

Transparent exopolymer particle (TEP) dynamics in the eastern Mediterranean Sea

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ABSTRACT: Depth and spatial distribution patterns of transparent exopolymer particles (TEP) were measured during 2 east to west cruise transects across the ultra-oligotrophic Levantine basin, eastern Mediterranean Sea (SESAME, September 2008; ISRLEV, July 2009), and at 2 stations 20 and 50 km offshore (2008). TEP distribution was examined relative to chlorophyll *a* concentrations, bacterial production, and alkaline phosphatase activity (APA), taken as a measure of phosphorus (P) stress. During the stratified period, TEP levels were highest in a 5 m surface layer and correlated positively with APA as P concentrations decreased. On the transects, TEP concentrations from both surface and depth (1000 m) samples increased from west to east, corresponding to a pattern of increasing oligotrophy. In pelagic waters, maximal rates of bacterial production and TEP concentrations were consistently measured in surface samples. These contained abundant large (170 µm) TEP with numerous associated bacteria, possibly derived from algal or bacterial release or detritus. Lowest TEP concentrations were found in the deep chlorophyll maximum layer where TEP appeared as small (~4 µm) particles with only few attached bacteria. In deeper (300 to 1000 m) samples, both TEP concentrations and size increased, with most particles having associated bacteria. Our data augment previous studies on the formation of TEP and the subsequent utilization of this material by heterotrophic bacteria both in the euphotic zone and in deeper waters. Moreover, our study suggests that in ultra-oligotrophic environments such as the Levantine basin, TEP plays an especially important role in carbon cycling and transportation.

KEY WORDS: TEP · Mediterranean Sea · Levantine basin · Bacterial production · Carbon cycling

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INTRODUCTION

Transparent exopolymer particles (TEP) are microscopic (~1 to >200 µm), organic particles consisting mainly of acidic polysaccharides and detected by staining with Alcian Blue (Passow & Alldredge 1955); they are ubiquitous in both marine and freshwater environments (Passow 2002). In the open ocean, TEP are derived via 2 pathways: (1) abiotically, by coagulation of colloidal precursors in the pool of dissolved organic matter (DOM) (Passow 2000, Verdugo & Santschi 2010) and of cellular debris from planktonic organisms, or (2) biotically, resulting directly from algal or bacterial ex-

tracellular excretion or mucilage, or from microbial breakdown of larger marine snow particles. TEP formation from dissolved precursors may be stimulated by turbulence or by bubble adsorption (Zhou & Mopper 1998, Passow 2002). Senescent or nutrient-stressed algae and cyanobacteria have been shown to generate TEP (Grossart et al. 1997, Engel 2004, Berman-Frank et al. 2007). Therefore, relatively greater amounts of TEP may be expected in late phases of algal blooms (Passow 2002) or with increasing oligotrophy of water masses (Engel 2004).

The gel-like structure, high surface area, and extremely sticky character of TEP support coagulation

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processes (Verdugo & Santschi 2010) and enhance the formation and sedimentation of larger aggregates such as marine snow (Passow 2002, Engel et al. 2004, Patara et al. 2009). In some marine environments, between 50 and 90% of the total bacterial marine community may be associated with these particles and form 'hot spots' of intense microbial activity and nutrient cycling that are significant in the recycling of particulate organic carbon (POC) to dissolved organic carbon (DOC) (Passow 2002, Verdugo et al. 2004, Azam & Malfatti 2007). Sedimentation of TEP-associated 'hot spots' from the surface may be important in the transport of particulate organic material and microorganisms to deeper waters (Smith & Azam 1992, Azam & Malfatti 2007, Bar-Zeev et al. 2009, Verdugo & Santschi 2010). During sedimentation, TEP can also function as a direct source of carbon and other nutrients for higher trophic level organisms such as protists, micro-zooplankton, and even nekton (Passow 2002, Engel et al. 2004).

The organic carbon pool in the ocean contains both POC and DOC. TEP microgels may act as a 'bridge' between DOC and larger particulates, thereby forming an important intermediary pool of organic matter (Beauvais et al. 2003, Engel et al. 2004, Verdugo et al. 2004). Thus, production of TEP may be critical in providing a source of available carbon to fuel microbial food webs (Azam & Malfatti 2007), especially in oligotrophic environments (Passow 2002, Beauvais et al. 2003).

