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Environmental sequencing of marine protistan plankton communities reveals the effects of mesoscale cyclonic eddy transport on regional protistan diversity in subtropical offshore waters

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Abstract

Mesoscale eddies which origin in Eastern Boundary Upwelling Systems (EBUS) such as the Canary Current System entrap nutrient rich coastal water and travel offshore while ageing. We have analyzed the protistan plankton community structures in the deep chlorophyll maximum (DCM), sub-DCM and oxygen minimum zone (OMZ) of three differently aged cyclonic EBUS eddies off Northwest Africa as well as of non-eddy affected reference sites using DNA metabarcoding. Throughout all water depths, we found that the investigated eddies generated local dispersal-driven hotspots of protistan plankton diversity in the naturally oligotrophic subtropical offshore waters off Northwest Africa. Based on the taxonomic composition of protistan plankton communities, these diversity hotspots are likely to play an important role in carbon sequestration and for regional food webs up to top predatory levels. Thereby, the life-span of an eddy emerged as an important criterion, how local offshore protistan plankton diversity is transformed quantitatively and qualitatively: each of the three eddies was characterized by notably distinct protistan plankton communities. This could be linked to the physicochemical water properties (predominantly macronutrients, temperature and salinity) of the eddies' cores and rings, which experience pronounced changes during the eddies' westward trajectories. Furthermore, we found evidence that eddy-specific deep-water protistan communities are relatively short-lived compared to the ones in the sunlit DCM. However, our results do not only witness from the importance of fine-scale physical ocean features for regional ecosystem processes, but they also show the complexity of these ocean features and that we are still far from understanding the biological processes and their driving forces in such features.

Keywords: cyclonic eddies; diversity hotspots; DNA metabarcoding; eastern boundary upwelling system; mesoscale ocean features; oceanic carbon pump; protistan plankton

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1. Introduction

Microbial eukaryotes (protists) play a vital role in the marine pelagic ecosystems. While marine phytoplankton account for only 1-2% of the total plant biomass globally, they contribute approximately 40% of the total fixed carbon on Earth (Falkowski, 1994). A key protistan plankton group is diatoms, which contribute ca. 40% of the primary production in the oceans (Falkowski et al., 1998). Bacterivorous protistan plankton (mostly heterotrophic nanoflagellates and small ciliates) are cropping bacterial production while herbivore protists (such as larger ciliates and heterotrophic dinoflagellates) routinely consume from 25% to 100% of the daily phytoplankton production, even in diatom-dominated upwelling blooms (Sherr and Sherr, 1994). Furthermore, protistan plankton channels carbon from lower trophic levels to multicellular organisms (Barber, 2007), explaining an aggregation of species of higher trophic levels in oceanic regions of high protistan plankton abundances. Not surprisingly, the diversity of protistan plankton strongly correlates with the diversity of consumers (García-Comas et al., 2016; Singer et al., 2021) making them key players in the success of fisheries (Chenillat et al., 2016). It has even been suggested that biogeographical diversity patterns estimated for primary producers may be used as a proxy of patterns for higher levels of the trophic chain (Duffy et al., 2007). Because of their high carbon transfer efficiency in the pelagic food web and their carbon export from the surface ocean to the deep-sea floor through sinking organisms, particles, aggregates or fecal pellets of primary consumers, oceanic protistan plankton is a pivotal component of the biological carbon pump. Because individual species of the protistan plankton have distinct carbon transfer or removal efficiencies (Degerman et al., 2018; Martin and Tortell, 2008; San Martin et al., 2006) the rate of carbon cycling and export from the ocean surface is heavily influenced by protistan plankton community composition (Brown et al., 2008; Legendre and Michaud, 1998; Michaels and Silver, 1988). Therefore, identifying patterns and hotspots of protistan plankton diversity in the global ocean is a cornerstone to improve our understanding of the local and global biological carbon pump.

Regions of highest protistan plankton productivity in the global ocean are areas with a high supply of nutrients, such as coastal upwelling regions (Pelegrí et al., 2005; Van Oostende et al., 2018; Vargas et al., 2007; Ward et al., 2012). But also, the oligotrophic open ocean may experience oscillations in the nutrient regime, which

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structure protistan plankton communities. As explained previously (Alexander et al., 2015) these oscillations may be driven by biological, anthropogenic or physical forcing. In the latter, meso- and sub-mesoscale physical processes such as eddies, fronts and filaments are of special importance as they are known to modulate the distribution and diversity of ambient plankton communities and their access to resources (Hernández-Hernández et al., 2020; Ramond et al., 2021).

The formation of eddies results mainly from baroclinic instability (Bibby et al., 2008; Kurian et al., 2011) due to e.g. the shearing from opposing currents, seafloor topology, upwelling filaments, wind forcing, coastline irregularities or a combination thereof (Batteen et al., 2003; McGillicuddy, 2016). In Eastern Boundary Upwelling Systems (EBUS) such as the Canary Current System (CanCS) off Northwest Africa, the formation of eddies is fueled by instabilities generated by velocity shear of the coastal current system and the Ekman circulation. In EBUS, eddies trap parcels of upwelled, nutrient-rich coastal water. Because the kinetic energy contained in the eddy field is often up to two orders of magnitude larger than the energy contained in the mean flow field (i.e. these eddies have a high rotational speed = high vorticity), the exchange between water masses trapped by the eddy and surrounding waters is severely limited (McGillicuddy, 2016). Thus, upwelled nutrients are advected offshore (up to several hundreds of kilometers) into the oligotrophic open ocean. Likewise, highly diverse plankton communities of upwelled costal water become entrained into eddies and are transported offshore. These communities can undergo ecological succession as the eddies travel offshore and age (Brown et al., 2008; Cesar-Ribeiro et al., 2020; Owen, 1980).

Our knowledge on how protistan plankton diversity is associated with such meso- and sub-mesoscale processes is, however, scarce, mainly because such highly dynamic hydrographic features are difficult to locate and to sample (Ramond et al., 2021). While tidal fronts in coastal areas are recurrent and (relatively) easier to target for the study of in-situ protistan plankton diversity (Ramond et al., 2021), eddies provide a more challenging structure. Furthermore, the few previous studies that were dedicated to the investigation of protistan plankton diversity in eddies relied on microscopy-based identification of protistan plankton (Hernández-Hernández et al., 2020) or used chemotaxonomic methods such as CHEMTAX (Barlow et al., 2017; Carvalho et al., 2019). Even though these studies provided invaluable resources, chemotaxonomic methods have a low level of taxonomic resolution and may be

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imprecise due the effects of nutrient availability on chlorophyll and other pigment ratios (Higgins et al., 2011). In microscopy-based diagnosis of protistan plankton community composition only a very limited number of plankton types was resolved (Abad et al., 2016; Eiler et al., 2013; Visco et al., 2015). Furthermore, classical taxonomic studies are extremely labor and time intensive and also expensive to acquire (Clayton et al., 2013). In addition, to the best of our knowledge, none of these previous studies that investigated protistan plankton community structure, appreciated the non-pigmented plankton types but focused exclusively on phytoplankton. The interrogation of taxonomic molecular barcodes obtained from environmental samples in combination with high-throughput sequencing and computational massive sequence data processing tools allows for in-depth insides into protistan plankton communities (Burki et al., 2021; Stoeck et al., 2010; Vargas et al., 2015). We here exploited this technology for the first study of protistan plankton diversity in three differently aged eddies originating in the Canary Current System off the Northwest African coast. In specific, we were asking how (quantitatively and qualitatively) cyclonic eddies affect the regional diversity of protistan plankton communities in a sub-tropical oligotrophic oceanic offshore region. Therefore, we analyzed samples from the deep-chlorophyll maximum (DCM), which holds a key role in ocean nutrient cycling, the biological carbon pump and the flow of energy (Cullen, 2015), from immediately below the DCM and from the oxygen minimum zone (OMZ), which is hypothesized to act as either a trap or as a sieve for the carbon export from eddies to the deep-sea (Chavez and Messié, 2009). Our results provide an unprecedented insight into the association of whole protistan plankton communities with ocean eddies in an eastern boundary region off Northwest Africa.

2. Methods

2.1Eddy descriptions and sample collections

We sampled three differently aged cyclonic mesoscale eddies off the Northwest African coast during two cruises with RV *Meteor* (Fig. 1). Eddy “CE_2019_19N_18W” (center coordinates: 18.73 N 18.03 W), which was closest to the coast, with an age of approximately two months, was sampled in June 2019 on cruise M156. Eddies “CE_2019_18N_20W” (17.73 N 20.43 W) and “CE_2019_14N_25W” (14.50 N 25.03 W) were sampled on cruise M160 in December 2019. Eddy CE_2019_18N_20W was

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four months old, while CE_2019_14N_25W was estimated to be six to seven months after formation while sampling. CE_2019_14N_25W also changed from a typical eddy-like shape into an ellipsoid form at the ocean surface during the sampling process. The age of the eddies was determined using satellite data of surface level anomalies by Copernicus Marine Environment Monitoring Service (CMEMS; http://marine.copernicus.eu).

