

OCEANOGRAPHY

Visualizing the barely detectable: Particle characteristics of the elusive aquatic gel phase in the northeastern subtropical Atlantic

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Marine gels constitute a large yet poorly explored ocean carbon pool. Shadow imaging was tuned to detect particles at two sensitivity levels: high-optical density particles (HODP), resembling the particles captured by most imaging systems, and low-optical density particles (LODP), representing a vastly more abundant and gel-dominated pool. This method enabled us to map and characterize a portion of oceanic gels without filtration and staining. In the subtropical northeast Atlantic, LODP outnumbered HODP of the same size by one or two orders of magnitude. Morphology and particle number spectra differed greatly between the two pools, with LODP showing greater morphological homogeneity and diel pulses in abundance. The inclusion of LODP is critical for the parameterization and quantitative interpretation of biogeochemical models that directly affect global carbon flux estimates. Due to a paucity of in situ observations, these particles are largely absent from even the most advanced ocean models.

INTRODUCTION

In the ocean biogeochemical system, the gel organic matter (GOM) pool consists of particles that bridge the critical transition from dissolved organic matter (DOM) to particulate organic matter (POM). Based on the type of stains used for their detection, GOM has been typically classified either as transparent exopolymer particles (TEP) (1, 2) or Coomassie brilliant blue-stained particles (CSP) (3–6), with unique features characterized as being highly transparent (7), sticky (8, 9), surface abundant (10), and fractal (11–15). For both particle classes, their visualization depends on retention onto membrane filters and staining, thus making their appearance different from what they are in situ.

The characteristics of these stained particle groups, especially TEP, have been extensively investigated in the oceanographic context. TEP precursors are released through biological processes dominated by phytoplankton and bacterial exudation, while abiotic mechanisms allow free acidic polysaccharides in the system to further coagulate into TEP once their concentration exceeds a threshold (4, 7, 14). Meanwhile, the spatial patterns of TEP remain a matter of debate. Several studies have reported that TEP concentrations are higher in eutrophic regions and in the upper, more productive layers of the ocean (7, 11, 16–20). Others have suggested that the production of TEP is not correlated with phytoplankton biomass, and TEP can contribute more to particulate organic carbon (POC) in oligotrophic environments than in other systems (21, 22).

GOM is estimated to account for 9 to 28% of the ocean's total carbon pool, which is markedly higher than the 4–11% contributed by POM (4, 14, 23–26). Other studies conducted across diverse oceanic systems inferred that 10% to nearly 100% of POC is contributed by TEP, with elevated variability observed in certain areas and in the presence of mesoscale structures [e.g., cyclonic eddies; 8, 22, 27–29].

While TEP have been investigated in most oceanic environments, the overall GOM pool may be understudied owing to the specificity of dyes, such as Alcian Blue (for acidic polysaccharides) and Coomassie brilliant blue (for proteins). Most in situ optical devices (e.g., Video Plankton Recorder, Underwater Vision Profiler, In Situ Ichthyoplankton Imaging System, Laser In Situ Scattering and Transmissometry, and the Imaging FlowCytobot) used in plankton research are insensitive to gels at their current settings. In contrast, the focused shadow imaging (FoSI) system is sensitive to a portion of the oceanic gel phase, depending on the setting of the illumination and the sensitivity in the postprocessing step of the image analysis (30). In nature, gel particles do not occur in pure form but on a continuum, depending on the degree to which they are colonized by microorganisms and have less transparent materials, such as sediments and phytoplankton, adhering to them. To our knowledge, no prior work has directly characterized the in situ distribution and properties of unmodified (i.e., unfiltered and unstained) aquatic gels.

Shadowgraphy is now the only available particle imaging technique that allows for a nonarbitrary setting of the detection threshold based on minimum absorbance [i.e., optical density (OD)] values. This calibration of digital grayscale values is achieved by a stepped neutral density filter consisting of 11 OD gradients from 0.04 to 1 (30, 31). Using this method, we assessed the spatial and temporal patterns of particles in the northeast Atlantic Ocean, categorizing them into low-optical density particles (LODP; OD > 0.1466) and high-optical density particles (HODP; OD > 0.2246), representing opaque particles that are detectable by conventional optical devices (31–33). By this definition, the LODP pool contains the HODP pool, and, strictly speaking, gels would comprise the difference between LODP and HODP. However, the separation by the difference is ambiguous because pure gels do not exist in nature as they are highly colonized by microbes (14), and many aquatic particles contain both opaque and transparent components (30, 34). Many small HODP can be held together by an almost invisible gel matrix and thus form one larger particle (30). The second reason for using LODP (and not the difference between LODP and HODP) is that a major goal of this study was to demonstrate the effect of the selection of the sensitivity threshold on particle characteristics including the particle

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number spectrum. As we will demonstrate by the estimation of the relative gel fraction (RGF), the LODP vastly outnumber the HODP, so that HODP have a negligible effect on the characteristics of the LODP pool. For the purpose of analyzing particle-size spectra and morphological characteristics, we approximate $\text{LODP} \approx (\text{LODP} - \text{HODP})$ and thus operationally associate the LODP with gels, which can be defined as three-dimensional networks of organic polymers with seawater as solvent entrapped in the network pores (25), fully acknowledging that shadow imaging (as all other methods) can only account for a portion of GOM. We also calculated the RGF as $(\text{LODP} - \text{HODP})/\text{LODP}$ to better capture subtle variations in the relative proportion of gel-like particles and the denser particles.

Our observations were conducted across a broad environmental gradient, including the upwelling region of northwest Africa influenced by some turbid coastal waters (CI), the oligotrophic open ocean (OO), and the Azores region (AZ), which exhibit distinct particle characteristics. By acquiring data across these diverse marine environments, we aimed to test whether the dominance of gel-like particles is restricted to specific conditions or represents a ubiquitous and quantitatively dominant feature of marine ecosystems. The primary hypotheses for this study are thus as follows: (i) Morphological characteristics differ between the two particle pools. (ii) These particle pools exhibit spatial and temporal patterns independent of each other. (iii) By including the gel pool, aggregate particle characteristics, such as the slope of the particle number spectrum, change.

RESULTS

Spatial differences in environmental parameters

A clear regional difference was observed in the physical water column characteristics spanning from the Azores (AZ) to the Canary Islands

(CI; Figs. 1 and 2). Both the AZ and CI epipelagic layers contained elevated beam attenuation, fluorescence, particle numbers, and total particle volume (TPV) in comparison with the oligotrophic ocean (OO) (Fig. 2). The effects of the Northwest African upwelling system and the geological region were noticeable around the CI with its elevated turbidity signal (beam attenuation), particle numbers, and TPV. The distribution patterns in HODP were much closer to the distribution patterns in chlorophyll fluorescence (Fig. 2).