The Levantine basin is considered P-limited and ultra-oligotrophic, being characterized by extremely low chlorophyll *a* (chl *a*) concentrations and primary production that is dominated by pico-phytoplankton populations. Chl *a* concentrations are typically $<0.5 \mu\text{g l}^{-1}$, with a deep (~80 to ~130 m) chl *a* maximum (DCM). Rates of primary and bacterial productivity are ~0.11 and ~0.05 $\mu\text{g C l}^{-1} \text{h}^{-1}$, respectively (Berman et al. 1984, Li et al. 1993, Tanaka et al. 2007).

Although increasing research attention focuses on the role of TEP and other transparent organic particles in marine ecology (Azam & Malfatti 2007), as yet there have been few studies on TEP in the Mediterranean Sea (Prieto et al. 2006, Radić et al. 2006). These were mainly conducted in the less oligotrophic western Mediterranean (but see Ortega-Retuerta et al. 2010) and were limited to samples within the euphotic zone. In contrast, the present study was carried out in the extremely oligotrophic Levantine basin with sampling down to 1000 m.

Our premise was that TEP might play an integral role in the cycling of organic matter from the surface to deeper (1000 m) waters in these ultra-oligotrophic waters. We measured depth and spatial distribution patterns of TEP during 2 east to west transects in the

summers of 2008 and 2009 across the ultra-oligotrophic Levantine basin, eastern Mediterranean Sea, and seasonally at 2 stations, 20 and 50 km offshore, during 2008. From our data, we deduce spatial and seasonal patterns of TEP formation and utilization in the Levantine basin and evaluate the role of TEP in carbon cycling and transport in these waters.

MATERIALS AND METHODS

Study area and sample collection. Offshore sampling: Offshore water samples were collected during daytime on monthly cruises in 2008 (the February/March mixed period and the late May/June stratified period) on board the RV 'Mediterranean Explorer'. We sampled 2 stations: a coastal station (T2, 200 m deep) above the continental shelf and an open-ocean station (T1, 1000 m deep) off the continental shelf (Table 1, Fig. 1). Water samples were collected for TEP, chl *a*, alkaline phosphatase activity (APA), and nutrients (nitrate + nitrite and phosphate) from different depths using Niskin bottles (8 l) mounted on a Rosette equipped with a CTD (Seabird 19 Plus), measuring temperature and salinity, and a PAR sensor (photosynthetically active radiation; Biospherical/Licor) for down-welling irradiance.

SESAME cruise: Aboard the RV Shikmona, we sampled 6 stations during September 2008 (stratified period) in the Levantine basin on an east to west transect (Table 1, Fig. 1) within the framework of the Southern European Seas: Assessing and Modeling

Table 1. Geographic locations and bottom depths of sampling stations described in the present study

| Station | Location | | Distance from shore (km) | Bottom depth (m) |
|--|-----------|-----------|--------------------------|------------------|
| | Lat° N | Long° E | | |
| Routine sampling, both stations from Feb to Mar and May to Jun 2008 | | | | |
| T2 | 34° 34.51 | 32° 09.98 | 20 | 200 |
| T1 | 34° 14.91 | 32° 10.06 | 51 | 1000 |
| SESAME cruise, sampling Sep 2008 | | | | |
| IL1 | 34° 52.03 | 32° 53.58 | 11.5 | 150 |
| IL2 | 33° 09.01 | 34° 08.51 | 82.1 | 1709 |
| IL4 | 33° 00.00 | 33° 14.69 | 180.9 | 1609 |
| IL9 | 33° 29.50 | 33° 28.90 | 227.8 | 2530 |
| IL13 | 28° 30.00 | 33° 40.13 | 359.3 | 2738 |
| IL20 | 25° 00.20 | 34° 00.80 | 916.2 | 2566 |
| ISRLEV cruise, sampling Jul 2009 | | | | |
| 1 | 34° 44.90 | 32° 56.90 | 24.1 | 1200 |
| 2 | 33° 38.90 | 33° 18.90 | 135.2 | 2000 |
| 3 | 32° 14.90 | 33° 41.90 | 240.4 | 1200 |
| 4 | 29° 29.86 | 34° 50.65 | 555.6 | 3000 |
| 5 | 29° 30.75 | 35° 28.98 | 579.6 | 1200 |

