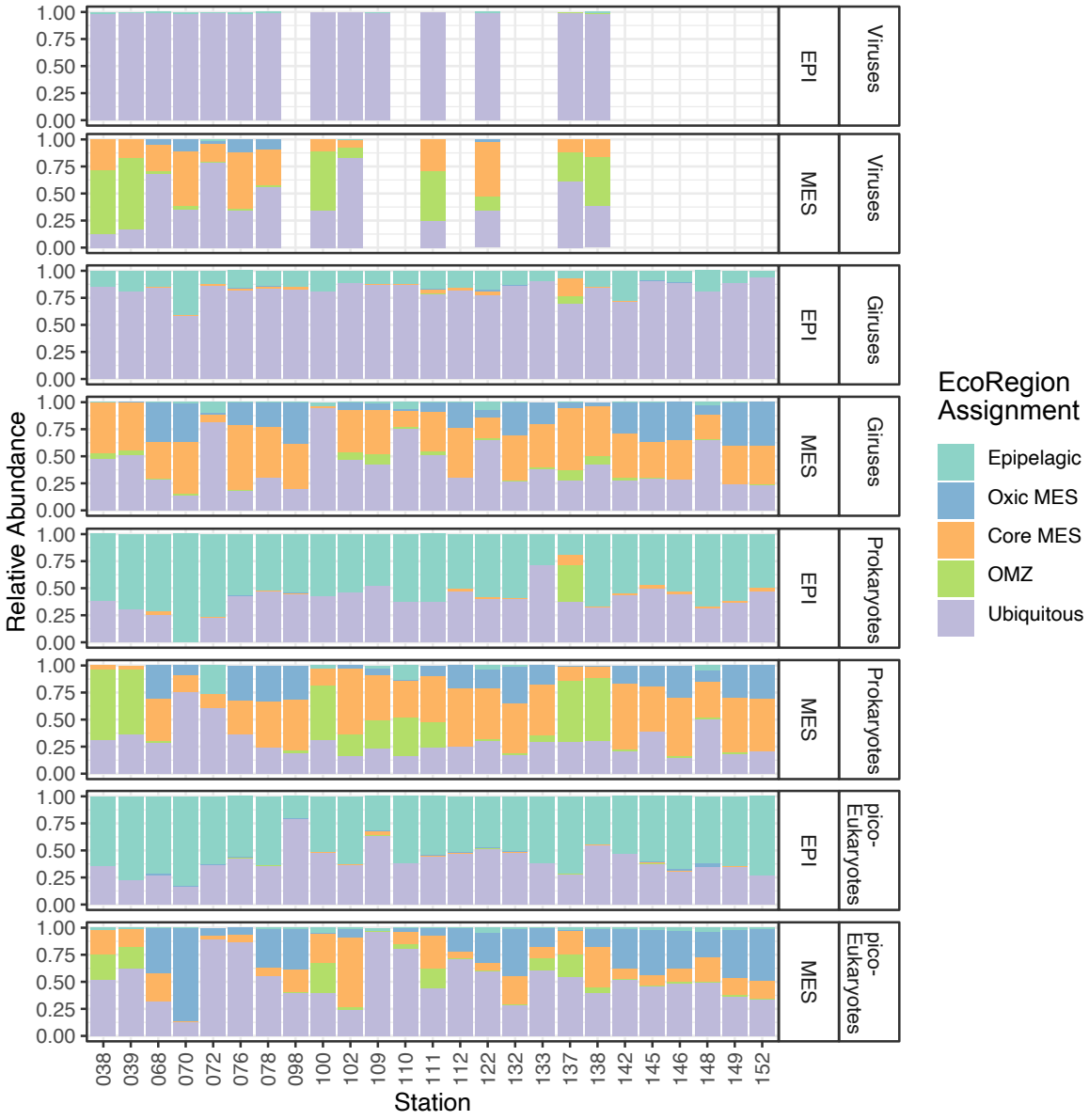
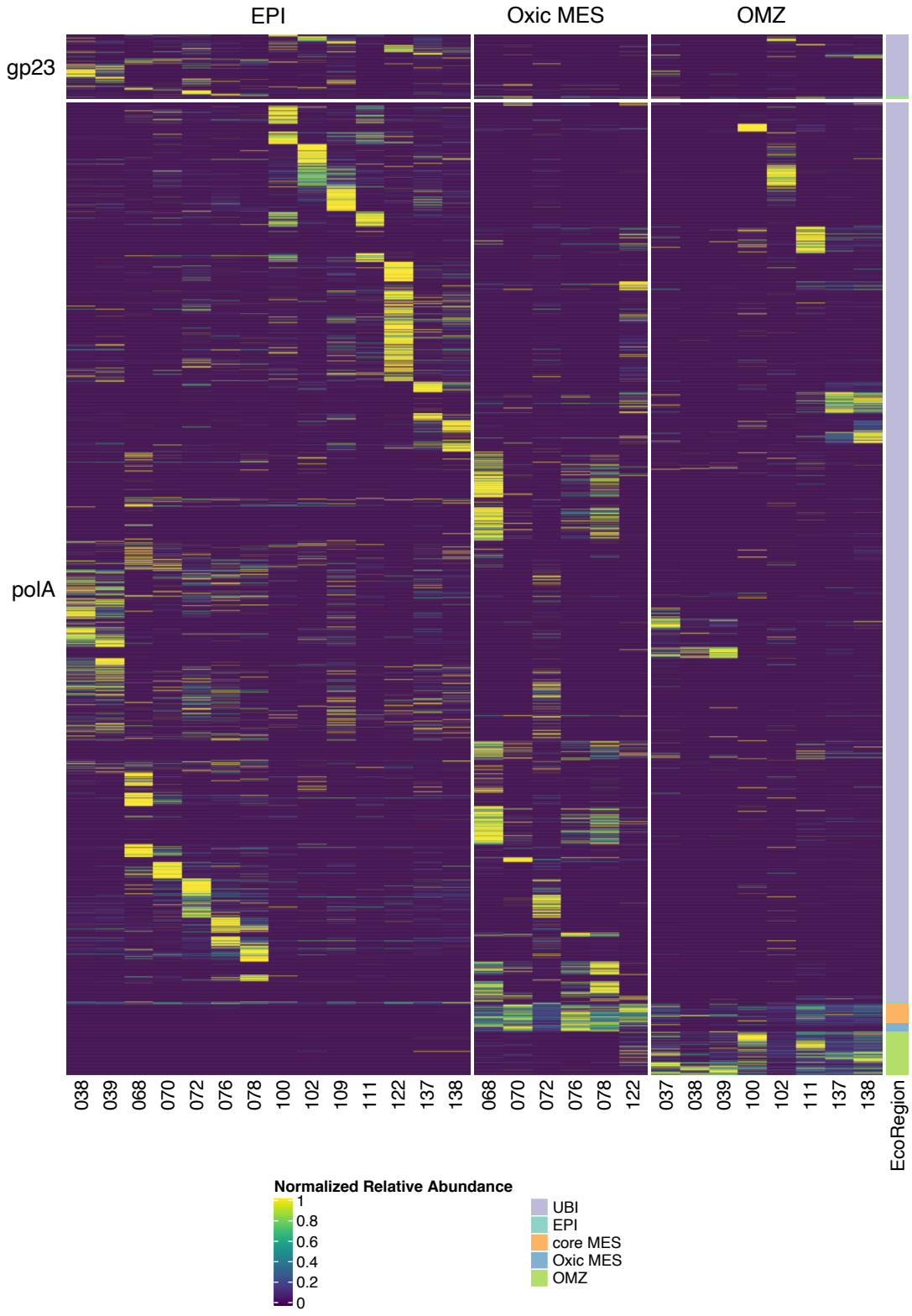
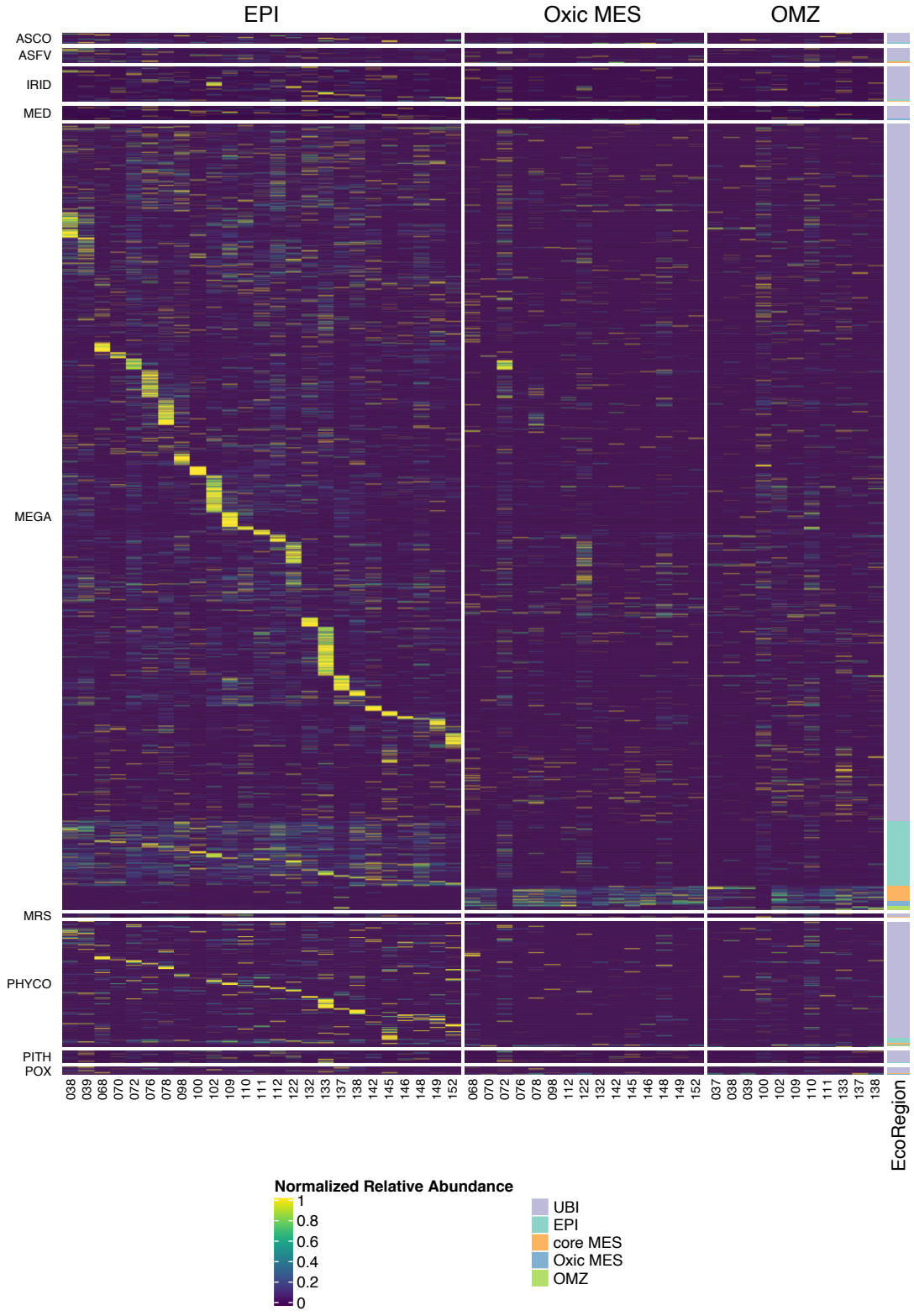