Sampling stations of the eddies were identified based on Archiving, Validation and Interpretation of Satellite Oceanographic data (AVISO) satellite images (Cesar-Ribeiro et al., 2020; Wu and Chiang, 2007). According to these satellite images sampling occurred along east-west and north-south transects through the eddy centers of CE_2019_18N_20W and CE_2019_14N_25W and an east-west transect only through eddy CE_2019_19N_18W. For each transect, three sampling sites were chosen: one in the middle of the center, one approximately half way to the center periphery and one in the periphery (i.e., five sites for CE_2019_18N_20W and CE_2019_14N_25W; three sites for CE_2019_19N_18W). The actual eddy dimensions and locations were later refined based on shipboard measurements. Based on these measurements, two sampling sites from CE_2019_18N_20W and CE_2019_19N_18W, each, and one sampling site from CE_2019_14N_25W had to be excluded post hoc. These original sampling stations were actually outside of the eddy's zero-vorticity ring. At each sampling station (Table 1), we collected 20 L of water from the deep chlorophyll maximum (DCM), from right below the DCM and from the oxygen minimum zone (OMZ) using a Niskin Rosette equipped with a Seabird 911 plus CTD system. The corresponding water depths are shown in Supplementary File 2. Conductivity, temperature, oxygen and turbidity were obtained from the CTD. To accurately determine the dissolved oxygen concentration, volumetric titration was performed using the Winkler method (Strickland and Parsons, 1968; Wilhelm, 1888). Nutrient concentrations (phosphate (PO_4), nitrate (NO_3), orthosilicic acid ($Si(OH)_4$)) of each layer were measured photometrically with an AutoAnalyzer (QuAAtro; Seal Analytical) using continuous flow analysis on unfiltered seawater for duplicate samples (Grasshoff et al., 2009).

To collect protistan plankton, water samples from Niskin bottles were drawn onto membrane filters (Durapore; Merck Millipore, Darmstadt, Germany; 0.65 µm pore size, diameter 47 mm; ca. 3-5 liters per filter) using a peristaltic pump. Filters were then immediately transferred into a cryovial containing 3 ml nucleic acid preservation

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solution (LifeGuard solution, Qiagen) and frozen at -20 °C until further processing in the laboratory. At each sampling site, three biological replicate filters were collected.

2.2 Sample processing and high-throughput sequencing

Total environmental DNA (eDNA) was extracted from each filter individually using Qiagen's DNeasy PowerWater kit according to the manufacturers protocol. From the extracted DNA we amplified the hypervariable V9 region of the small subunit ribosomal RNA (SSU rRNA) gene as a molecular taxonomic barcode following a standard protocol (Stoeck et al., 2010). The protocol employed 1391F as forward primer (5'-GTACACACCGCCCGTC-3'; (Lane, 1991)) and EukB as reverse primer (5'-TGATCCTTCTGCAGGTTCACCTAC-3'; (Medlin et al., 1988)). The PCR protocol consisted of an initial denaturation step at 98°C for 30 s, followed by 30 cycles of 10 s at 98°C, 20 s at 61°C, 25 s at 72°C and a final five-minute extension at 72°C. The reactions volumes amounted to 50 µl and included 0.5 µl Phusion polymerase (New England Biolabs (NEB), Ipswich, MA, USA), 10 µl 5xPhusion GC buffer (NEB), 1 µl 10 mM dNTPs, 0.5 µl template DNA, 32.5 µl PCR grade water, and 0.5 µl of each forward and reverse primer. Triplicate PCR reactions were run for each DNA extract to minimize PCR bias. Prior to purification (MinElute Kit; Qiagen), PCR sample replicates were pooled.

To prepare the resulting PCR products for high-throughput sequencing (HTS), sequencing libraries were constructed using the NEB Next Ultra DNA Library Prep Kit for Illumina (NEB). Library quality was assessed with an Agilent Bioanalyzer 2100 system (Agilent, Santa Clara, CA, USA). Illumina MiSeq sequencing was conducted by SeqIT GmbH & Co. KG (Kaiserslautern, Germany). The sequence data files are deposited at the Sequence Read Archive of the National Center for Biotechnology Information under project number PRJNA795916.

2.3 Sequence quality control, clustering and taxonomic assignment

Illumina libraries were split based on the specific barcode identifiers of each individual sample and primers were removed from sequences using CUTADAPT v1.18 (Martin, 2011). Following, sequences were sorted in the same read direction with the FASTX toolkit (RRID: SCR_005534). Sequences were then processed using the Divisive Amplicon Denoising Algorithm (DADA2, (Callahan et al., 2016)) using the DADA2 package v1.8 in R v4.0.5 as described for hypervariable taxonomic marker

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genes from metabarcoding studies (Forster et al., 2019) with the model trained on Illumina runs and the following criteria: V9 SSU rRNA gene sequences were filtered using *filterAndTrim* with truncLen=80 and maxEE=1. The truncation length criterion was determined by choosing the sequence position at which Phred assigned a quality score of ≥30 (Q3) for at least 51% of all reads in a dataset (=base call accuracy 99.9%, (Ewing and Green, 1998)). For maxEE we chose the most stringent value, to maximize the quality of the final sequence reads used for downstream analyses. Sequences from Read1 and Read2 were merged with the function *mergePairs* in DADA2 with a minimum overlap of 20 nucleotides and an allowed mismatch of 2. Potential chimeras were removed using the *uchime-denovo* algorithm (Edgar et al., 2011) in VSEARCH. Taxonomy was assigned with VSEARCH using the PR2 reference database for eukaryotic SSU rRNA gene sequences (Guillou et al., 2013), with the last common ancestor (LCA) as a decision criterion and a syntax cut-off of 0.8. To minimize ecologically uninformative noise, only ASVs with at least 2 reads were maintained for downstream analyses. The resulting ASV-to-sample matrix was then used for all statistical analyses. In the first step, we have analyzed the taxonomic composition on phylum level to identify the dominant phyla (in terms of assigned reads). For a higher taxonomic resolution, we then performed a more detailed analysis of these dominant phyla on family level. We refrained from increasing the taxonomic resolution beyond the family level because the proportion of unassignable ASVs on genus or species level was too high.

2.4 Statistical analyses

Data analyses were conducted in R v. 4.0.5 using the program packages vegan (Oksanen et al., 2020) and ggplot2 (Wickham, 2016) for graphical visualization. Similarity of sampling sites based on their physico-chemical properties was calculated and visualized by a principal component analysis (PCA) using the *rda* function. Physico-chemical parameters were scaled to a 0-1 value range using the *rescale* option of the scale package. Correlation of physico-chemical parameters with ordination axes were extracted from the PCA output.

Rarefaction analysis for each protistan plankton sample was conducted to assess the degree of sample saturation. Prior to the calculation of alpha- and beta-diversity measures, the number of sequences per sample was rarefied (normalized)

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to the smallest sample size with the *rrarefy* function. In case of the complete dataset this was 131 108 sequences. We calculated the Shannon-Wiener Index H' and ASV richness as measures of alpha-diversity. The Bray-Curtis Index (BC) was used to calculate the similarity between samples based on the normalized ASV-to-sample matrix. BC similarity values were transformed to a distance matrix for a non-metric multidimensional scaling (NMDS) analysis using the metaMDS function with default settings. Vectors were fitted to the ordination using the *envfit* function of the vegan package in R. The fit (R^2) of each variable to the ordination using the *envfit* function was assessed with a Monte Carlo analysis of 1,000 permutations. In addition to a beta-diversity analysis of all samples, we conducted a beta-diversity analyses for each individual sampling depth (DCM, below DCM and OZM). Therefore, we normalized each of the three datasets to the smallest sample size within these datasets (214,045 for the DCM dataset, 131,108 for the below DCM dataset and 237,637 for the OMZ dataset). This allowed to exploit the maximal information included in each individual dataset. BC similarity for the individual depth layers was then visualized in distance dendrograms.