Particle quantification of two particle pools and the effect of spatial factors

The mean LODP abundances across the entire water column were 1×10^4 to $35 \times 10^4 \text{ liter}^{-1}$ in CI, 1×10^4 to $11 \times 10^4 \text{ liter}^{-1}$ in the OO, and 1×10^4 to $40 \times 10^4 \text{ liter}^{-1}$ in AZ. The comparison of LODP and HODP abundance showed that particle abundance in both pools significantly decreased with depth and leveled at $1 \times 10^4 \text{ liter}^{-1}$ for LODP in the bathypelagic zone, a trend that was consistent across all regions (Fig. 3). Particle abundance and TPV significantly differed between geographic regions and vertical depth strata (epi-, meso-, and bathypelagic zones), with LODP consistently vastly more abundant than HODP by one or two orders of magnitude ($P < 0.0001$; Table 1 and Fig. 3, A to C). While the two pools were highly correlated, substantial variability was found around the regression lines, even at log-log scale, indicating that abundances in one pool cannot be precisely predicted by the abundances in the other pool (Fig. 3). The slopes of the log-scale abundance between HODP and LODP ranged from 0.6 to 1.66 and thus frequently differed from 1, with only one case falling close to 1. This is indicative of curvilinear relationships in abundance between the two particle pools (Fig. 3). The examination of the spatial factors affecting particle distributions via aligned

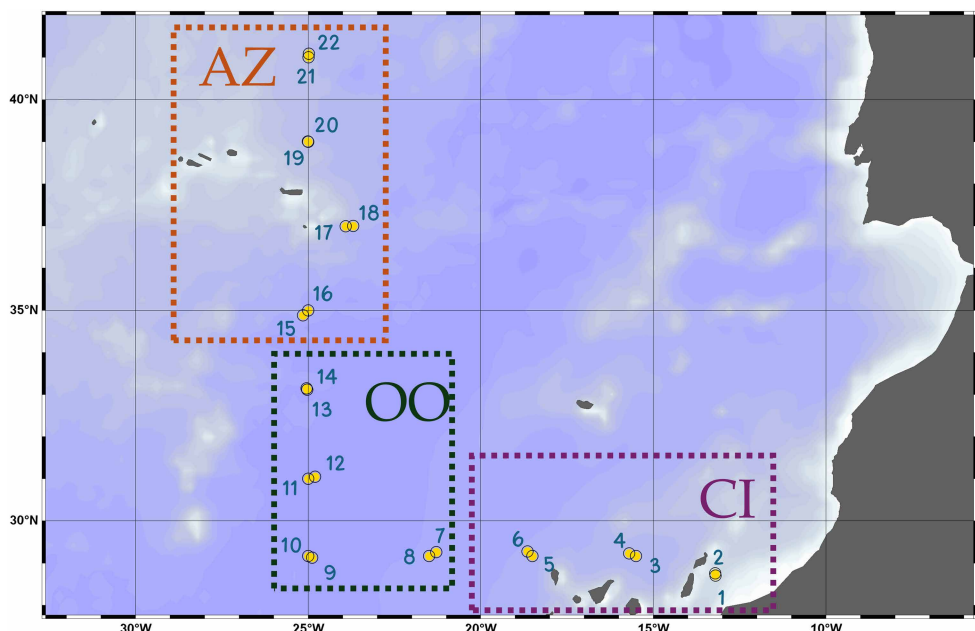


Fig. 1. Station map of the 22 casts during the DisEntangling Seasonality of Active Flux in the Ocean (DESAFÍO) expedition. The transect was divided into three regions based on the surface total particle volume (TPV): the Canary Islands region (CI; casts 1 to 6), the oligotrophic ocean region (OO; casts 7 to 14), and the Azores region (AZ; casts 15 to 22). For each station, CTD casts with the FoSI system were performed during the day (odd numbers) and at night (even numbers). Stations were visited in reverse numerical order.

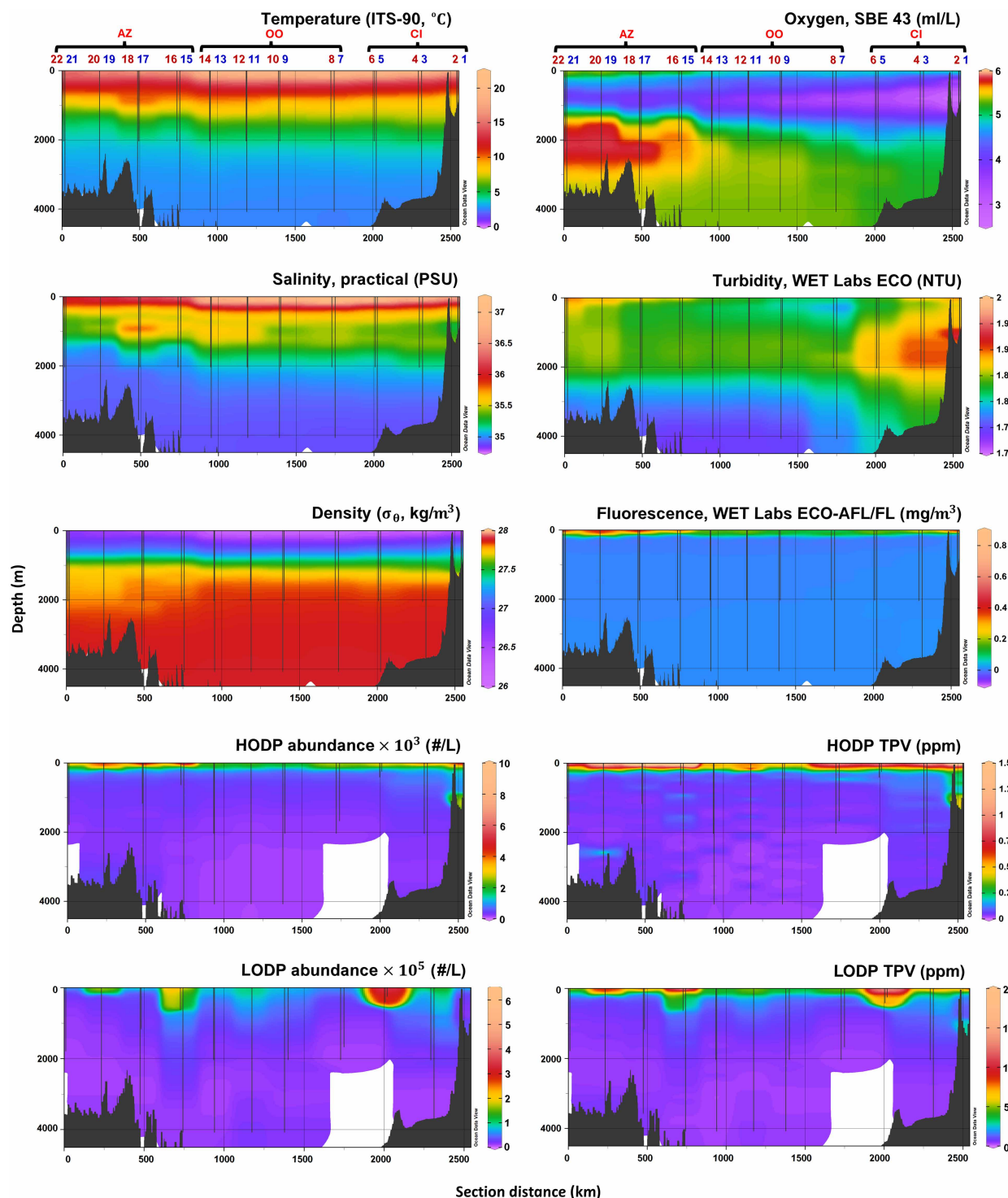


Fig. 2. Section profiles of the temperature, oxygen, salinity, turbidity, density, fluorescence, particle abundance, and TPV of HODP and LODP (size range, 23.5 to 1000 μm). The cast numbers and regions were marked along the top horizontal axis, with the vertical profiles of each cast shown by the solid vertical lines. L, liter.