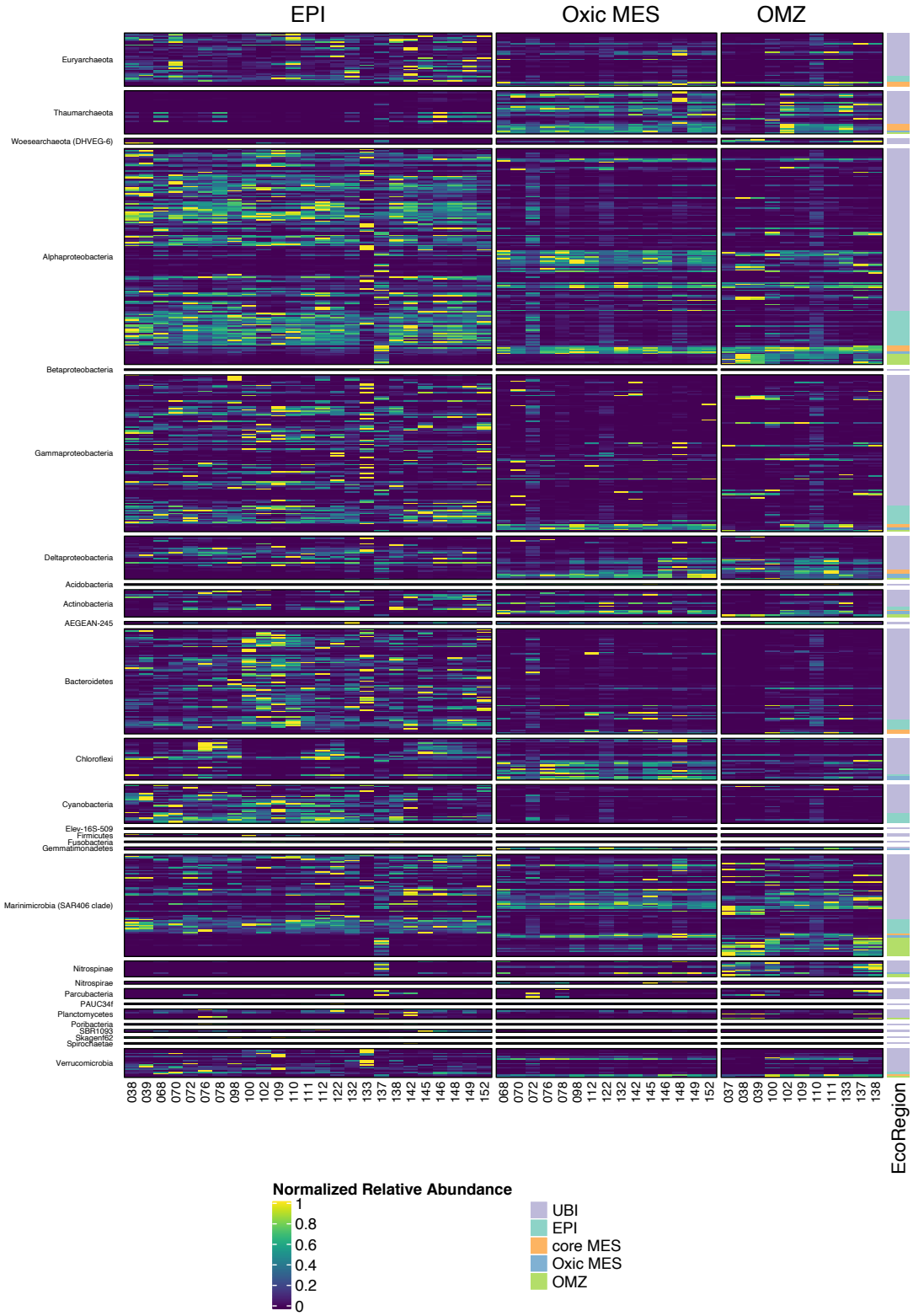
Supplementary Figure 3: Relative abundance of OTUs classified into different eco-regions in to ocean layers