3. Results

3.1 Physicochemical structures of the eddy and reference samples

Vertical CTD profiles of physico-chemical data as well as nutrients at the sampling sites (eddies and reference stations) are provided as Supplementary Files 1 and 2. Here, we focus on the most prominent physico-chemical differences between the three eddies CE_2019_19N_18W, CE_2019_18N_20W and CE_2019_14N_25W and the two reference sites. In a principal component analysis (PCA) based on the physico-chemical data, the three depth layers (DCM, sub-DCM, OMZ) followed a gradient along PCA axis 1, while the three eddies had a notably stronger association with an axis-2 gradient (Fig. 2). Axis 1 explained as much as 93.2% of the observed variation. As a rule, concentrations of macronutrients and silicate increased from DCM to sub-DCM and OMZ, while salinity, fluorescence, temperature and dissolved oxygen decreased. Differences in physicochemical parameters were notably more pronounced in the DCM samples. The sub-DCM and the OMZ, were increasingly more similar in their physicochemical properties. In the OMZ, even the reference samples clustered with the eddy samples. We were not able to identify the physico-chemical parameters that separated the samples within each depth layer and eddy along PCA

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axis 2. It is noteworthy, that among all DCM samples, the youngest eddy CE_2019_19N_18W which was still closest to the coast was characterized by the lowest concentrations of macronutrients and silicate. Furthermore, temperature and salinity were notably lower compared to the other DCM samples.

3.2 Sequence data overview

After cleaning of the obtained raw sequence datasets, we retained between 131 108 (min) and 1 2619 83 (max) high quality (HQ) sequences (SI Table 3). Subsequently, normalization of read counts to the minimum sequence number was applied for the complete dataset and also for each depth layer separately to account for differences in sequencing depth. This resulted in 131 108 (complete data set), 214 045 (DCM), 131 108 (sub-DCM) and 237 637 (OMZ) reads per sample. Rarefaction profiles showed that all samples were sequenced to near saturation (SI Fig. 4).

3.3 Alpha diversity of protistan plankton communities

The normalized ASV richness as well as the Shannon Index *H'* as measures of alpha diversity were largely congruent for all samples (Fig. 3). Eddy CE_2019_18N_20W had a significantly lower *H'* compared to the oldest CE_2019_14N_25W eddy across all samples $p < 0.05$). The same eddy had significantly lower ASV richness and *H'* compared to the background refence waters of CB and CVOO (across all depth, Bonferroni corrected $p < 0.005$ in both cases). Thus, eddy CE_2019_18N_20W stood out as the eddy with the lowest overall alpha diversity. We did not find significant differences in ASV richness and *H'* when comparing DCM, sub-DCM and OMZ with each other. In more detail, the overall highest ASV richness was obtained for a sample from the sub-DCM of the six to seven months old CE_2019_14N_25W eddy (sample 1: 4454 ASVs) and the lowest for an OMZ sample from eddy CE_2019_18N_20W (4: 1037). The overall highest *H'* was obtained for an OMZ sample of eddy CE_2019_14N_25W (3: 6.53) and the lowest for an OMZ sample of eddy CE_2019_18N_20W (4: 3.27). In the DCM (Fig. 3a), none of the eddy protistan plankton communities was as diverse (*H'*) and ASV-rich as any of the two background reference waters. This was in sharp contrast to the OMZ (Fig. 3c), in which plankton communities of the CE_2019_14N_25W eddy were notably more diverse compared to the ones of the two other eddies and also to both reference sites (except *H'* for sample CE_2019_14N_25W(1) compared to CVOO reference).

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Plankton communities of eddy CE_2019_18N_20W were the least diverse in the OMZ. In the sub-DCM (Fig. 3b) lowest ASV richness was measured in the youngest eddy CE_2019_19N_18W and lowest H' in eddy CE_2019_14N_25W.

In the second step, we analyzed to what extent the three different eddies increased the regional protistan plankton diversity of the background waters (reference samples) (Fig. 4) by the eddy-induced transport of eddy-specific protistan communities into offshore regions under study. In terms of ASV richness (Fig. 4a), all eddies increased the regional diversity notably. The six to seven months old eddy CE_2019_14N_25W increased regional diversity the most (220 % integrated over all depth for CB background waters and 171% for CVOO background waters). The least impact on protistan ASV richness was obtained for the youngest eddy CE_2019_18N_20W (178 % integrated over all depth for CB background waters and 146% for CVOO background waters). Interestingly, the increase of regional ASV richness was typically higher in deeper waters (sub-DCM and OMZ) compared to the sunlit DCM. The Shannon diversity *H'* did not mirror the ASV richness pattern (Fig. 4b). As a rule, *H'* increased only marginally due to the introduction of a new community into the CB and CVOO background waters, or even decreased slightly, especially in case of the four-months aged eddy CE_2019_18N_20W in all depths.

3.4 Beta diversity

In a nonmetric multidimensional scaling (NMDS) of Bray Curtis (BC) distances of all samples, protistan plankton communities showed a clear pattern (NMDS stress: 0.121, Fig. 5). Samples from the different depth layers DCM, sub-DCM and OMZ (including the reference samples) were distributed predominantly along NMDS axis 1, with a gradient from the DCM towards the OMZ from lower to higher axis 1 values. Samples from the individual eddies were largely following a gradient along axis 2, with samples from the youngest eddy CE_2019_19N_18W in the lower axis 2 value range. Protistan communities from the oldest CE_2019_14N_25W eddy appeared as an intermediate between the young CE_2019_19N_18W eddy and the four-month-old CE_2019_18N_20W eddy. Within the individual depth layers, the protistan plankton communities of the two reference sites were as dissimilar to each other as were the protistan plankton communities of two different eddies. It is noteworthy that in the OMZ, the protistan plankton community structure of one individual sample from the eddy CE_2019_14N_25W (1) was remarkably different from the two other OMZ

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samples of this eddy. Several environmental parameters were strongly correlated with the NMDS axes with significant p-values (Table 2). The gradient along axis 1 from the shallower DCM towards the deeper OMZ was positively correlated with nutrients (nitrate, phosphate, orthosilicic acid, all of which had a high correlation coefficient), and negatively with temperature, salinity, oxygen (low correlation coefficient) and density, all of which were functions of the water depth. *Chla*-fluorescence and turbidity (low correlation coefficient) were also negatively correlated with the gradient from the DCM towards the OMZ. None of the tested environmental parameters were particularly strongly correlated with NMDS axis 2. Therefore, the parameters structuring protistan plankton communities of the individual eddies within each of the three depth layers remained largely obscured.

For a more detailed comparison of protistan plankton communities within each of the three depth layers individually, we constructed BC-based dendrograms for the DCM (Fig. 6a), the sub-DCM (Fig. 6b) and the OMZ (Fig. 6c). These analyses made obvious that the structuring of protistan plankton communities followed different patterns in the three depth layers. In the DCM, protistan plankton communities trapped in all three eddies as well as the ones of the two reference stations formed individual clusters. Thereby, the DCM protistan plankton community of the youngest eddy CE_2019_19N_18W was most similar to the ones of the two reference sites. In the next deeper water layer, the sub-DCM, both reference sites fell into the same cluster as the samples from the CE_2019_14N_25W eddy, indicating the relatively high similarity of the protistan plankton communities in the deeper water layers of the CE_2019_14N_25W eddy to the non-eddy-disturbed open ocean. Protistan plankton communities trapped in eddy CE_2019_19N_18W and the aged eddy CE_2019_18N_20W each remained as separate clusters. In the deepest water layer, the OMZ, only the protistan plankton communities trapped in the aged eddy CE_2019_18N_20W formed an eddy-specific cluster, while all other samples merged in the same cluster. This indicates that in the young CE_2019_19N_18W and the oldest CE_2019_14N_25W eddy, the protistan plankton community structures were relatively similar to the ones in the non-eddy-disturbed open ocean.

3.5 Taxonomic composition of protistan plankton communities

In the DCM of all samples (SI Fig 5 a), sequences assigned to dinoflagellates had the highest relative abundance. As a rule, these were followed by radiolarians,

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Discoba and haptophytes. Anomalies on phylum-level taxonomy in the DCM worth emphasizing were that the youngest eddy CE_2019_19N_18W stood out for its higher proportion of chlorophyte-assigned sequences and lower proportion of sequences assigned to Discoba compared to all other samples. Likewise, sub-DCM and OMZ samples were dominated by sequences assigned to dinoflagellates, radiolarians, Discoba and haptophytes (SI Fig 5 a). In the following we describe the taxonomic inventory of these four most abundant taxon groups in a higher taxonomic resolution. Because the variation of the taxonomic assignments of sequence reads was typically notably smaller among samples of the same eddy and depth compared to the inter-sample comparison we averaged the taxonomic assignments for the following sub-groups per eddy and depth layer, which facilitates the diagnosis of differences and similarities among eddies, different water depth layers and the reference background waters.