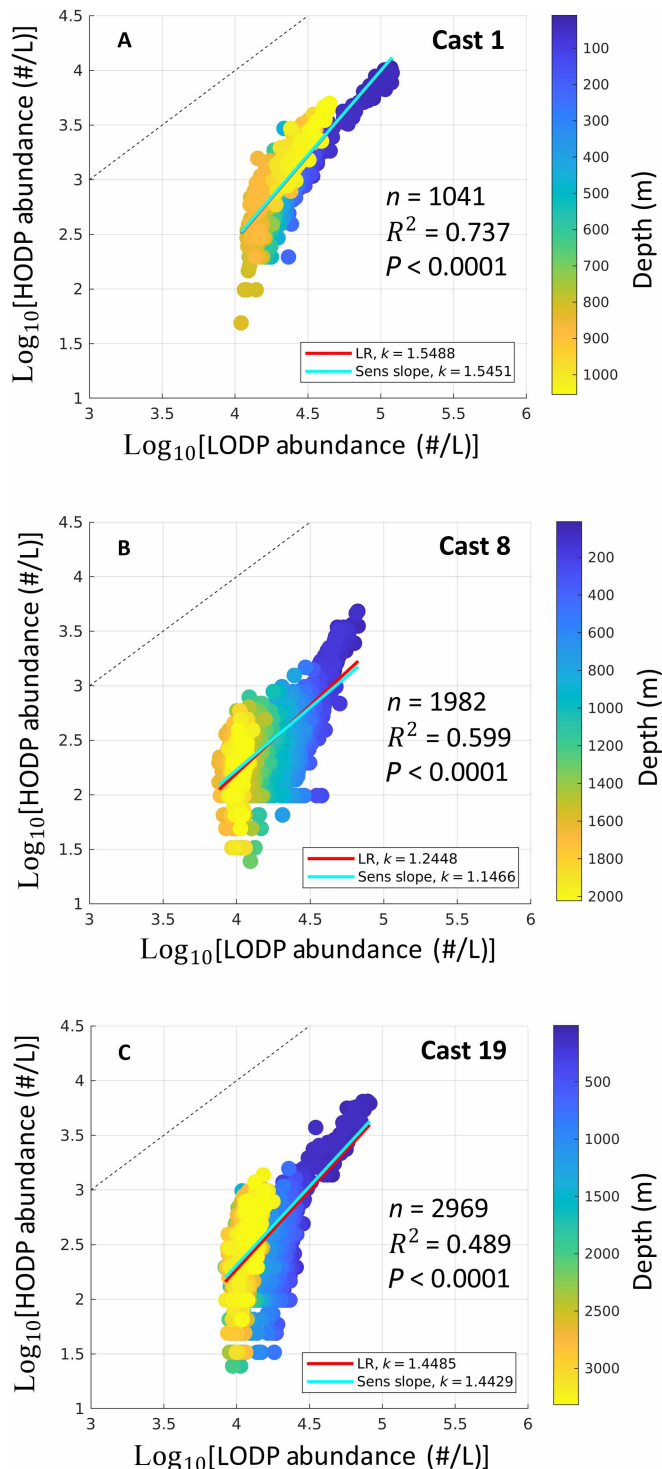


Fig. 3. Comparison of particle abundance between HODP and LODP. Three examples of HODP and LODP abundances at (A) cast 1, (B) cast 8, and (C) cast 19 (representing the three regions, respectively) showing the $\log_{10}$ -transformed particle abundance [# / liter (L)], where the dashed lines represent the 1:1 line. LR are the least-squares linear regressions; "Sen's slope" denotes the Theil-Sen analysis; k denotes the corresponding slopes. n , R^2 , and P denote the sample sizes, coefficients of determination, and P values of the linear regressions, respectively.

rank transform (ART) tests showed that the distribution of particle abundance and the TPV were significantly affected by the depth strata and geographic regions and their interaction terms (Table 1).

RGF with depth

Calculating the RGF as the total abundance of particles [i.e., $(\text{LODP} - \text{HODP}) \text{ LODP}^{-1}$] showed that most variability among stations occurred in the epipelagic layer (0 to 200 m) and the benthic nepheloid layer. These lower fractions of gels were due to the presence of optically denser particles, such as phytoplankton, at the surface and sediments in the nepheloid layer that decreased the proportions of gels slightly by 2 to 6% (Fig. 4, A to C).

Particle morphological characteristics in two particle pools

In most cases, the distributions and mean of the equivalent spherical diameter (ESD), OD, aspect ratio, and the circularity of particles changed significantly with depth (Fig. 5 and Table 2). However, while the means of ESD were significantly different, the mode of the distributions of particle sizes remained closely aligned. This indicates that the observed differences in means and medians were mostly due to the long tail in the distribution of HODP (i.e., the occurrence of rare but very large particles in the HODP pool). On the vertical scale, the distributions of both particle pools were significantly different across the three depth strata in respect to aspect ratio, OD, and circularity (Fig. 5 and Table 2, LODP by depth stratum and HODP by depth stratum). Both particle pools become smaller, less elongated, optically denser, and more circular with depth (P^+ , $P^\# < 0.0001$; here, the Kolmogorov-Smirnov distributional test is indicated with the superscripted +, and the Wilcoxon rank sum test for comparison of the means is indicated with the superscripted #). Comparisons of the two particle pools showed that, within the same depth zone, LODP were significantly smaller in size, more transparent, and less circular than the HODP (P^+ , $P^\# < 0.0001$; Table 2 and Fig. 5). The ESD was significantly different between the epipelagic and mesopelagic zones, as well as the epipelagic and bathypelagic zones; however, it was not significantly different between the mesopelagic and bathypelagic zones, a pattern that applied to both particle pools (LODP: $P^+ = 0.1897$, $P^\# = 0.4508$; and HODP: $P^+ = 0.1606$, $P^\# = 0.4020$). While HODP circularity increased with depth, it also exhibited a bimodal distribution in the epipelagic zone (Fig. 5 and Table 2, HODP by depth stratum). The aspect ratios of LODP were highly constrained around the averages at all depths, whereas the aspect ratios of HODP were highly variable (Fig. 5).

Regional trends in particle morphological characteristics were weaker than vertical trends across both particle pools. No significant difference was found in LODP size across the three regions, whereas HODP size increased significantly as it was measured from CI to AZ (Table 2, LODP and HODP by region). The aspect ratio of LODP was higher in CI than the OO, with the OO and AZ being quantitatively close; however, the latter was still significantly lower (Table 2, LODP by region), the reverse spatial trend of which was observed regarding HODP. In AZ, LODP were significantly more transparent in comparison with those in CI and the OO (Table 2, LODP by region), with a substantial reduction of OD being observed from CI to AZ in the HODP (Table 2, HODP by region). The circularity of HODP from CI to AZ (Table 2, HODP by region) differed from that of LODP, whose most circular particle clusters were found in the OO and least in AZ (Table 2, LODP by region).

Table 1. Statistics for both particle pools across space (by vertical zone and by geographic region). The effect of spatial factors on particle abundance and TPV and their combined effect (i.e., the interaction term, Geo * Vert) for both LODP and HODP using aligned rank transform (ART) analysis of variance (sample size per group, $n = 47,023$). Bold face indicates significance at $\alpha = 0.05$.