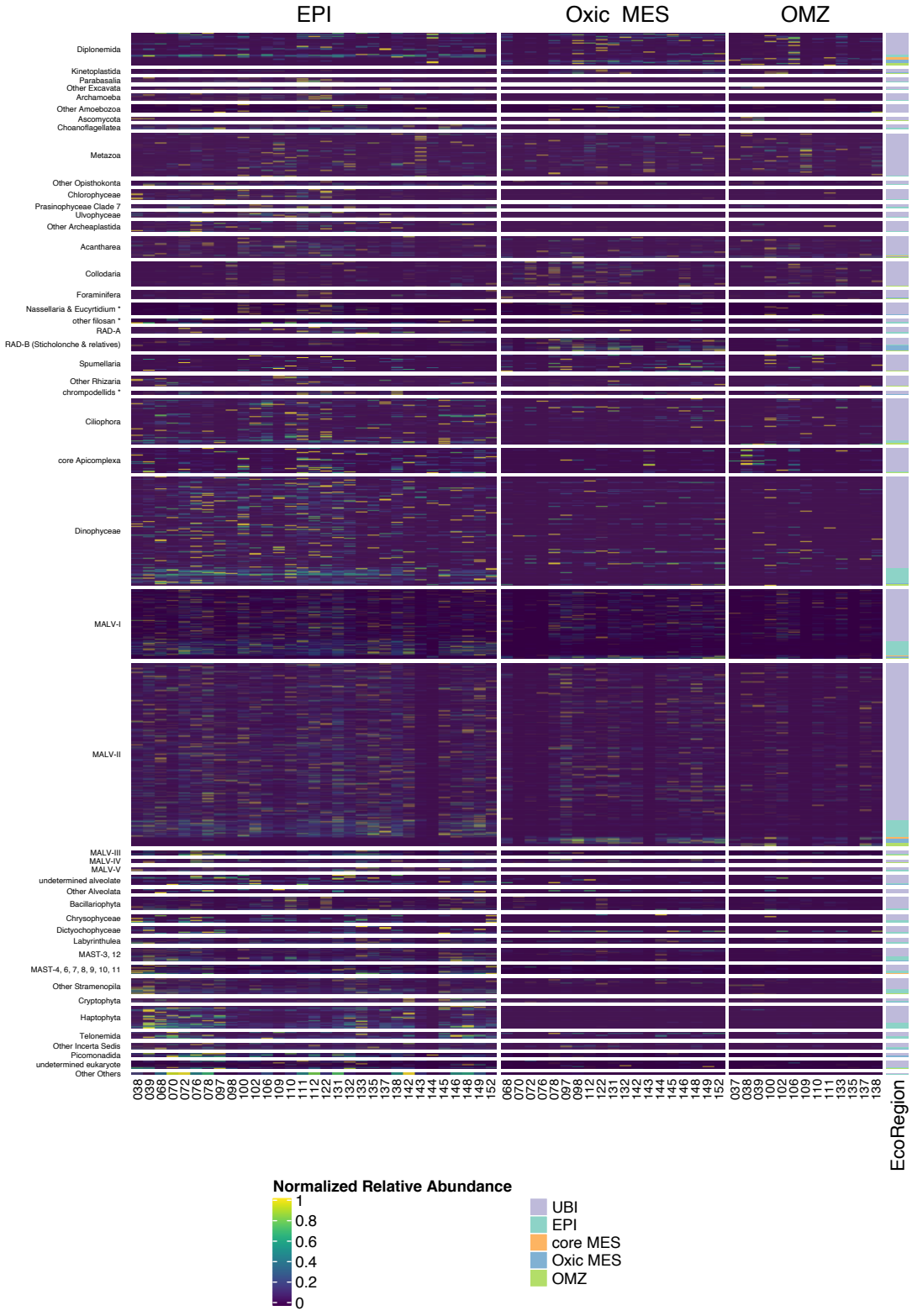
Supplementary Figure 4: Normalized Relative abundance of Phages and their preferred eco-region