Haptophyta (SI Fig 5 b). In the DCM, haptophytes were dominated in all samples by Chrysochromulinaceae and Phaeocystaceae. In the youngest eddy CE_2019_19N_18W, the relative read abundance of Chryoschromulinaceae was notably higher compared to all other samples, accounting for nearly 90% of all haptophyte-assigned sequence reads. Phyecoycstaceae were accordingly less abundant in the DCM core of CE_2019_19N_18W compared to all other samples. This also applied to unassigned Prymnesiophyceae, which accounted for roughly a quarter of the sequence reads in both background waters (reference samples) and for 10% and 20% in eddies CE_2019_18N_20W and CE_2019_14N_25W, respectively. Furthermore, it is noteworthy that HAP-clade II sequences were negligible in all eddy DCM samples, but accounted for 5% and 14% in references Cape Blanc and CVOO. In the deeper sub-DCM waters, the proportion of the HAP-clade 4 increased remarkably, particularly in the two background waters Cape Blanc and CVOO and the cone shaped eddy CE_2019_14N_25W, but also, to a notably lesser extent, in the other sub-DCM samples. In the young eddy CE_2019_19N_18W, HAP-5 clade assigned sequences accounted for the majority (43%) of haptophyte sequences at the cost of Chrysochromulinaceae, which declined vastly from DCM to sub-DCM. In the sub-DCM of the four-months aged eddy CE_2019_18N_20W, Phaeocystaceaea were dominating and accounted for 81% of all haptophyte sequence reads.

Radiolaria (SI Fig 5 c). Spumellarida (unassigned and Spumellarida Group I) belonged to the most abundant radiolarians in terms of relative sequence reads with

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a remarkable increase of Spumellarida Group I from the DCM to the sub-DCM and the OMZ in all samples. In the DCM, the young eddy CE_2019_19N_18W had by far the highest proportion of Arthracanthida-Symphyacanthida- and Collospheridae-assigned sequences, accounting together for 63% of all radiolarian sequences in the DCM of CE_2019_19N_18W. The aged eddy CE_2019_18N_20W stood out from the other samples because of its relatively high proportions of acantharean and RAD-B group 1 sequence reads in the DCM. The DCM of the oldest eddy CE_2019_14N_25W as well as the CVOO background waters had relatively high proportions of Polycystinea (including Collodaria and Nasselaria) compared to all other samples (21% and 16% respectively).

Discoba (SI Fig 5 d). The vast majority of Discoba-assigned sequence reads in all samples (up to 98%, Cape Blanc OMZ reference) belonged to unidentified Diplonema that could not be further classified. The DCM-and sub-DCM samples of the young eddy CE_2019_19N_18W as well as the deep-water OMZ samples of the eddy CE_2019_14N_25W stood out from the other samples by their relatively high proportions of DSPD-1 (deep-sea pelagic diplonemids group 1).

Dinoflagellata (SI Fig 5 a).The composition of the dinoflagellate communities is relatively consistent across all eddies, the background waters and also across all vertical water layers. The vast majority of sequence reads in all samples belong to the two taxonomic clades Dino-Group I and Dino-Group II (also termed Marine Alveolates Group I and II or Syndiniales Group I and II). The youngest eddy CE_2019_19N_18W stands out from all other samples because of its relatively high proportion of Dino-Group III sequence reads, particularly in the sub-DCM. The high proportion of unassigned Dinophyceaea, which included numerous ecologically distinct orders, decreased remarkably from DCM to sub-DCM and OMZ. Similarly remarkable is the high proportion of unassigned dinoflagellates which increased from DCM to sub-DCM and OMZ in all samples. In sum, both groups of sequence reads that escaped further taxonomic classification accounted for up to 43% (DCM of Cape Blanc background water) of all dinoflagellate-assigned sequence reads in the oceanic region under study.

Supplementary File 6 provides a higher-resolution taxonomy assignment for all samples (ASV-to-sample matrix).

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4. Discussion

4.1 Effect of eddies on regional protistan plankton diversity

A previous field survey demonstrated that the diversity of protistan plankton in ocean surface waters (including the DCM) follows a pronounced, mainly temperature-driven, latitudinal diversity gradient with an increasing Shannon diversity from polar regions to the equatorial tropics (Ibarbalz et al., 2019). This agrees well with an in silico obtained global pattern for marine phytoplankton (Barton et al., 2010). Thus, both studies identify the subtropical, low-seasonality oceanic region as locations of moderate protistan plankton diversity. This agrees well with the findings of this study (see Shannon diversity and ASV richness of reference samples, Fig. 3). Our results showed that dispersal through eddies enhanced this moderate regional diversity of protistan plankton communities in a subtropical oceanic offshore region. When combining protistan communities from reference samples with the protistan communities introduced to the oligotrophic offshore regions, the regional diversity even increases the global diversity peaks of whole protistan plankton communities in tropical oceanic regions (Ibarbalz et al., 2019).

This finding is consistent with predictions from ecological theory, which considers the role of dispersal in relation to diversity patterns: with increasing rates of dispersal, the corresponding model predicts an increase in alpha-diversity, while regional beta-diversity decreases (Cadotte, 2006). Global numerical simulations investigating the effects of eddies on marine phytoplankton diversity predicted that eastern boundary regions such as the one under study are dispersal driven hotspots of diversity. This is in contrast to western boundary regions such as the Agulhas system, which is a locally driven plankton diversity hotspot (Clayton et al., 2013). These numerical simulations by Clayton and colleagues (2013) provided modelled predictions of patterns in diversity and hypotheses regarding the mechanisms that control them, which prior to our study has gone untested for whole protistan plankton communities in eastern boundary regions. Our field study provides support for these simulated predictions for eastern boundary regions.

In general, the richness of plankton communities at any location is a composite of the richness of immigrant plankton types (a_i) plus the richness of locally adapted

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types (α_{la}). Locally adapted types thrive in the local environment with a net population growth, which is balanced by export through advection or mixing processes. Conversely, immigrant types are maintained by a source due to transport from elsewhere, such as eddies. They are not ideally adapted to local environmental conditions. Loss due to competitive exclusion balances the source, and immigrant types would disappear from the local populations if transport were shut off (Clayton et al., 2013; Ward et al., 2021). Because the regional protistan plankton diversity in the subtropical offshore regions under study was driven by the transport and convergence of plankton types from different regions, we can clearly confirm model predictions which suggest that eastern boundary regions are dispersal-driven plankton diversity hotspots rather than locally driven hotspots. This corroborates with the hypothesis that α_i is quantitatively of higher importance ($\alpha_i > \alpha_{la}$) for the regional diversity in sub-tropical and tropical regions compared to temperate and polar regions (Clayton et al., 2013). In contrast, the four western boundary regions are confluences of different water masses, each bringing their resident phytoplankton populations (d'Ovidio et al., 2010). One would therefore expect a similar immigration-driven diversity hotspot scenario in western boundary regions compared to the sub-tropical eastern boundary regions. However, most of these immigrants are able to thrive in the confluence regions, because the local growth of the immigrants after shutoff from transport is supported by an enhanced supply of nutrients associated with the typical confluence of western boundary systems. Therefore, eddy-fueled dispersal effects on regional protistan diversity in western boundary systems, such as differently aged Agulhas rings (Cesar-Ribeiro et al., 2020) is substantially different from the one in eastern boundary regions (this study).

The increase of regional protistan ASV richness through eddy dispersal occurred in all three water layers sampled (DCM, sub-DCM and OMZ). An at first sight peculiar finding was that the relative change in the Shannon diversity index *H'* was to a much lesser extent affected by immigration than the regional ASV richness. Furthermore, differences became evident in a direct comparison between these two quantities. Such observations, however, are not unusual and can be explained as follows: *H'* (Shannon and Weaver 1949) is a measure of both, the protistan plankton ASV richness and the evenness of the protistan plankton communities. This is an index of species diversity and not of species richness. The latter is just one component

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of species diversity and refers to the number of species. A detailed discussion on this subject was published previously by (Spellerberg and Fedor, 2003). An increase in regional protistan plankton richness introduced by immigrants of the aged eddy CE_2019_18N_20W is therefore not in contradiction to a decreased *H'* relative to both reference samples, but highlights a community with a high species (ASV) richness and a low evenness, i.e., few ASV are highly dominant in their sequence read abundance, outnumbering the vast majority of other less-abundant species in the protistan community. This is most likely an effect of the evolution of protistan communities in ageing eddies.