	Part. no.				TPV			
	LODP		HODP		LODP		HODP	
	F	P	F	P	F	P	F	P
Geographic region	3414.63	<0.0001	1244.13	<0.0001	2642.94	<0.0001	141.95	<0.0001
Vertical zone	9245.16	<0.0001	25210.79	<0.0001	18196.89	<0.0001	1689.83	<0.0001
Geo * Vert	628.88	<0.0001	550.86	<0.0001	649.76	<0.0001	65.04	<0.0001

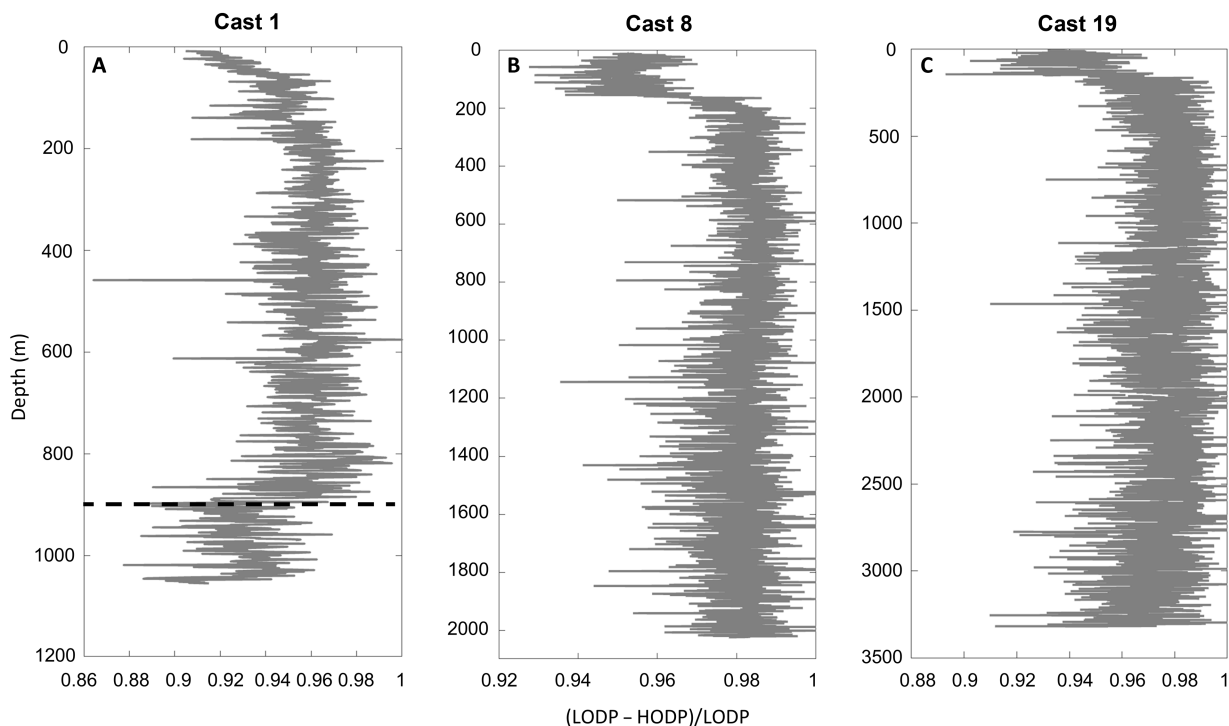


Fig. 4. Comparison of the RGF. Three examples of the profiles at (A) cast 1, (B) cast 8, and (C) cast 19 (representing the three regions, respectively), the same stations as Fig. 3. RGF was computed by normalizing the difference by low density-particle abundance: $(LODP - HODP)/LODP$. The dashed line in (A) represents the extent of the benthic nepheloid layer as determined by beam transmissometry. No nepheloid layers were detectable in the other two casts.

To better demonstrate the variation in particle shapes, Fig. 6 shows examples of particles falling within the various intervals of circularity and aspect ratios. Notably, a predominant number of particles with low circularities, especially less than 0.2, were classifiable as plankton (e.g., diatoms and copepods). By contrast, fecal pellet-shaped particles exhibited higher circularity but varied in their aspect ratios.

Diel particle dynamics

The two particle pools behaved differently with respect to the diel cycle. LODP abundance increased during the day and decreased during the night in the upper 50 m, likely due to increased photosynthates that promote gel production during the day and digestion in the dark (Fig. 7A). Notably, only some casts were useful for diel comparisons, as the daytime casts needed to be taken sufficiently late in the day to observe the buildup and sufficiently late at night to

observe the decrease. The Wilcoxon signed rank test showed significant diel differences in LODP abundance in the upper 50 m ($P_L = 0.0156$), but not in HODP abundance ($P_H = 0.4688$). None of the particle pools exhibited significant diel differences in the 50- to 100-m layer ($P_L = 0.0781$ and $P_H = 0.5781$, respectively). It is noteworthy that the particle abundance was not consistently higher during the day for the HODP, again indicating distinct differences in dynamics between the two particle pools (Fig. 7, B and D).

Particle number spectra

We have already demonstrated that the LODP vastly outnumbered the HODP; thus, the intercepts of the particle number spectra across all casts were much higher for LODP. However, we also observed significant variations in the slope of the particle-size spectra between both the particle pools and vertical depth strata (Fig. 8). The average slopes of LODP were -4.55 ± 0.26 (means $\pm$ SD) in the epipelagic