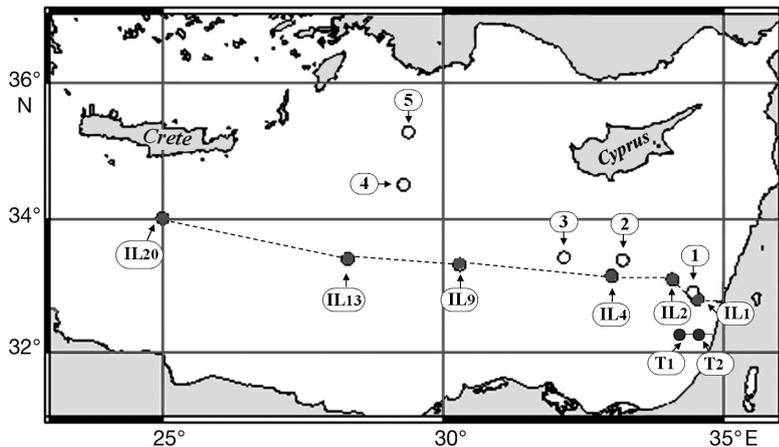


Fig. 1. Eastern Mediterranean Sea. Sampling locations from different cruises: ISRLEV—Stns 1 to 5 (July 2009); SESAME—Stns IL1, IL2, IL4, IL9, IL13 and IL20 (September 2009); and the 2 routine stations west of Herzlia—T1 and T2, which were sampled monthly between January and November 2008

Ecosystem Change (SESAME) program. At each station, 5 depths were sampled: the surface layer (~5 m), mid-euphotic zone (~50 m deep), DCM (~120 m), below the DCM (300 m), and at depth (1000 m). Samples were analyzed for chl *a*, TEP, POC, and nutrient concentrations.

ISRLEV cruise: Samples were collected aboard the RV 'Mediterranean Explorer' during July 2009 (stratified period). We sampled 5 stations along an east to northwest transect (Table 1, Fig. 1). Each station was sampled at 5 depths as in the SESAME cruise. On this transect, we measured the same parameters as during the SESAME cruise, with the addition of TEP visualization in the microscope and bacterial productivity (BP).

Nutrient analyses. Triplicate water samples were collected in 15 ml acid-washed plastic scintillation vials. Nutrients were determined using a segmented flow Skalar SAN^{plus} System spectrophotometer as detailed in Kress & Herut (2001). The precision for nitrate + nitrite and dissolved inorganic phosphate (DIP) was 0.02 and 0.003 μM , respectively, with detection limits of 0.02 and 0.008 μM , respectively.

Alkaline phosphatase activity (APA). APA was measured fluorometrically with 3-O-methylfluorescein-phosphate (MUF) as a substrate using the method of Ammerman (1993). APA ($\text{nmol PI}^{-1} \text{h}^{-1}$) was calculated by using a linear regression of fluorescence values versus the measured incubation time (~24 h incubations at ~25°C and pH of 8.2) and correcting the fluorescence values of samples to the blank sterile seawater (SSW) samples (excitation: 364 nm; emission: 448 nm).

Particulate organic matter (POC). Seawater (4 l) was filtered through pre-combusted (4 h, 450°C) GF/F filters and stored at -20°C until analysis within 1 mo after the cruise. Prior to analysis, the filters were dried

overnight at 60°C. POC was determined using a Perkin-Elmer Model 240 CHN analyzer after carbonate was removed from the filters by HCl fumes overnight. Blank pre-combusted filters were used for calibration.

Chl *a* as an algal proxy. Chl *a* was measured in duplicate by means of a Turner Designs fluorometer calibrated with pure chl *a* (Sigma C6144, from *Anacystis nidulans*). Seawater samples (300 ml) were filtered through Whatman GF/F fiber filters, and pigments were extracted on-board in cold acetone (90%) for 24 h in the dark (Holm-Hansen et al. 1965).

TEP concentrations, visualization of TEP, and associated bacteria. Water samples (100 ml) were filtered onto 0.4 μm polycarbonate filters, and TEP concentrations (μg gum xanthan [GX] equivalents l^{-1}) were measured according to Passow & Alldredge (1995). We used a conversion factor of 0.51 to convert from micrograms of GX equivalents to micrograms of carbon (Engel & Passow 2001).

To visualize TEP, samples (150 ml) were filtered gently (~100 to 150 mbar) onto 0.4 μm polycarbonate filters, stained for 10 s with freshly prepared, 0.2 μm pre-filtered Alcian Blue (AB) solution (0.02%), and rinsed with 5 ml of double distilled water (DDW). The filter was then stained with 30 μl DAPI (250 $\mu\text{g ml}^{-1}$) for 12 min in the dark and rinsed with 5 ml DDW to remove excess DAPI (Passow & Alldredge 1995). The filter was then placed on a coated Cytoclearslide (Clearing Slides, GE Osmonics Lab store) with 10 μl of glutaraldehyde (0.5%) to fix the sample and a drop of immersion oil, and was covered with a cover slip. Slides were kept in a dark, humid container until examined at the laboratory. TEP images were taken under bright-field (Nomarski) illumination; bacteria were visualized with an UV epifluorescent microscope (Nikon 80i microscope) equipped with a specific DAPI filter (excitation: 350 nm; emission: 450 nm).