4.2 Species (ASV) richness, diversity and taxonomic composition of protistan plankton communities in eddies propagating from the Northwest African coast

All three eddies investigated in this study are different in their ASV richness, diversity and taxonomic composition, and thus, affected regional protistan plankton diversity qualitatively and quantitatively in different ways. We here discuss the differences in protistan plankton community structures among the three differently aged eddies to assess their individual effects on regional offshore waters along their trajectories. We, however, refrain from discussing our results in the context of succession patterns of ageing eddies. Such a study would require the sampling of the same eddy on a high temporal and also spatial scale.

We found the most pronounced differences in protistan plankton communities among the three eddies under study in the DCM, while in sub-DCM and the OMZ the communities become increasingly more similar, which corroborated with the physicochemical profiles of the eddies. The DCM of the youngest eddy CE_2019_19N_18W, sampled closest to the Mauritanian coast, had the least diverse protistan plankton community and lowest ASV richness, enhancing the plankton diversity of the regional offshore background water to a lesser extent than eddies CE_2019_18N_20W and CE_2019_14N_25W. Its physicochemical properties with relatively low temperature, salinity and chlorophyll fluorescence in the DCM are typical properties of a younger cold core eddy. Warmer higher-salinity surface water masses form a ring-like structure around colder upwelled nutrient-rich water that is feeding the Canary Current System. This forms a structure consisting of the center of the eddy (cold core), an outer swirling ring, and the surrounding background water

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(Dilmahamod et al., 2021). Such eddies typically contain a variety of waters with differing temperature-salinity characteristics, and the fine-scale variability is especially high in the core of the eddy (Leach and Strass, 2019). Due to a strong stratification between these water masses, the core usually maintains its distinctive physicochemical properties after propagation from the coast and during its trajectory towards offshore regions. Even though leakage or intrusion of background water is not unusual, depending on changes in velocities across the vertical eddy profile, seasonal and hydrographic effects, split, merge and linking events, eddy core and ring structures, and eddy shapes (Hall and Lutjeharms, 2011; Lamont and Barlow, 2017; Liu et al., 2019). This may further influence not only physicochemical properties but also protistan plankton communities in the eddy core.

We found some evident taxonomic signatures that distinguished the DCM of eddy CE_2019_19N_18W from the offshore reference sites and also from the other two eddies. These signatures strongly indicate the confluence of (near-coastal) sunlit surface waters and upwelled deep-water masses. Such a relatively high abundance of Chlorophyceae as we detected in the DCM of eddy CE_2019_19N_18W is atypical for offshore as well as for deep waters from the non-photic zone. This class of green algae prefers colder and low salinity waters, such as we found in the DCM core of CE_2019_19N_18W, as well as sunlit surface and coastal waters (Tragin and Vaulot, 2018). Likewise, the relative to other samples higher abundance of sequence reads assigned the radiolarian Arthracanthida-Symphyacanthida clades in the DCM of CE_2019_19N_18W witnesses from a seed community in the eddy core that is partially recruited from surface waters. This taxon group is photosymbiotic and possesses robust skeletons withstanding turbulent waters (Decelle et al., 2012). Arthracanthida and Symphyacanthida complete their full life cycles in the photic zone because they are not capable of forming cysts and each new generation is forced to recruit new phototrophic endobionts (Decelle et al., 2013; Martin et al., 2010). Thus, their abundances typically decrease with water depth (Mars Brisbin et al., 2020). The notably lower abundances of Arthracanthida and Symphyacanthida in the DCM of other samples analyzed in this study may point to the low competitiveness of these taxon groups when nutrients and micronutrients (such as strontium needed for the skeleton) become depleted. Another noteworthy signature is the high read abundance of the haptohyte Chrysochromulina in CE_2019_19N_18W compared to all other DCM samples. Chrysochromulina is a globally distributed marine pico- to nano-sized

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phototrophic flagellate (Estep and MacIntyre 1989). Some species are also capable of phagotrophy and the balance between phagotrophy and photosynthesis seems to be influenced by environmental factors (Flynn and Mitra, 2009; Wilken et al., 2020). Favorable conditions to trigger growth of Chrysochromulina include a combination of lower salinities, higher macronutrient concentrations, also higher concentrations of nitrogen compounds and higher N:P ratios (Dahl et al., 2005), all of which are the case for the DCM core waters of CE_2019_19N_18W compared to the other eddies and the reference stations. Chrysochromulina species can form massive blooms and produce hypertoxins as a metabolic response to cellular stress triggered by environmental and physiological factors, presumably to outcompete other phytoplankton (Johansson and Granéli, 1999). Such toxins can cause massive fish kills and severe economic damage (Anderson et al., 2000). On the other hand, Chrysochromulina species are major players for the ocean carbon cycle and carbon sequestration (Fixen et al., 2016; Hovde et al., 2015), but also contribute to methane oversaturation in marine waters, and, thus, are important organisms when studying climate-relevant processes in marine surface waters (Klintzsch et al., 2019). To the best of our knowledge, Chrysochromulina blooms have thus far not yet been reported from marine waters off West Africa (Hallegraeff et al., 2021). The higher proportion of the Discoba group DSPD-1 (deep-sea pelagic diplonemdis) in the DCM of CE_2019_19N_18W is biological evidence for the entrapping of relatively recent upwelled nutrient rich water from deeper water layers. This remarkably diverse group of heterotrophic excavate flagellates from mesopelagic layers has come to attention relatively recently through massive environmental sequencing surveys (Lara et al., 2009; López-García et al., 2001; Scheckenbach et al., 2010). Reports from the photic zone are rare (Flegontova et al., 2016) and the formation of eddies in upwelling regions provides an hitherto undescribed mechanisms to transport these typical dark ocean inhabitants to the sunlit surface layers. Flegontova et al. (2016) emphasized in their detailed study about diplonemids that “So far, no DSPD I diplonemid has been formally described, and we know nothing about their biology.” The observation that other DCM samples have much lower proportions of DSPD indicates that DSPDs are no successful competitors while residing in the DCM as the eddies age and travel further offshore. The extremely high proportion of unassigned diplonemids in all DCM samples shows that we are still far from knowing and understanding the diversity and ecology of these enigmatic organisms. The offshore waters of the Northwestern

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African coast are a new valuable source for further diplonemid-focused studies. Diplonemid communities are stratified according to depth(Flegontova et al., 2016). Given the high proportion of these new diplonemid sequences in all DCM samples it is reasonable to assume that the corresponding organisms may belong to the photic zone diplonemid community (Flegontova et al., 2016; Vargas et al., 2015) and have corresponding metabolic and competitive properties. This, however, remains to be validated in further studies of diplonemids in this oceanic offshore region.

In contrast, macronutrients in the DCM of the four-months aged eddy CE_2019_18N_20W were largely depleted, selecting for taxa better adapted to low-nutrient conditions. The haptophyte algae Phaeocystaceae is most abundant in the nutrient-depleted DCM of eddy CE_2019_18N_20W. *Phaeocystis* is the only described genus in this family (Adl et al., 2019), and, like other haptophytes, plays a key role in ocean carbon cycling (Smith et al., 1991), but also in the sulfur cycle because of its significant dimethyl sulfide (DMS) production (Liss et al., 1994). *Phaeocystis* species evolve in nutrient, especially nitrogen rich waters (Schoemann et al., 2005; Smith et al., 1991), as can be found in freshly upwelled and trapped nutrient rich water in younger eddies such as CE_2019_19N_18W. This alga is a major consumer of nitrogen, and may have contributed significantly to the nitrogen depletion in the DCM of eddy CE_2019_18N_20W, while fixing inorganic carbon. A very similar scenario was described for *Phaeocystis* in polar waters that started to bloom at high nitrogen concentrations and then consumed the nitrogen in the water to depletion (Smith et al., 1991). However, *Phaeocsytis* species are also among the dominant endosymbionts of actantharean Radiolaria in warm oligotrophic oceanic regions (Decelle et al., 2012; Mars Brisbin et al., 2018). The symbiosis between *Phaecoystis* and acanthareans is an ecologically relevant mechanism to enable primary production hotspots in low-nutrient regions (Decelle et al., 2013). This corroborates well with our finding that the relative proportion of (unidentified) acanthareans is also highest in the eddy CE_2019_18N_20W DCM samples compared to all other DCM samples. Acanthareans belong to the microplankton and have a mixotrophic lifestyle as an adaptation to low-nutrient conditions. They provide their algal symbionts with nutrients, which they acquire through predation. In return, the phototrophic symbiont provides carbohydrates produced during photosynthesis. The energy that acanthareans gain from these carbohydrates does not only make them strong competitors in nutrient depleted environments such as in the core of eddy CE_2019_18N_20W, but also