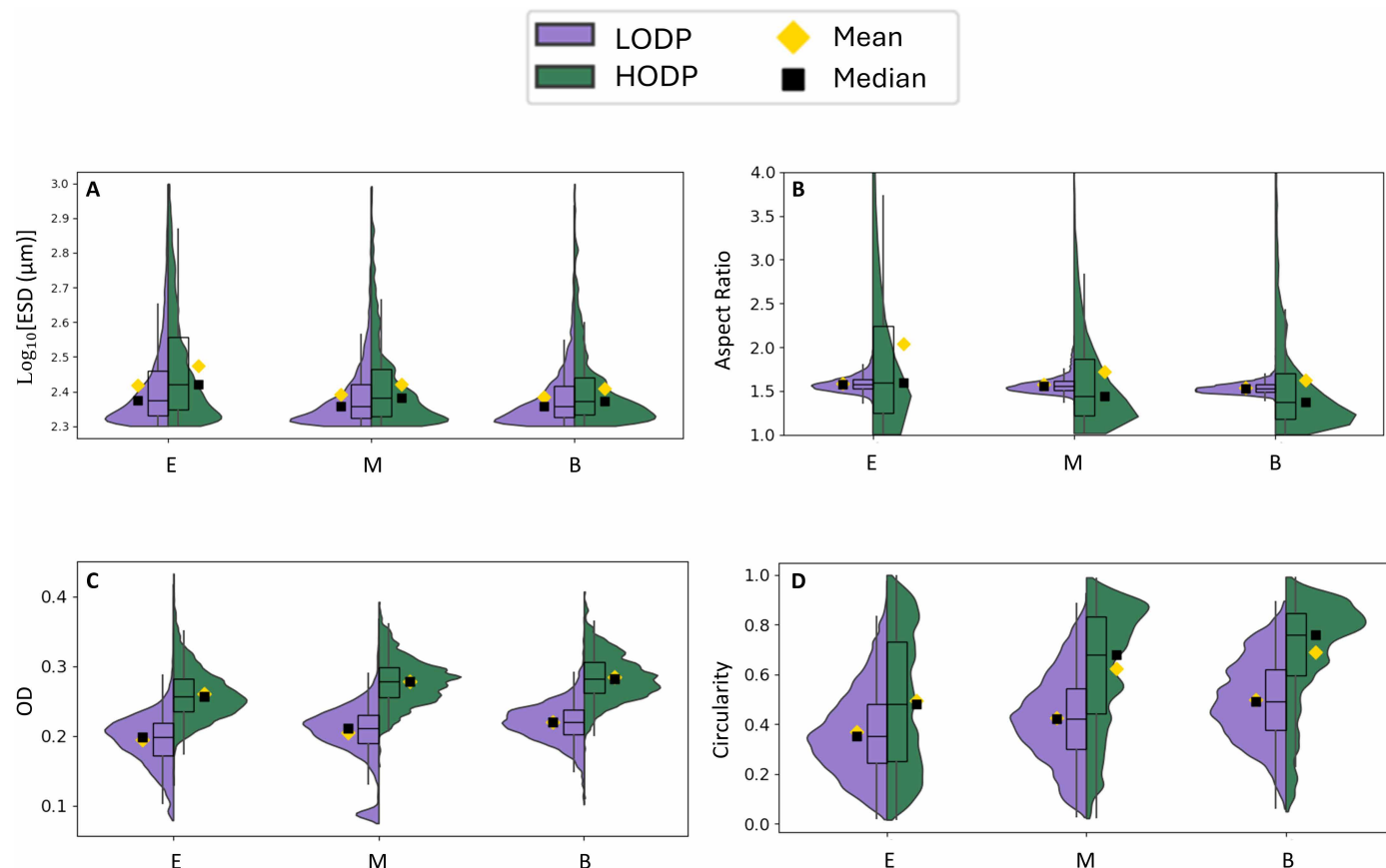


Fig. 5. Distribution of particle morphological characteristics in each depth stratum. Violin plots were overlaid with box plots for particle characteristics in different depth strata. Particle size range: 200 to 1000 μm . Epipelagic (E; ≤ 200 m), mesopelagic (M; 200 to 1000 m), and bathypelagic (B; > 1000 m). Violin plots were based on kernel density estimates using Gaussian smoothing and normalized to the same maximum widths. (A) The logarithm base 10 of the equivalent spherical diameter (ESD), (B) aspect ratio, (C) OD, and (D) circularity. Purple (left) represents LODP. Green (right) represents HODP.

zone and -5.11 ± 0.45 in the mesopelagic zone, whereas those of the HODP were -3.68 ± 0.23 and -3.90 ± 0.17 in the epipelagic and mesopelagic zones, respectively. The slopes were significantly different among the four groups in all pairwise comparisons [the analysis of covariance (ANCOVA) homogeneity of slopes tests; $P < 0.0001$].

DISCUSSION

Optical devices to study particle inventories and ocean carbon flux have been deployed for decades; however, the substantial impact of analytical thresholds on the particle morphological characteristics and their quantification remains largely neglected. Our study comprehensively investigated the vertical and horizontal distribution of gel-like (the numerically dominating LODP) and opaque (HODP) particles in the northeast Atlantic Ocean using shadowgraphy. We consistently observed the regional and vertical spatial patterns of both particle pools, with the abundance of LODP being ~ 1 to 3 orders of magnitude greater than HODP. The abundance of LODP decreased with depth at a lower rate than HODP, resulting in a higher proportion of LODP in the interior of the ocean. The decrease in both number (Figs. 2 and 3) and size (Fig. 5) of both pools is consistent with fragmentation by zooplankton and microbial digestion. The exceptions are benthic nepheloid layers that contribute optically dense

sediments to the HODP, thereby slightly decreasing the relative proportion of LODP (Fig. 4). Similarly, pigmented particles, such as phytoplankton, make particles more optically dense on average and contribute to a decrease of LODP in the epipelagic layer. We demonstrated significantly different trends of the two pools in terms of aspect ratio, OD, and circularity, whereas no significant difference was observed in terms of the average particle size across geographic regions.

Effect of spatial factors on particle morphological characteristics

The higher variability in morphological characteristics of HODP compared with that in LODP illustrates how the choice of thresholds markedly affects aggregate quantities, such as the average sizes and the morphological shapes of aquatic particles, because the cluster of opaque (higher OD) particles captured at a higher threshold is likely to consist of components attached to barely visible exopolymer matrices (30, 35, 36). Several studies have discovered microdomains contributing to the various levels of structures in the in situ natural exopolymer matrices, suggesting that exopolymer gels are more than merely heterogeneous random aggregates (37, 38). Although LODP abundance was orders of magnitude greater than HODP abundance, the total abundance of gels may still be underestimated. The documented gel abundance in seawater based on the abundance of TEP

Table 2. Statistics of morphological characteristics. Summary of *P* values from the Wilcoxon rank sum (comparing means) and Kolmogorov-Smirnov (K-S) test (comparing distributions) on four parameters: equivalent spherical diameter (ESD), aspect ratio, OD, and circularity. The *P* values indicate pairwise comparisons between three vertical depth strata [epipelagic (E), mesopelagic (M), and bathypelagic (B) zones] or between regions [Canary Islands (CI), the oligotrophic ocean (OO), and Azores (AZ)]. The *P* values in italics indicate results that were not significant at the α level of 0.05. Sample sizes *n* for each morphological characteristic are 16,421 (LODP, E), 3787 (LODP, M), 3215 (LODP, B), 7701 (LODP, CI), 8080 (LODP, OO), 7642 (LODP, AZ), 2054 (HODP, E), 723 (HODP, M), 672 (HODP, B), 1179 (HODP, CI), 1092 (HODP, OO), and 1178 (HODP, AZ).

LODP by depth stratum						
	Rank sum			K-S test		
	E/M	E/B	M/B	E/M	E/B	M/B
ESD	<0.0001	<0.0001	<i>0.4508</i>	<0.0001	<0.0001	<i>0.1897</i>
Aspect ratio	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
OD	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Circularity	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
HODP by depth stratum						
	Rank sum			K-S test		
	E/M	E/B	M/B	E/M	E/B	M/B
ESD	<0.0001	<0.0001	<i>0.4020</i>	<0.0001	<0.0001	<i>0.1606</i>
Aspect ratio	<0.0001	<0.0001	0.0023	<0.0001	<0.0001	0.0018
OD	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Circularity	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
LODP by region						
	Rank sum			K-S test		
	CI/OO	CI/AZ	OO/AZ	CI/OO	CI/AZ	OO/AZ
ESD	0.3972	0.5023	0.8818	0.2448	0.2236	0.7324
Aspect ratio	0.0259	<0.0001	<0.0001	0.0109	<0.0001	<0.0001
OD	0.6758	<0.0001	<0.0001	0.0089	<0.0001	<0.0001
Circularity	0.0080	<0.0001	<0.0001	0.0137	<0.0001	<0.0001
HODP by region						
	Rank sum			K-S test		
	CI/OO	CI/AZ	OO/AZ	CI/OO	CI/AZ	OO/AZ
ESD	0.0359	<0.0001	0.0139	0.0986	<0.0001	0.0066
Aspect ratio	0.7076	0.0039	0.0225	0.1918	0.0009	0.0028
OD	0.0012	<0.0001	0.0001	0.0009	<0.0001	0.0075
Circularity	0.0027	<0.0001	0.0287	0.0008	<0.0001	0.0242

and CSP was $\sim 10^6$ to 10^8 liter⁻¹ (14, 39). In contrast, LODP concentrations in our analysis only ranged from 10^4 to 4×10^5 liter⁻¹. However, at least some of the difference in numbers between LODP and previously observed TEP abundances can be explained by the differences in observable size ranges as a simple calculation will illustrate. Enumeration using Alcian Blue staining, for instance, detects particles as small as 3 μ m (14). The power-law spectrum of particle numbers with size is

$$\frac{dN}{dl} = al^{-b} \tag{1}$$

where *N* is the particle number, *l* is the linear dimension of the particles, *a* is scalar, and *b* is the slope of the number spectrum. Integration of particle numbers from a minimum size (*l*_{min}) to infinity is therefore

$$N(>l_{\min}) = \int_{l_{\min}}^{\infty} al^{-b} = \frac{a}{b-1} \times l_{\min}^{(1-b)} \tag{2}$$

The ratio of integrated TEP particle numbers for the two size ranges thus collapses to

$$\frac{N_{>23.5}}{N_{>3}} = \left(\frac{23.5}{3}\right)^{(1-b)} \tag{3}$$

Using a frequently observed slope of -3 for TEP particles (14), the proportion of particle numbers in the >23.5- μ m-size fraction is therefore 0.016 or 1.6% of the numbers in the >3- μ m-size fraction. Based on the previously mentioned range of 10^6 to 10^8 TEP particles liter⁻¹, the estimated abundance for TEP larger than 23.5 μ m then becomes 1.6×10^4 to 1.6×10^6 liter⁻¹, which broadly overlaps with our observed LODP concentrations of 10^4 to 4×10^5 liter⁻¹.