Bacterial production. In the present paper we use the term 'bacteria' to include both bacteria and archaea within the microbial population. BP was estimated using the ^3H -leucine incorporation method (Kirchman et al. 1985, Simon & Azam 1989) as modified by Smith & Azam (1992). Triplicate (1.7 ml) samples were incubated with 200 nmol l^{-1} of ^3H -leucine (Amersham; specific activity: 160 Ci mmol^{-1}) for a period lasting from 4 to 14 h at *in situ* (~25°C) temperatures in the dark. (Preliminary experiments indicated that this was a saturating level of ^3H -leucine and that

incorporation was linear during this time period.) Trichloroacetic acid (TCA)-killed samples served as zero time controls. The incorporation was stopped with the addition of 100 μl of cold 100% TCA to the vials, and samples were kept at 4°C until processing, which was carried out several days after the cruise. After addition of 1 ml scintillation cocktail (Sigma No. 1008300), the samples were counted in a scintillation counter (Tri-Carb 2100TR Packard). Leucine incorporation was converted to BP using a factor of 3.1 kg C mol⁻¹ with an isotope dilution factor of 2.0 (Smith & Azam).

RESULTS

Distribution of TEP under mixed and stratified conditions

To follow seasonal changes in TEP depth distribution, 2 pelagic stations close to the Israeli coast (T2 and T1) were sampled in the winter and summer of 2008. During winter mixing (February to March), the season with generally the highest phytoplankton standing stocks, temperature ($18 \pm 1.4^\circ\text{C}$), salinity (39.35 ± 0.5 psu), chl *a* (range: 0.12 to 0.99 $\mu\text{g l}^{-1}$), TEP (range: 120 to 290 $\mu\text{g GX l}^{-1}$ on different sampling dates) and APA (0.5 ± 0.05 MUF nM h⁻¹) were uniformly distributed down to ~200 m (mean \pm SD) (Fig. 2A,B).

During the stratified period (May through June) the water column was characterized by high surface temperatures ($24 \pm 3^\circ\text{C}$), with a sharp thermocline at ~150 m at both stations (representative data from Stn T2 are shown in Fig. 2C, D). The euphotic depth (taken as 0.1% surface irradiance) reached ~150 m. Chl *a* concentrations were low in near-surface waters (0.02 to 0.1 $\mu\text{g l}^{-1}$), with a DCM (0.27 ± 0.05 $\mu\text{g chl l}^{-1}$) at ~125 m and no apparent algal bloom. TEP concentrations were maximal near-surface (225 to 275 $\mu\text{g GX l}^{-1}$) and minimal at the DCM (51 to 82 $\mu\text{g GX l}^{-1}$). Below the DCM, TEP concentrations increased with depth to 200 m (100 to 132 $\mu\text{g GX l}^{-1}$). Significantly higher rates of APA were measured near the surface, 0 to 10 m (1.4 ± 0.5 nM MUF h⁻¹), than at 200 m (0.2 to 0.4 nM MUF h⁻¹).

During the stratified period, both TEP and APA exhibited significant correlations with chl *a* (Fig. 3). A significant negative correlation ($R^2 = 0.48$, $p = 0.004$, $n = 14$) was found between TEP and chl *a*, while a significant positive correlation ($r^2 = 0.71$, $p = 0.0002$, $n = 14$) was found between TEP and APA (Fig. 3A,B). During winter mixing, no such correlations were obtained between the different parameters.