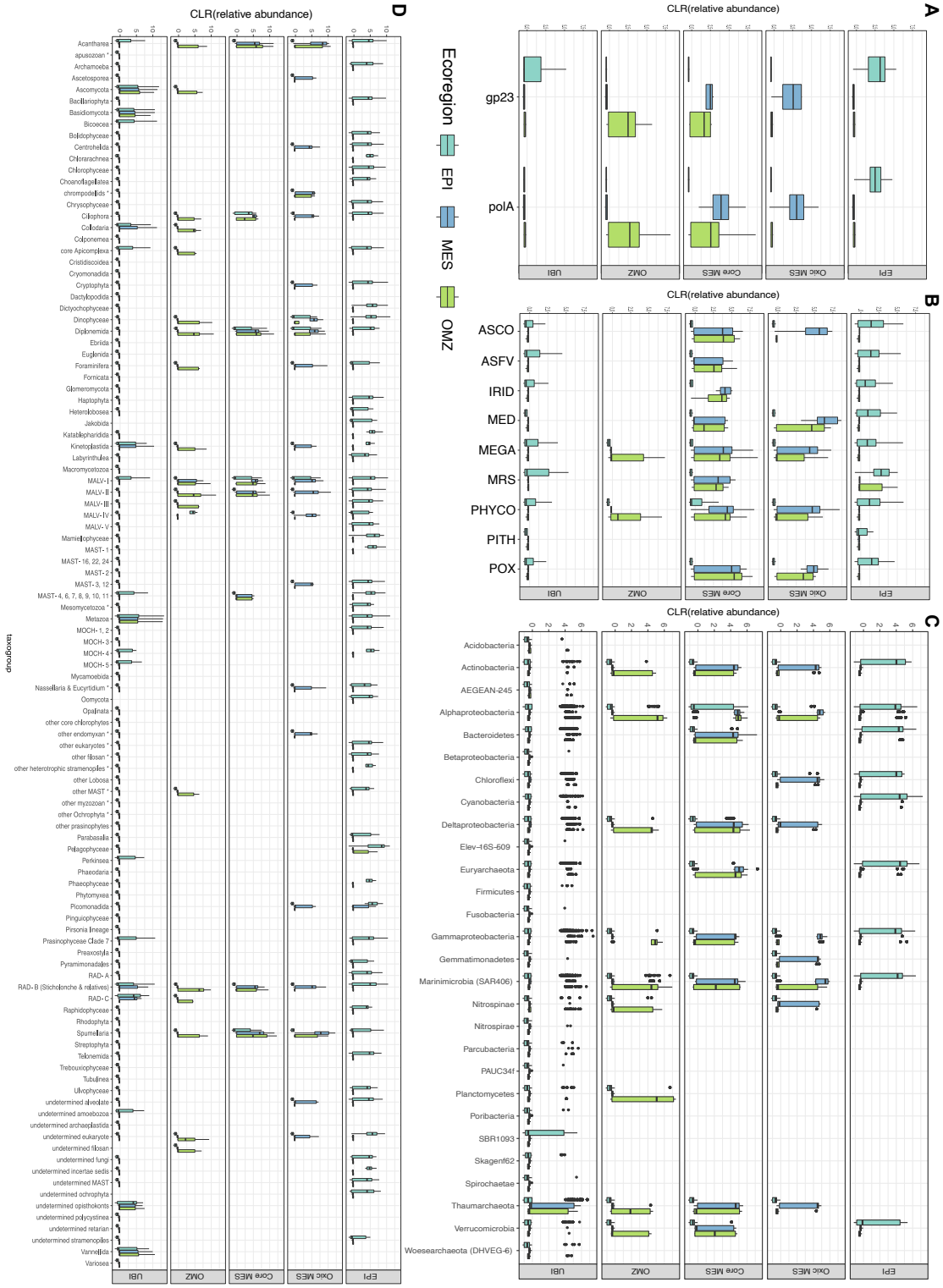
Supplementary Figure 5: Normalized Relative abundance of giruses and their preferred eco-region



Supplementary Figure 6: Normalized Relative abundance of Prokaryotes and their preferred eco-region



Supplementary Figure 7: Normalized Relative abundance of pico-Eukaryotes and their preferred eco-region



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