A considerable proportion of TEP recognized by the Alcian Blue staining approach thus remained undetected by FoSI due to the size resolution limit. However, despite their higher abundance, the overall contribution of those “missing” TEP less than 23.5 μ m is minor to the TPV and carbon pool compared to larger particles (14). Our findings therefore suggest that, although the FoSI system undersampled

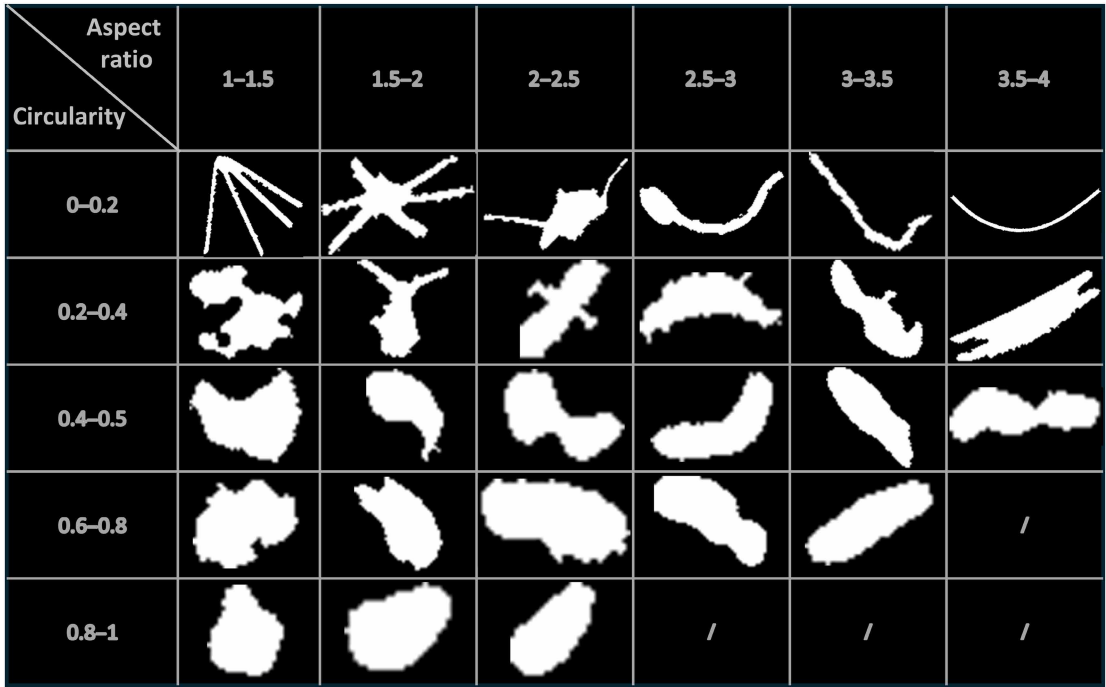


Fig. 6. Examples of binarized particle reconstructions. The particles are displayed in a sequence of different aspect ratios and circularities. The aspect ratio is defined as that of an ellipse with the same second moment as the particles. “/”, no examples found.

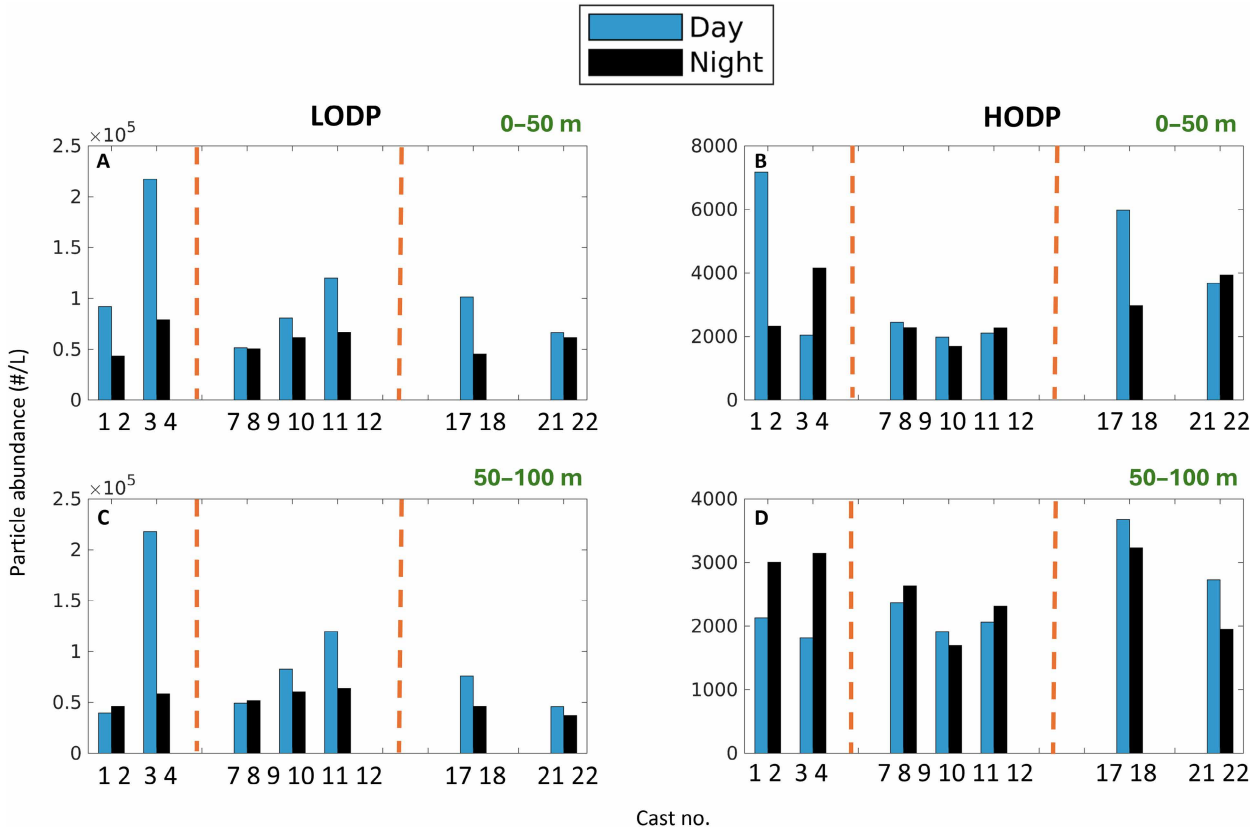


Fig. 7. Diel patterns of particle abundances by depth strata and particle pools. (A and B) 0 to 50 m and (C and D) 50 to 100 m for LODP [(A) and (C)] and HODP [(B) and (D)] with size ranging from 23.5 to 1000 μm . The blue bars represent day abundances, and the black bars signify night abundances. The dashed lines delineate the three regions [Canary Islands (CI), the oligotrophic ocean (OO), and Azores (AZ)].