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provides additional energy which is required to maintain the strontium sulfate skeletons of acanthareans (Decelle et al., 2013, 2012). Furthermore, the DCM core of eddy CE_2019_18N_20W had higher proportions of the dino-group II, a taxonomic group consisting of diverse Syndiniales phylotypes with *Amoebophrya* being the only described genus. Syndiales group II (also called marine Alveolates Group II (MALV II, (Groisillier et al., 2006)) is one of the dinoflagellate groups that is responsible for the overwhelming numerical dominance of dinoflagellate sequences in all samples analyzed in this study, the other one being MALV I. All Syndiniales have a parasitic lifestyle are parasitoids and obligately kill their hosts. Hosts of Group II include predominantly other dinoflagellates and also acanthareans, as well as other radiolarians and Cercozoa (Guillou et al., 2008; Siano et al., 2011). Thus, Group II phylotypes may play an important role in the top-down control of plankton communities. Survival of the dinospores in the water after release from the killed host lasts only for few days (Coats and Park, 2002). Therefore, it is reasonable to assume that at least most of the recorded sequence reads come from actual host infections. Most sequences obtained from environmental studies that were assigned to Group II originated from waters of the oxygenated photic zone (Guillou et al., 2008). In the winter months, Syndiniales Group II phylotypes were so abundant in oligotrophic DCM offshore waters of South Benguela that they were the most dominant taxon group in the plankton (Rocke et al., 2020). But also in other oceanic regions, MALV II phylotypes belong numerically to the most dominant sequences in protistan plankton metabarcoding datasets (Vargas et al., 2015). MALV I ASVs were in contrast predominantly (but not exclusively) retrieved from low-oxygen or anoxic marine waters (Guillou et al., 2008). Based on the results obtained in this study, we find no support of the hypothesis that MALV I ASVs prefer oxygen-depleted environments. Our study is the first one to report such a dominance of dinoflagellates from the oceanic region under study. A remarkably high proportion of the detected dinoflagellate communities in this oceanic region seems morphologically unknown as we conclude from the high proportion of taxonomically unassigned dinoflagellate sequences. Furthermore, MALVs, which account for the vast majority of detected dinoflagellate sequences in our study, usually escape light microscopy diagnosis. To better understand the ecological role of these abundant organisms in the DCM core of eddy CE_2019_18N_20W and in the oceanic region under study, future research should focus on the identification of host-parasite relationships and their spatio-temporal

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patterns. But also more detailed morphological inventories of protistan plankton in this subtropical oligotrophic ocean region should be a future research focus. This is also evidenced in the RAD-B radiolarians, which we found in higher sequence read abundances in the DCM of eddy CE_2019_18N_20W compared to all other samples. This entity is also known exclusively from its sequence reads that were retrieved from a variety of different oceanic regions, globally distributed, from surface waters to the deep-sea (Sandin 2019). There is unfortunately no detailed knowledge about these RAD-B radiolarians which prevents us from further exploiting our finding in an ecological context. However, previous reports suggested that a high abundance of RAD-B radiolarians may be linked to low-predation pressure (Sandin et al., 2019; Giner et al., 2020), which, however, still remains to be tested.

The collapse of a cyclonic eddy is initiated by a loss of velocity and is initially characterized by ultra-oligotrophy, followed by leakage and intrusion events (Cesar-Ribeiro et al., 2020; Liu et al., 2019). Considering these properties, eddy CE_2019_14N_25W is assumed to be in a late stage of collapse because nutrients were higher as in the aged eddy CE_2019_18N_20W and plankton communities that have evolved in the eddy core start to mingle with plankton communities of the background water. The result is a hybrid protistan community that we obtained from our samples of eddy CE_2019_14N_25W. In the DCM, this eddy had higher relative proportions of unassigned Polycystinea compared to the other eddies, emphasizing our knowledge gaps when it comes to the diversity of these radiolarians with their opaline silica skeleton in oligotrophic offshore waters. One order within Polycystinea is Collodaria, of which the DCM of eddy CE_2019_14N_25W had noteworthy higher relative proportions compared to all other samples. Collodaria are mixotrophs that flourish in calm and oligotrophic surface waters (Swanberg, 1979). They can prey on a range of different organisms and size classes, including bacteria, phytoplankton, ciliates and copepods, and thus, play an important role in the pelagic food web (Biard et al., 2015). But they also contribute notably to carbon fixation due to their phototrophic endosymbionts (Michaels et al., 1995), the most common of which is a dinoflagellate (Probert et al., 2014).

In the deeper waters (sub-DCM and OMZ) the protistan communities were vastly different from the DCM. With increasing depth, the protistan communities of the youngest eddy CE_2019_19N_18W and the oldest eddy CE_2019_14N_25W became more similar to the protistan communities in the background waters

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(references samples). In contrast, the seemingly more robust eddy CE_2019_18N_20W had distinctively different protistan communities in the deeper waters compared to the other two eddies and to the reference samples. A characteristic feature of the background waters was as remarkable increase in the relative abundance of Haptophyte Clade HAP-4 in the sub-DCM, which was also mirrored in eddies CE_2019_14N_25W and, to a lesser extent, in eddy CE_2019_19N_18W. Furthermore, the Haptophyte Clade HAP-5 increased notably in the sub-DCM in all samples. Both HAP-clades were defined in Egge et al. (2015) based exclusively on environmental sequences. These authors reported that ASVs of both clades were previously detected predominantly in deeper, mostly oligotrophic ocean waters. The ecology of HAP-4 and HAP-5 haptophytes is enigmatic. Our finding that in the OMZ both hapthophyte clades were numerically negligible in all samples, suggests a sensitivity to oxygen-depletion. Likewise, the composition of radiolarians changed remarkably from the DCM to the sub-DCM and the OMZ. Changes in Radiolarian communities with increasing depth are well known, and particular Spumellarida were preferentially recovered from deeper ocean waters (Not et al., 2007). The extremely high proportions of Spumellarida in the aged eddy CE_2019_18N_20W suggests that this taxon group outcompetes other radiolarians and is a very successful species in the eddy-entrained deep-water masses, including low-oxygen conditions. Also, in the deeper waters of all samples, Discoba ASVs are by far dominated by taxonomically unassigned diplonemids. This makes a discussion about specific differences in the composition of the abundant Discoba sequences and ASVs impossible. Likewise, we are not capable of linking the higher sequence read abundances of Dino-Group III in CE_2019_19N_18W to specific properties of this eddy, because this group consists entirely of environmental sequences from a variety of different oceanic ecosystems and we have no further information available about the taxa belonging to this taxonomic unit (Guillou et al., 2008).

This emphasizes once more the shortcomings resulting from our extremely scarce knowledge about protistan diversity and their ecology in the oceans. It is essential to reveal the identity behind taxonomically unassigned ASVs and to study the ecology of these organisms if we want to understand ecosystem function(ing) and processes that lead to selective developments in ecosystems. Interestingly, even though dinoflagellates also accounted for the numerically most abundant sequence reads in the sub-DCM and the OMZ, they hardly contributed to the observed changes

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in beta-diversity patterns across vertical depth profiles within each eddy and the reference sites. On higher taxonomic levels, the dinoflagellate diversity was surprisingly uniform across all samples. The best explanation is that the vast majority of dinoflagellates observed had a parasitoid lifestyle and that they typically infest a diverse range of hosts such as for example a variety of different radiolarians (Guillou et al., 2008), which were dominant in all samples. However, we also observed ASVs taxonomically assigned to free-living dinoflagellates, such as Gymnodiniaceae, Protoceratiaceae and Heterocapsaceae. These taxon groups, however, did not notable contribute numerically (in terms of sequence reads) to the obtained dinoflagellate community and they were also relatively equally contributed across all samples and depths. This is explained by their high versatility due to their mixotrophic life style: dinoflagellate can cope with a range of different nutrient regimes and are among others typical inhabitants of oligotrophic offshore and also coastal waters, including eddy entrained water masses (Cesar-Ribeiro et al., 2020). Therefore, dinoflagellates, mixotrophs as well as parasites do not provide a unique signature for specific eddy conditions such as eddy age, origin or depth.