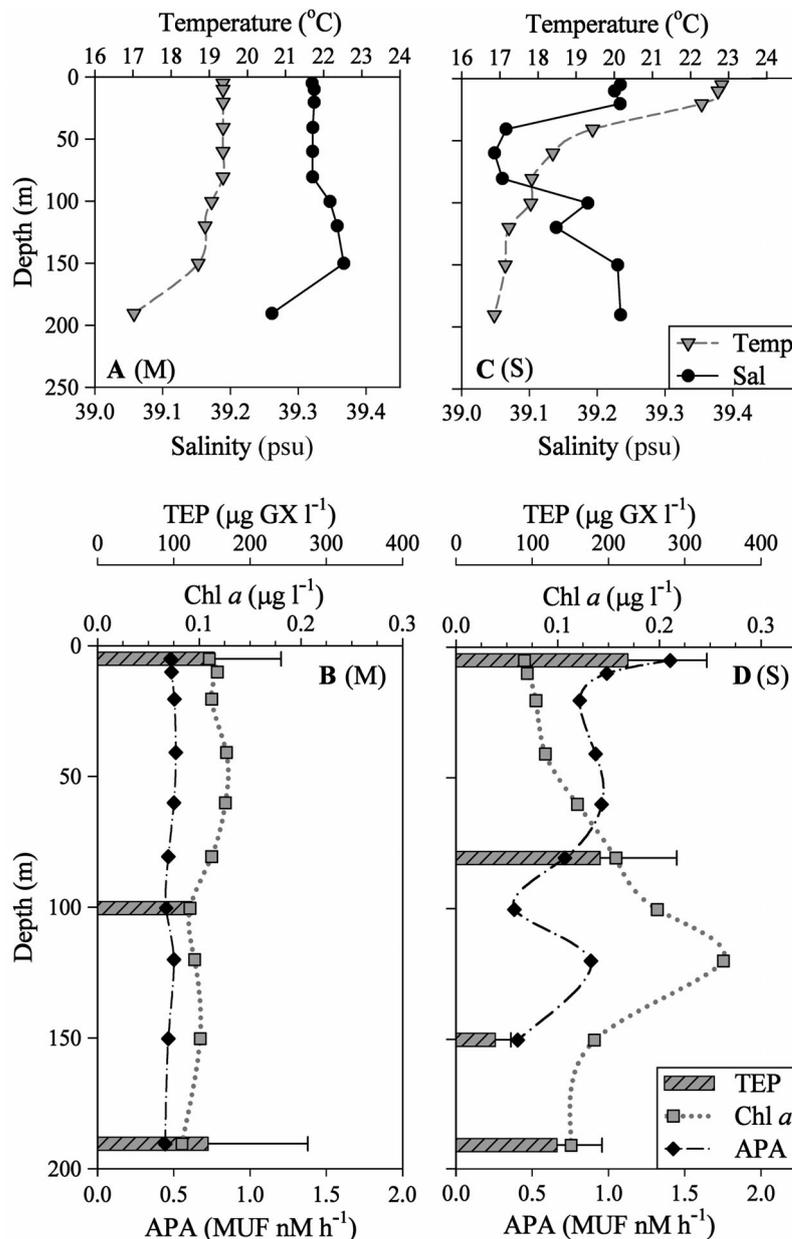


Fig. 2. Representative depth profiles sampled during the mixed, M, period (A,B) and the stratified, S, period (C,D) from Stn T2. (A,C) temperature (∇) and salinity (\bullet). (B,D) transparent exopolymer particles (TEP, bars), chlorophyll *a* (chl *a* \blacksquare), and alkaline phosphatase activity (APA \blacklozenge). GX: gum xanthan; MUF: 3-0-methylfluorescein-phosphate

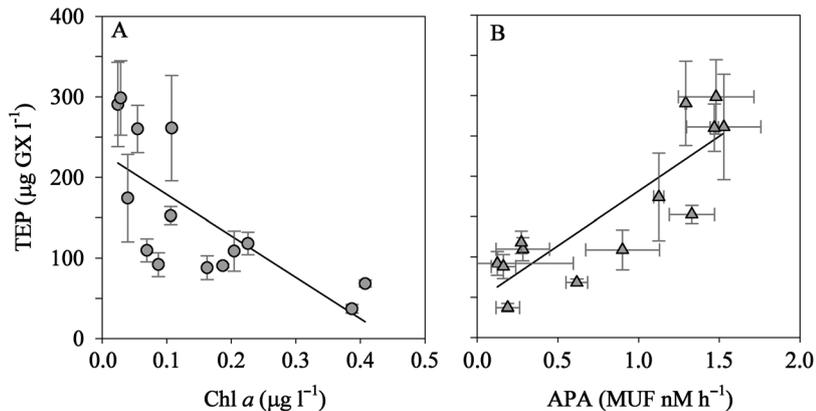


Fig. 3. Relationship (Pearson correlation test) between transparent exopolymer particles (TEP), chlorophyll *a* (chl *a*), and alkaline phosphatase activity (APA) (data from monthly sampling at Stns T1 and T2). Linear regression is shown of: (A) TEP versus chl *a* ($r^2 = 0.48$, $p = 0.004$, $n = 14$) and (B) TEP versus APA ($r^2 = 0.71$, $p = 0.0002$, $n = 14$). All means \pm SD

Spatial distribution of TEP and chlorophyll in the Levantine basin

Data obtained during 2 transects across the Levantine basin (SESAME and ISRLEV), made during the stratified summer seasons of 2008 and 2009, respectively, enabled us to generate a more comprehensive view of the role of TEP in these waters. Sea-surface temperatures in summer ranged from 25 to 29°C. In both transects, nitrate + nitrite and phosphate concentrations were extremely low in the euphotic zone (~ 0.1 and ~ 0.02 $\mu\text{M N}$, respectively). Integrated concentrations (to 200 m) of nitrate + nitrite and DIP (35.4 to 330.2 and 1.4 to 11.3 $\mu\text{M m}^{-2}$, respectively) showed a decreasing gradient (i.e. increasingly oligotrophic conditions) from west to east that was significantly correlated with distance from shore (E. Rahav pers. comm.).