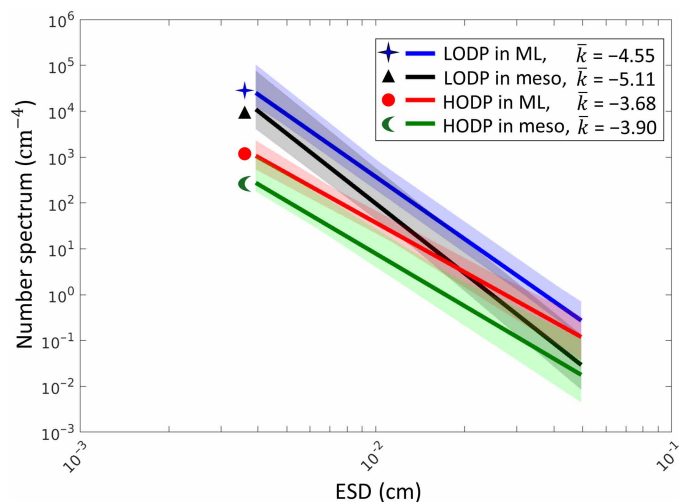


Fig. 8. Particle number spectra in vertical depth strata. The blue star represents the mean of particle spectrum regression model slopes for LODP in the epipelagic layer (ML) with a mean slope $k = -4.55$ (95% confidence limits, ± 0.26). The black triangle represents the mean of LODP in the mesopelagic zone with a slope $k = -5.11 \pm 0.45$. The red circle represents the mean slope of HODP in the ML with slope $k = -3.68 \pm 0.23$. The green crescent represents the mean of HODP in the mesopelagic zone with the slope $k = -3.90 \pm 0.17$. The background color bands illustrate the ranges of the particle spectra of all casts. The slopes were significantly different for each group at $P < 0.05$ level according to an analysis of covariance (ANCOVA) homogeneity of slopes test.

a significant portion of ultratransparent and small gel particles, it captured most of the bulk carbon pool associated with larger aggregates. Another challenge in comparing our results with stain-based assays is that TEP represent only one subset of the entire GOM pool. Oceanic gels identified by our protocol at threshold 11 (OD of 0.1466) likely included not only TEP but also other macrogels within the same OD interval (e.g., CSP).

Similarly to the LODP, the TEP concentration varied across some oceanic systems. Several studies have observed substantially higher TEP levels in coastal regions, especially within upwelling systems (22, 25, 40–44), whereas others have found no significant regional differences when comparing the Arctic Ocean with the Pacific Ocean or comparing various parts of the Pacific Ocean (9, 45). The particle distributions in casts 1 and 2 were affected by the extension of Northwest African upwelling systems toward the ocean and its proximity to the CI, which is indicated by the boost of mesoplankton biomass around the Canary upwelling system (46). The vertical distribution of LODP also aligned closely with TEP distribution patterns investigated previously (2, 11, 18, 22, 40, 47). Most reported TEP concentrations were highest in the sunlit zone; however, a few studies in the Pacific have documented relatively uniform vertical profiles (18, 48). The particle size in both pools decreased with greater depth (Fig. 5A), which is also consistent with previous studies (25, 47). High photosynthetic rates, combined with enhanced heterotrophic bacterial metabolism, typically boost the release of exopolymers and thus the formation of particles. The diel patterns of LODP were notably similar to those observed on marine snow, with abundances rising during the day and declining at night (49). In our study, the pattern was observed only in LODP and not in HODP and only in the upper 50 m.

It is self-evident that the LODP have a lower average OD than the HODP; however, the difference in average OD (i.e., above the threshold cutoff) is still interesting to examine (Fig. 5C). For instance, a gel-like particle can have few or many dense inclusions. Although no documented data were available for direct comparisons, the correlation between OD and depth in the current study appears to corroborate observations of the variability of density-induced settling velocities with depth (50). It is also unsurprising that gels are more homogeneous in aspect ratio, featureless, and “blobby,” because the formation of gel matrices is determined by the interaction of polysaccharides (51).

Particle shape determines particle settling velocity in fluid systems with low Reynolds numbers, especially for plankton (52). As both the number of particles and their average morphological characteristics change depending on the sensitivity threshold levels of the imaging systems, it is important to report this information in future studies. The thresholds used in the analysis of previous studies were either subjectively selected via visual inspection or varied depending on the neighboring pixels. Taking our images as an example, the average particle ESD of LODP and HODP was 270.4 and 308.8 μm , respectively, in the euphotic zone, which means that they differed by $\sim 15\%$. Whether particle sinking velocity is a function of the square of particle radius as in the original Stokes’ law (53) or is further modified by taking the fractal dimensions of particles into account (54), the different average particle sizes can lead to biases in the estimation of particle fluxes. The bias is further exacerbated when the density differences between the particle pools are considered. For instance, gels are typically considered neutrally and, in some cases, even positively buoyant (55). The problem of arbitrary detection thresholds, which, in turn, affects particle numbers and characteristics, is not restricted to shadowgraph systems; scattering sensors are similarly affected by the optical densities of particles (56–58). However, no imaging system or staining protocol can capture the entirety of the gel pool to date, and more work is needed to fully account for GOM in aquatic systems.

Low relative fraction of gels in the top and bottom of the ocean

The LODP have less vertical attenuation compared to the HODP, which is in line with previous observations of TEP (9, 11). In addition, relatively higher abundances of HODP were found in the top and bottom layers of the ocean, showing a similar pattern in the deeper waters, as described by Santschi *et al.* (59). However, the relative change in the proportion of LODP was only $\sim 10\%$ overall.

Previous studies have reported contrasting patterns in the TEP to POC ratio: Some have documented low ratios in the epipelagic layer and high ratios at depth (25, 29, 60), whereas other studies have suggested the opposite trend (43, 61). However, any conclusion about the relative contribution of gel particles to POC has to be reached with caution as the carbon density of gels is much lower than that of optically denser particles.

In the productive mixed layer (ML), where strong photosynthesis is supported by ample sunlight, the RGF was low owing to the predominant biomass contributed by pigmented phytoplankton, as was apparent in the fluorometer data. In the bottom nepheloid layer, sediment resuspension due to turbulence can lead to the mixing of marine particles with opaque sediments, such as clay minerals and biogenic sediments [e.g., diatom frustules and coccoliths; (62)].

Diel patterns of particles from 23.5 to 1000 μm

The diel pulses in HODP exhibited high variability, likely affected by the vertical migration of zooplankton and associated increases in biomass and fecal material (63). Migrating zooplankton and, thus, their fecal pellets fall mainly within the HODP pool and could contribute to some of the higher observed values at night. In contrast, the daytime peak in gel abundance may reflect the accumulation of photosynthates produced by phytoplankton in the photic zone. A similar diurnal pattern was reported for marine snow in the California upwelling system, in which nocturnal declines, however, were attributed primarily to grazing by migrating zooplankton (49).

Effects of the gel pool on the particle-size spectrum

Previously reported slopes of particle size distribution (PSD), ranging from -4 to -2 worldwide (64, 65), were similar to our observations of HODP but notably shallower than the LODP number spectra. As previously suggested by Buonassissi and Dierssen (66), even a trivial difference in the estimation of small particles can alter the overall slope of the particle-size spectrum. This is an important result as the PSD slope has been used diagnostically in aquatic systems. Studies suggest that oligotrophic systems are characterized by steeper slopes than eutrophic areas such as upwelling systems (32, 65, 67). In our PSD analysis, the PSD slopes were always steeper when the gel fraction was included, which indicates that a high proportion of LODP at the lower end of the spectrum went undetected by other methods (Fig. 8). Using conventional optical devices could thus lead to an underestimation of small particle fluxes in the oligotrophic regions (68).

Mari and Burd (69) inferred that the PSD slope of non-TEP particles within 1 to 200 μm was steeper than that of TEP in the same size range. This apparent contradiction with our results may have several causes. By using lower thresholding in combination with our image analysis protocol, we included the more abundant small and optically less dense particles that are typically overlooked at higher detection thresholds. In shadowgraphy, small particles are inherently optically less dense because the path length of the light is shorter and less attenuated when passing through the particles. By reducing the threshold to obtain higher sensitivity, the algorithm detects a higher fraction of smaller particles and consequently increases the steepness of the particle number slope. Another possible reason for the discrepancy of slopes is that gels may have aggregated and stacked during the filtration and staining process and smaller gels now appear as larger aggregates, thus decreasing the apparent slope. Therefore, more studies are needed to provide a systematic inter-comparison of in situ optical and staining methods.