Conclusion

Cyclonic eddies that form in the upwelling region of the Northwest African continental shelf are unique ecosystems with trapped water masses within which distinctive protistan plankton communities evolve while the eddies age and travel westwards into sub-tropical oligotrophic offshore waters. A large proportion of the taxonomic metabarcodes from the protistan plankton communities in the eddies under surveillance and in the oligotrophic background waters could not be assigned to ecologically informative taxonomic entities. This demonstrates our knowledge gaps when it comes to the identification of presumably important members of the protistan plankton communities in the oceanic region under study. These gaps impede us from deeper interpretation of our massive sequence data sets to infer further information about eddies as unique ecosystems. Nevertheless, the data obtained in this study increased our current knowledge on the effect of mesoscale oceanic features in offshore waters off Northwest Afrika. Eddies increase the plankton diversity in these offshore waters to a remarkable extent, thus, providing hotspots in oligotrophic waters that may play an important role in carbon sequestration and for regional food webs (including top predators). The three eddies under study harbored distinctively different

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protistan plankton communities, which is most likely a result of the different developmental stages of the eddies. But also, seasonal effects as well as the location of the eddy's formations may play a role for recruiting the initial seed community in the eddy core and, hence, the further evolution of this community. Unique eddy-specific communities seem to evolve much faster in the photic zone (DCM) of the eddy cores compared to deeper layers. This became evident in our observation that even the youngest eddy CE_2019_19N_18W harbored already a plankton community that was notably different from the background water community. In the deeper water layers of the non-photic zone and the OMZ, it takes a longer time until a unique eddy-specific ecosystem has established and a characteristic protistan plankton community has evolved. This became evident in our observation that in deeper water layers of the youngest eddy the protistan plankton community was relatively similar to the one of the background waters. The same applied for the oldest eddy, where the deeper water masses could mingle with background waters, while in the surface waters of this eddy, plankton communities could still be separated from background waters (even though there was already a relatively high similarity). This observation seems logic as cyclonic eddies are typically conical structures that are tapering in the deeper water layers. Accordingly, the tapering end is the first where intrusions and leakages occur as soon as the decrease in vorticity heralds in the collapse of an eddy. Thus, we can conclude that eddy-specific deep-water communities are relatively short lived. Therefore, the life-span of a mesoscale eddy, which may typically take from few weeks to many months (Duo et al., 2019) is an especially important criterion for the evolution of protistan plankton communities in the deep-water layers of an eddy and the way how eddies influence regional diversity patterns in deeper waters. Only little is known from previous studies as most investigations focused on eddy surface waters. More research is needed to better understand the effects of eddies on ecosystem diversity and function in deeper water layers.

Our results showed impressively that the core of mesoscale eddies can support vastly different ecological key players that have the potential to influence a regional offshore ecosystem in different ways. Major mechanisms include top-down control through parasites, carbon fixation through free-living microalgae or phototrophic endosymbionts, or phagotrophy across different domains of life and organismic size classes. Concerted efforts are now to reveal whether the numerical dominance of different taxon groups (in terms of sequence reads) is also mirrored in the functional

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diversity of the eddy cores, which will reveal further information on the mechanisms how mesoscale ocean features alter sub-tropical oligotrophic offshore ecosystems.

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Data availability

The sequence data files are deposited at the Sequence Read Archive of the National Center for Biotechnology Information under project number PRJNA795916. All remaining data will be made available at the PANGEA database.

Author contribution

SK and TS designed the scientific study, analyzed the data and wrote the paper. MN and HB contributed in the scientific study design and commented on the paper. TF, did the eddy reconstruction, sampling site alignment and commented on the paper.

Competing interests

The authors declare that they have no conflict of interest.

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Figure legends

Figure 1. Schematic representation of eddy locations at the time of sampling, of individual sample locations within each eddy (CE_2019_19N_18W, CE_2019_18N_20W, CE_2019_14N_25W) and at the two background waters (reference sites CVOO and CB).

Figure 2. PCA of eddy stations and background waters (reference sites CVOO and CB) based on physico-chemical parameters

Figure 3. ASV richness and Shannon Index *H'* as measures of alpha-diversity of protistan plankton communities in the DCM (a), sub-DCM (b) and OMZ (c) of the three eddies under study (CE_2019_19N_18W, CE_2019_18N_20W, CE_2019_14N_25W) and the background reference waters (CVOO and CB).

Figure 4. Effect of the three eddies under study (CE_2019_19N_18W, CE_2019_18N_20W, CE_2019_14N_25W) on regional ASV richness and Shannon Index *H'* of protistan plankton communities in the DCM (a), sub-DCM (b) and OMZ (c). The y-axis shows a decrease of richness and diversity in the two background waters CVOO and CB resulting from the eddy-specific communities that were transported into the offshore background waters during the westward-bound eddy trajectories.

Figure 5. Beta-diversity (NMDS based on BC-distances of ASV-to-sample matrix) of protistan plankton communities in the DCM (red), sub-DCM (green) and OMZ (blue) of the three eddies under study (CE_2019_19N_18W, CE_2019_18N_20W, CE_2019_14N_25W, coded by symbol shape). NMDS stress = 0.121. Correlation results of environmental parameters with NMDS axes are shown in Table 2.

Figure 6. Partitioning of diversity (Bray Curtis distance-based dendrograms) of protistan plankton communities in the three eddies under study and the two background waters (reference sites CVOO and CB) for the DCM (a), the sub-DCM (b) and the OMZ (c).

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Tables

Table 1. Coordinates of sample sites located inside the mesoscale eddy structures and the two reference sites with the corresponding sampling dates and ship cruises.

Sampling station	latitude	longitude	Reference/Eddy	date	cruise
Reference_CB	21.17	-20.92	Reference	07.12.2019	M156
CE_2019_19N_18W	18.58	-18.08	Eddy	07.22.2019	M156
Reference_CVOO	17.59	-24.28	Reference	12.09.2019	M160
CE_2019_14N_25W(1)	14.44	-25.25	Eddy	12.13.2019	M160
CE_2019_14N_25W(2)	14.62	-25.07	Eddy	12.13.2019	M160
CE_2019_14N_25W(3)	14.72	-25.49	Eddy	12.15.2019	M160
CE_2019_18N_20W(1)	17.61	-20.60	Eddy	11.30.2019	M160
CE_2019_18N_20W(2)	17.81	-20.80	Eddy	12.02.2019	M160
CE_2019_18N_20W(3)	17.82	-20.60	Eddy	12.03.2019	M160
CE_2019_18N_20W(4)	17.81	-20.41	Eddy	12.03.2019	M160

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Table 2. Correlation results from *envfit* analyses of environmental parameters and NMDS axes 1 and 2 of NMDS analyses (beta diversity) of protistan plankton communities in the DCM, sub-DCM and OMZ of the three eddies under study (see Fig. 4).

Parameter	NMDS1 axis	NMDS2 axis	R^2	p-value
Nitrate	0,85	-0,41	0,90	1,00E-04
Phosphate	0,84	-0,43	0,88	1,00E-04
Orthosilicic acid	0,85	-0,40	0,87	1,00E-04
Temperature	-0,84	0,48	0,93	1,00E-04
Salinity	-0,76	0,45	0,77	1,00E-04
Diss. Oxygen	-0,66	0,49	0,68	1,00E-04
Density	0,81	-0,47	0,88	1,00E-04
Fluorescence	-0,86	0,33	0,84	1,00E-04
Turbidity	-0,78	-0,12	0,62	2,00E-04