Phytoplankton standing stocks were minimal during the summer, with no apparent algal bloom. Thus, chl *a* concentrations were extremely low, but nevertheless decreased from 0.07 to 0.03 $\mu\text{g l}^{-1}$ from west to east, indicative of a gradient of rising oligotrophy. A significant positive correlation between chl *a* and distance from the Israeli coast was observed for surface and DCM samples (Fig. 4).

The relationship between TEP and trophic status of the phytoplankton (using chl *a* as a proxy) with distance from the Israeli shore was examined at 3 key depths; near the surface (5 to 80 m), the DCM (~ 120 m) and in deep water (300 to 1000 m) across the Levantine basin. Significant, negative correlations between TEP concentrations and distance from shore were observed for near-surface and deep waters ($r^2 = 0.62$, $p = 0.003$, $n = 9$ and $r^2 = 0.72$, $p = 0.004$, $n = 10$, respectively). No significant relationships were found between TEP and distance from shore at the DCM.

Depth distribution of TEP and related parameters

In Table 2 we list the average, near-surface (5 m) concentrations of TEP, chl *a*, and POC, and the rates of BP across the Levantine basin based on data from both the SESAME (September 2008) and ISRLEV (July 2009) cruises. Averaged depth profiles of TEP, BP, POC, and chl *a* are shown in Fig. 5 and Table 2.

During these cruises, irradiance was very high (~ 1400 $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$) at 5 m depth across the Levantine basin, while nutrients and chl *a* concentrations were extremely low (nitrate + nitrite: ~ 0.1 μM ; phosphate: ~ 0.02 μM ; chl *a*: 0.04 to 0.07 $\mu\text{g l}^{-1}$). A consistent peak of chl *a* (~ 0.32 $\mu\text{g l}^{-1}$) was observed in the

DCM located at ~ 120 m (Table 2, Fig. 5).

The highest concentrations of TEP ranging from 116 to 420 $\mu\text{g GX l}^{-1}$ were recorded in surface waters, while minimum TEP concentrations, 48 to 189 $\mu\text{g GX l}^{-1}$ (about 40% of the near-surface values) were consistently observed in the DCM (~ 120 m). At greater depths (300 to 1000 m), TEP concentrations increased again to about 70% of near-surface values, ranging between 83 and 386 $\mu\text{g GX l}^{-1}$ (Fig. 5).

The highest levels of BP (0.3 to 2.5 $\mu\text{g C l}^{-1} \text{d}^{-1}$) were also detected in the surface waters. BP decreased by $\sim 70\%$ in the mid-euphotic zone (60 m), but increased to 40% (~ 0.9 $\mu\text{g C l}^{-1} \text{d}^{-1}$) in the DCM. Below the DCM, BP levels were low, with minima at 200 and 1000 m.

Visualization of TEP in the water column

Microscopic images of TEP from near-surface samples frequently showed large (176 ± 105 μm maximum length \pm SD) AB-stained material sloughing off from various algal cells, as well as detached sheet-like material (Figs. 6A,B & 7). Although we found no significant correlation between TEP and BP at any of the depths sampled (data not shown), some of these particles appeared to be heavily colonized by bacteria (Fig. 6A,B).

In samples from the DCM, most TEP were much smaller, 4.4 ± 4 μm (maximum length \pm SD) and more irregular in shape than those from near the surface (Figs. 6C & 7). Bacteria were only occasionally associated with these TEP (Fig. 6C).

TEP from 1000 m tended to be of an irregular, amorphous shape and larger (60 ± 33 μm , maximum length \pm SD) than in DCM samples (Figs. 6D & 7). Bacteria almost always appeared to be associated with these particles (Fig. 6D).