The significance of including the gel fraction in biogeochemical flux models

The substantial increase of particle abundances when more gel-like particles (missed by conventional optical methods) are included has fundamental implications for understanding the ecology of aquatic ecosystems. First, amorphous aggregates provide surfaces that are always more densely populated by microbes than ambient water (14). Concentrations of microbes on aggregates, including even the faintest gels, exceed those living freely suspended 100 to 10,000 times (16, 70). The dense collocation of microbes on aggregates, combined with distinct microbial communities on particles, allows for processes that are not present in the surrounding water. Depending on how particles are enumerated, the proportion of particle-associated microbes

and processes shifts greatly. Now, we know little about the types of particle-associated processes due to methodological constraints and even less about their rates and overall contributions to pelagic processes. The second major implication of the large pool of LODP is that these particles serve as an important “glue” in the formation of larger, fast-sinking particles that contribute substantially to global carbon fluxes. Particle stickiness has long been suspected as a reason why simulated carbon flux poorly agreed with observations (70). A more accurate assessment of true particle numbers and size and shape characteristics of more transparent particles is thus important for calculating more realistic encounter rates in coagulation and vertical flux models (71–73).

Our findings aid in the exploration of in situ dynamics of an understudied particle pool in aquatic systems that are likely to directly affect the estimates of the magnitude of carbon pools and fluxes (70). Therefore, optimizing the quantitative analysis of particle information collected by optical devices becomes imperative to reduce the uncertainty in model predictions and to enhance our understanding of the biological carbon pump and how the ocean regulates the Earth's carbon cycle (74, 75).

In conclusion, we found that the morphological characteristics differed between the LODP and the HODP pools and that the two pools showed considerable independence from each other with depth and geographic regions. By including the more transparent particle pool (or the “gel pool”), aggregate particle characteristics, such as the slopes of the particle number spectra, also changed, reiterating the fundamental necessity of applying objective criteria to define what constitutes a particle in various imaging platforms. To fill the gap in our knowledge of marine gel distribution, enhanced cross-calibration among imaging platforms and increased image data in various aquatic systems are needed to thoroughly investigate the characteristics and distribution patterns of marine gels and their contribution to biogeochemical cycles.

MATERIALS AND METHODS

Sampling sites and spatial divisions

Data were collected during the DisEntangling Seasonality of Active Flux in the Ocean (DESAFIO) expedition (PID2020-118118RB-I00) from 31 January to 2 March 2023. The project was conducted in the northeastern subtropical Atlantic Ocean, spanning the region between the AZ ($41^{\circ}5'32.39''\text{N}$, $24^{\circ}58'41.00''\text{W}$) and the CI ($28^{\circ}42'0.00''\text{N}$, $13^{\circ}12'2.40''\text{W}$; Fig. 1). Vertical profiles of conductivity, temperature, and fluorescence were recorded from the sea surface to 4000-m depth using a CTD Seabird SBE911 Plus equipped with a dissolved oxygen sensor Seabird SBE43 and a fluorometer and turbidimeter Wet Labs ECO-NTU-RTD mounted in the rosette sampler. The vertical profiles of temperature, salinity, oxygen, fluorescence, and turbidity were binned to 1 dbar.

The FoSI described in detail by Boichdanský *et al.* (30) and Huang and Boichdanský (31) was directly attached to the bottom section of the CTD rosette and thus collected particle image data simultaneously with other sensors on the CTD. The incomplete particle profiles in casts 5, 6, 18, and 20 were caused by unexpected camera shutdowns most likely precipitated by low battery voltage.

We separated particles according to geographic regions: the CI (near the CI) consists of casts 1 to 6; the OO (to the west of CI and the south of the Azores) of casts 7 to 14; and AZ (near the Azores) of casts 15 to 22. The vertical spatial groups were the epipelagic

(≤ 200 m), the mesopelagic (200 to 1000 m), and the bathypelagic zones (> 1000 m).

Image processing of two particle pools

The image analysis protocol implemented in our study followed the pipeline detailed in Huang and Bochsansky (31). The resolution of the FoSI image collected during DESAFÍO was $11.75 \mu\text{m}/\text{pixel}$; however, to avoid single-pixel noise, analysis was performed on particles larger than $23.5\text{-}\mu\text{m}$ linear dimension. Changing the threshold settings leads to particle pools with different OD values. We defined the particle pools based on absorbance, where $A(\lambda) = OD(\lambda)$. For the detection of LODP, the threshold was set to 11, corresponding to an absorbance (OD of 0.1466), and, for the detection of HODP, the threshold was set to 29 (OD of 0.2246) in the image analysis protocol. The LODP thus represent all particles including the vastly more abundant gel particles, whereas the HODP referred to more opaque particles (i.e., with a higher OD) typically captured by other optical devices (31). The rest of the parameters remained consistent during the image analysis.

Comparing particle characteristics in two particle pools

The LODP and HODP data, which were resolved to 1-m-depth bins, were analyzed using the Theil-Sen estimator (76, 77), followed by the Mann-Kendall test (78). In contrast to a linear regression model that assumes that the residuals are normally distributed, the Theil-Sen estimator is based on the distributions of the medians of the pairwise slopes, which is suitable for datasets that deviate from normality, such as in the current study.

To examine whether the spatial factors (i.e., horizontal regions and vertical depth strata) had significant effects on particle abundance and TPV, a nonparametric factorial analysis, the ART analysis of variance, was performed to test the effects of multiple factors on the particle data (79). This test relaxes the normality and homoscedasticity assumptions of the residuals required for parametric tests.

The RGF

While the LODP is used as an approximation for gels for morphological characterization and for the particle number spectrum (see below), we also calculated the relative fraction of gels of the total particle numbers to capture subtle spatial variations. RGF was estimated by

$$\text{RGF} = \frac{N_{\text{LODP}} - N_{\text{HODP}}}{N_{\text{LODP}}} \quad (4)$$

where N_{LODP} and N_{HODP} denote the particle abundance of LODP and HODP. The analysis was conducted on the spatial resolution of particle abundance in 1-m-depth bins.

Particle morphology and abundance analysis

The MATLAB function `regionprops()` was used to measure particle morphological characteristics, including ESD, area, perimeter, aspect ratio, circularity, and OD. The OD value with respect to each particle was converted from brightness using the calibration approach with the ensemble model described in the image analysis protocol (31). The aspect ratio was calculated from the axes of the ellipse with the same second moment as the particle

$$\text{Aspect ratio} = \frac{\text{Major axis}}{\text{Minor axis}} \quad (5)$$

The Kolmogorov-Smirnov and Wilcoxon rank sum tests were implemented to examine the difference in distribution and median value, respectively, regarding ESD, aspect ratio, OD, and circularity. The Wilcoxon signed-rank test (a paired test) was used to examine significance levels in the diel changes in particle abundance in the day-night cast pairs.

Particle number spectrum analysis

Particle number spectrum analysis across regions and depths was conducted to investigate the spatial variability of the PSD. The particles were sorted into different size classes before further enumeration and normalization. The formula used to calculate the particle number spectra n was

$$n = \frac{N}{(S_u - S_l) \cdot V} \quad (6)$$

where n represents the number size spectrum, N represents the total number of particles in a specific size bin, S_u represents the upper end of the size bin, S_l represents the lower end of the size bin, and V represents the sample volume of water. The homogeneity of slopes was evaluated using ANCOVA (i.e., the significance of the interaction term in the ANCOVA model).

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