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1298 **Figures**

Figure 1

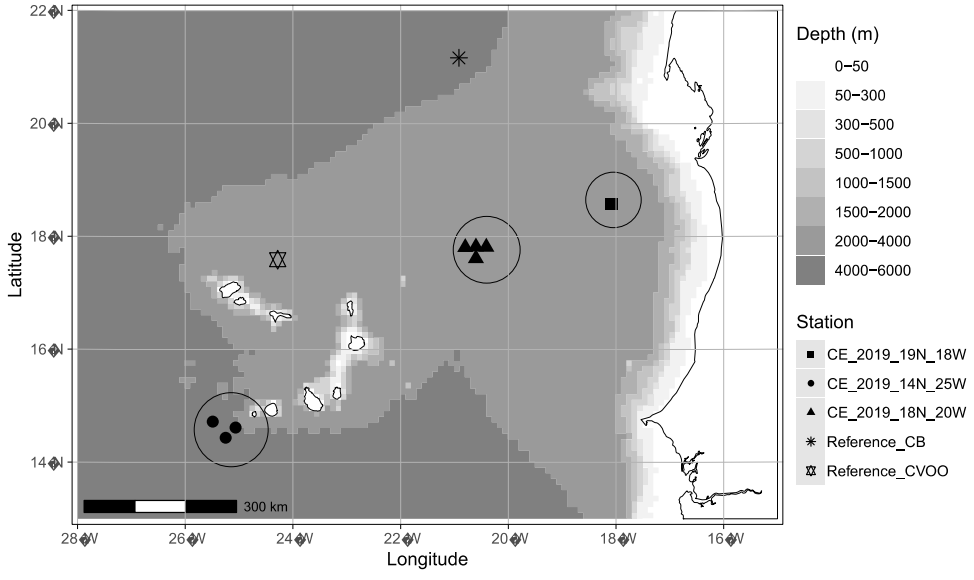

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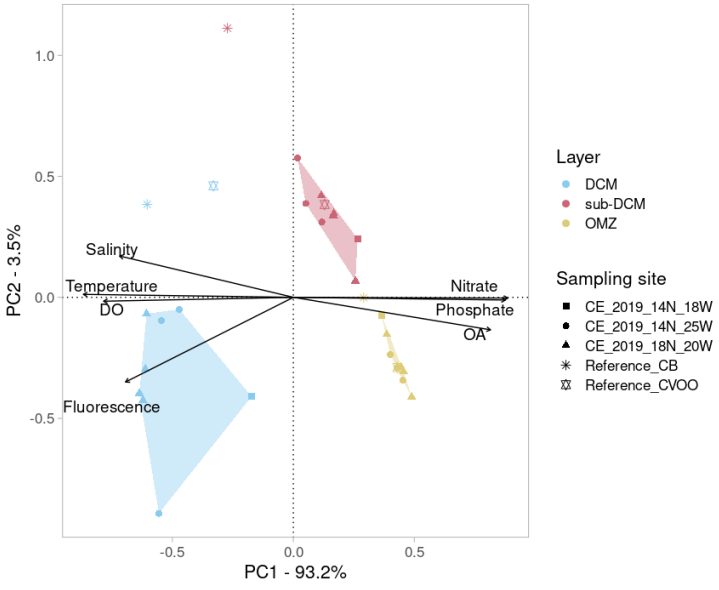
Figure 2



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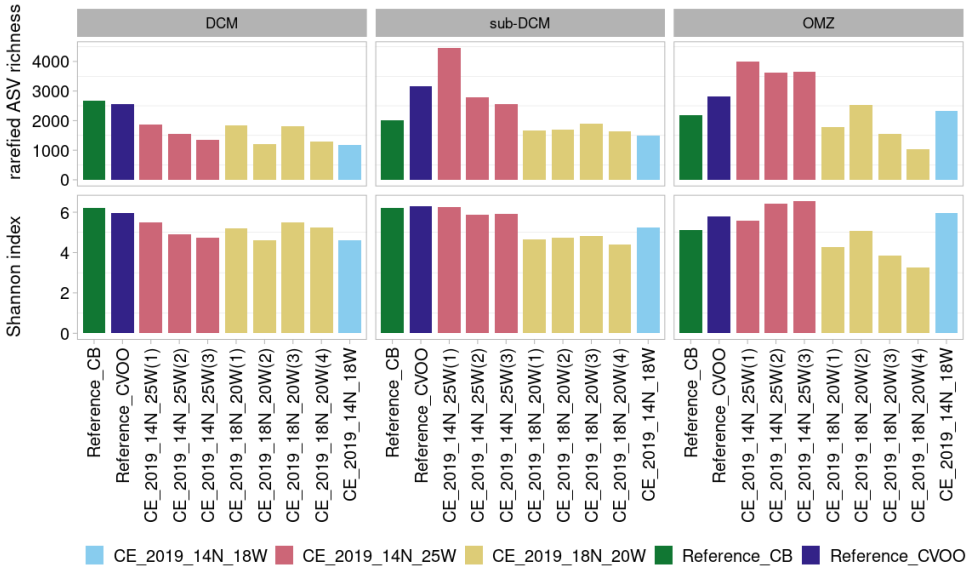

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Figure 4

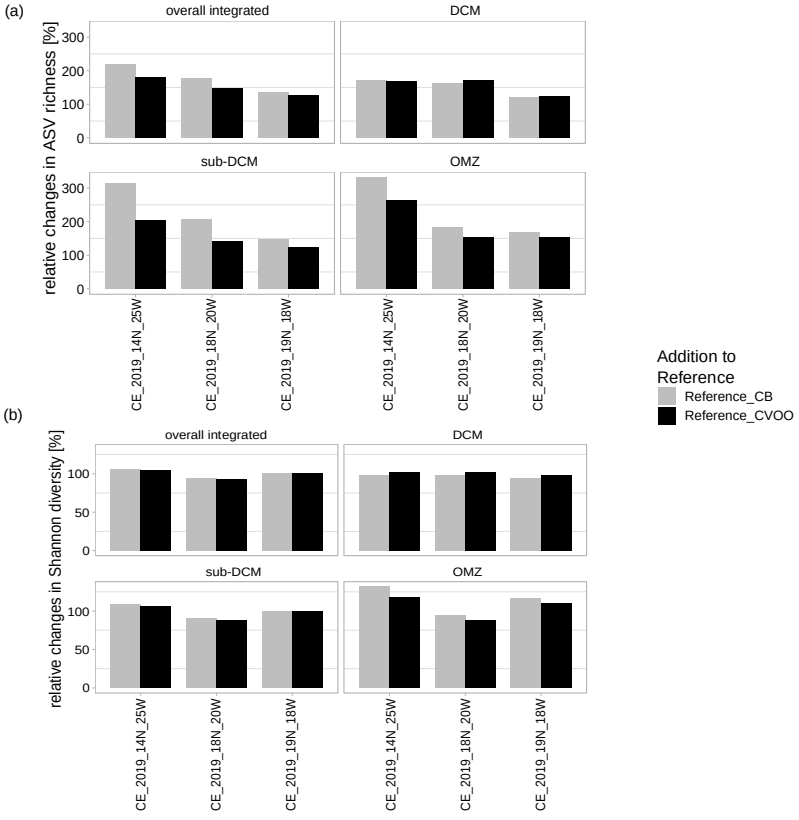

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Figure 5

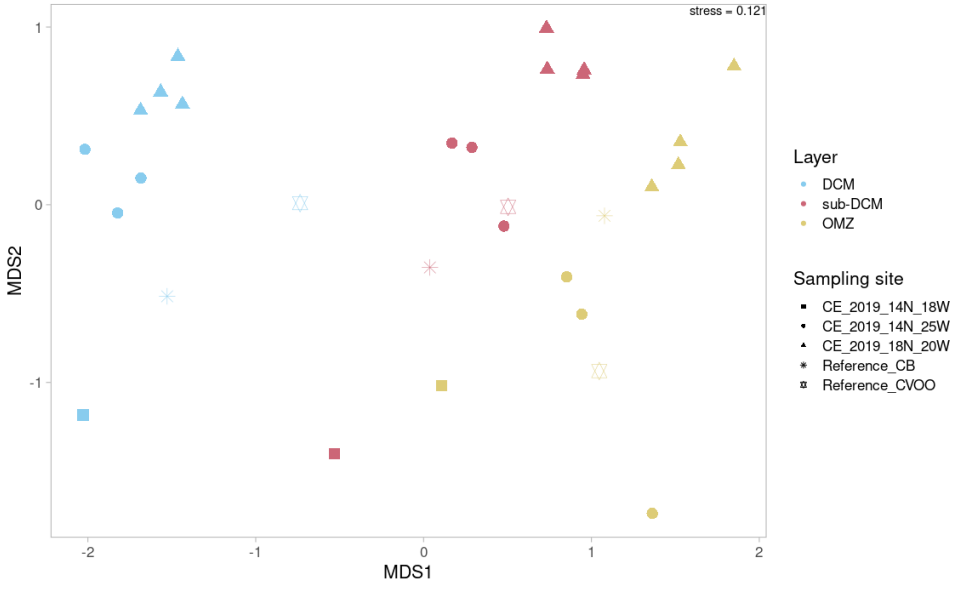

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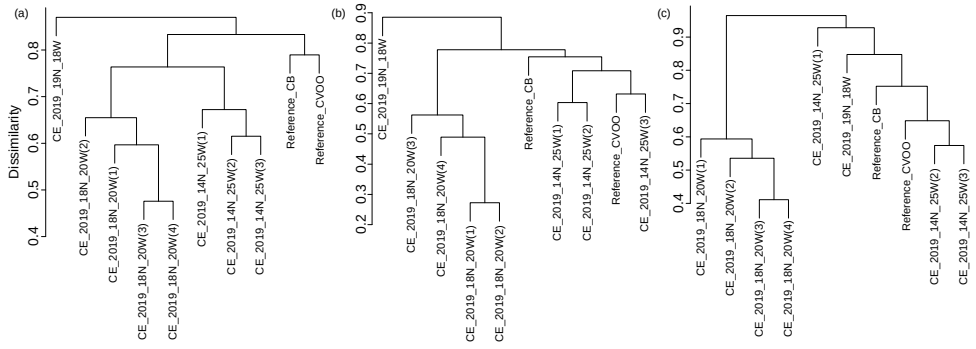
Figure 6



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