TEP carbon in relation to total POC

Across the pelagic Levantine basin, POC concentrations varied from 12 to 250 $\mu\text{g C l}^{-1}$. In contrast to TEP and chl *a*, POC did not correlate with distance from

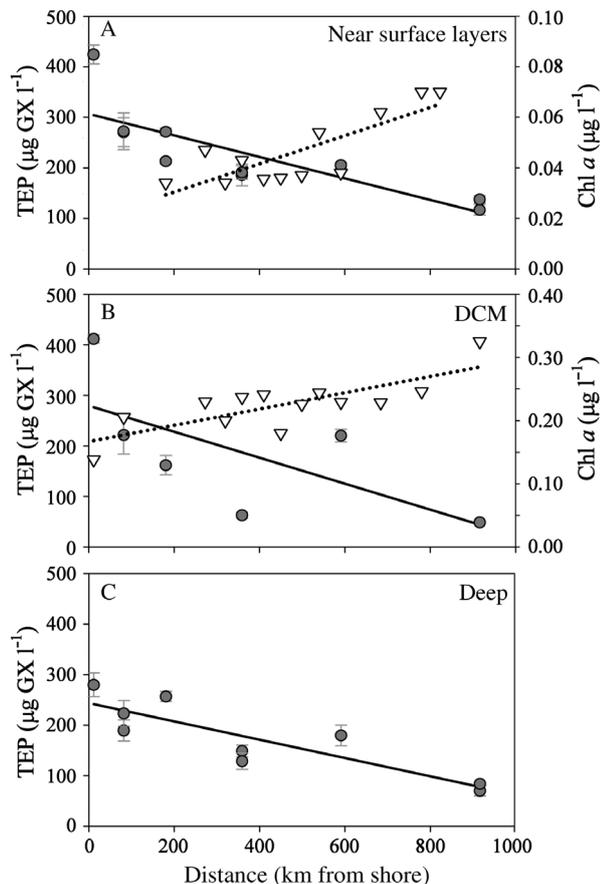


Fig. 4. Relationship between transparent exopolymer particles (TEP, ●) and chlorophyll *a* (chl *a*, ▽) concentrations (means \pm SD) and the distance from shore; SESAME and ISRLEV transects: (A) 5 to 80 m depth. TEP: $r^2 = 0.62$, $p = 0.004$, $n = 9$; chl *a*: $r^2 = 0.615$, $p = 0.0002$, $n = 12$. (B) Deep chlorophyll maximum (DCM). TEP: $r^2 = 0.3$, $p = 0.15$, $n = 6$; chl *a*: $r^2 = 0.57$, $p = 0.002$, $n = 13$. (C) 250 to 1000 m. TEP: $r^2 = 0.72$, $p = 0.003$, $n = 10$

Table 2. Surface (5 m) concentrations of transparent exopolymer particles (TEP), chlorophyll *a* (chl *a*), particulate organic carbon (POC) and the rates of bacterial productivity (BP) from the eastern Levantine basin (all means \pm SD). Values were averaged from replicated measurements at 5 m from different stations (n : number of stations). These average values were then used to represent the 100% baseline for each parameter plotted in Fig. 7. GX: gum xanthan

| Parameter | Concentration or rate | n |
|---|-----------------------|-----|
| TEP ($\mu\text{g GX l}^{-1}$) | 345 ± 143.2 | 12 |
| Chl <i>a</i> ($\mu\text{g l}^{-1}$) | 0.04 ± 0.01 | 12 |
| BP ($\mu\text{g C l}^{-1} \text{d}^{-1}$) | 2.24 ± 0.86 | 5 |
| POC ($\mu\text{g C l}^{-1}$) | 165 ± 38 | 7 |

shore for any of the sampled depths and did not exhibit any significant west–east gradient.

To estimate TEP carbon content (TEP-C) we used the lowest TEP ($\mu\text{g GX l}^{-1}$) to C ($\mu\text{g l}^{-1} \text{C}$) conversion factor (0.51) suggested by Engel & Passow (2001). Based on this conversion factor, we estimated concentrations of TEP-C ranging from 38 to 178 $\mu\text{g C l}^{-1}$. Thus, at all stations sampled, a substantial portion of POC appeared to consist of TEP-C. At near-surface depths, TEP-C contributed $63 \pm 3\%$ of the total POC, increasing to $83 \pm 36.6\%$ at the DCM, with large variability between different sampling stations. In deeper waters, we obtained an apparent value for TEP-C that was $\gg 100\%$ of the measured POC pool. A significant ($r^2 = 0.5$, $p = 0.003$, $n = 14$) negative correlation was observed between POC ($\mu\text{g C l}^{-1}$) and TEP ($\mu\text{g GX l}^{-1}$) concentrations for all stations and depths measured (Fig. 8).

DISCUSSION

Within the voluminous literature on TEP, relatively few reports document TEP concentrations in the pelagic of different seas (Table 3 and references therein). These show considerable range in TEP concentrations between oceanic regions, with large variability and impact on carbon cycling attributed to spatial, temporal, and depth factors (Table 3). Most investigations to date have examined TEP variability only within the upper photic zone, and we are not aware of any published studies of TEP levels in deeper water (> 200 m). Also, to the best of our knowledge, no studies from extreme oligotrophic environments showing the depth, spatial, and seasonal distribution of TEP have yet been published.

Our results from the ultra-oligotrophic Levantine basin show a large spatial and temporal variability of both TEP morphology and concentrations. TEP concentrations in the present study varied from 19 to 600 $\mu\text{g GX l}^{-1}$, which is within the range of 23 to 791 $\mu\text{g GX l}^{-1}$ of TEP reported from many marine environments (Table 3).

Seasonal changes of TEP concentrations in an ultra-oligotrophic environment

The release of mucoid substances such as TEP from phytoplankton is often the result of seasonal changes in irradiance and nutrient availability (Passow 2002, Berman-Frank et al. 2007). At the offshore stations (T1 and T2), there were marked differences in nutrient (nitrate + nitrite and DIP) concentrations in near-surface waters between the mixed and strati-

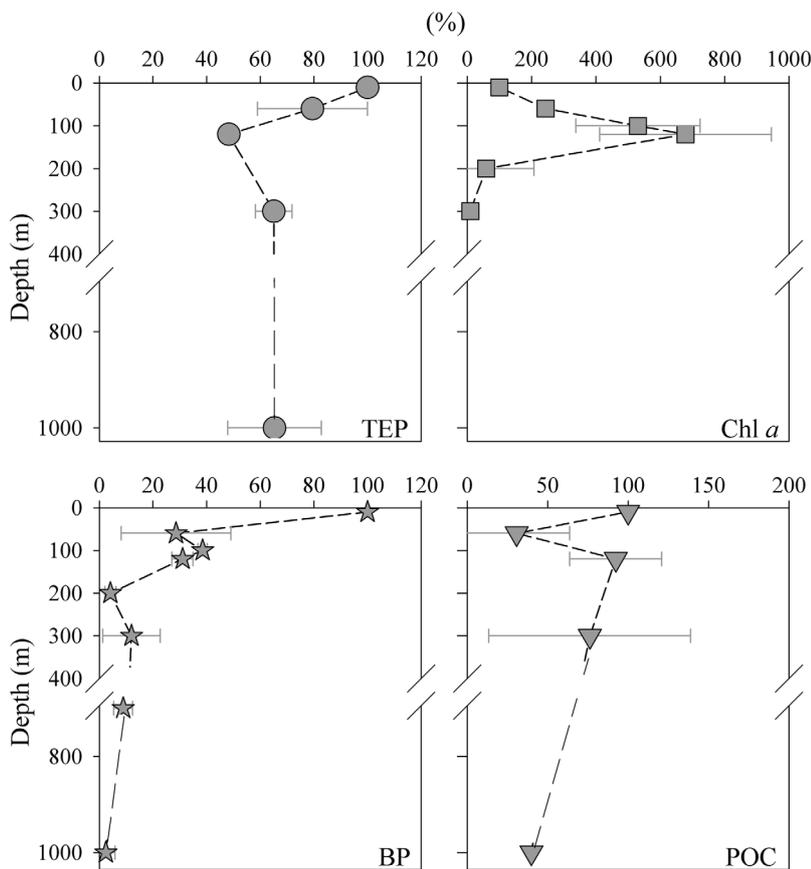


Fig. 5. Representative depth profiles (averaged from data measured at 7 stations during the SESAME and ISRLEV cruises) of transparent exopolymer particles (TEP), chlorophyll *a* (chl *a*), bacterial productivity (BP), and particulate organic carbon (POC), normalized to surface (5 m) samples taken as 100%. Actual concentrations and rates are given in Table 2. Means \pm SD

fied seasons; these influenced the levels of chl *a*, APA, and TEP (Fig. 2). During the stratified period, high APA rates, indicative of P stress in both bacteria and phytoplankton, were measured in the near-surface layer. Limited P availability, combined with high solar irradiance, probably led to an uncoupling in the phytoplankton of photosynthetic carbon uptake and the release of 'excess carbon' as TEP, with algal biomass remaining low (Berman-Frank & Dubinsky 1999, Corzo et al. 2000). The relationships found between APA, chl *a*, and TEP concentrations over the course of sampling at Stns T1 and T2 were compatible with this scenario (Fig. 3A,B). TEP and APA were significantly positively correlated (Fig. 3B), suggesting that increasing amounts of TEP were released when P stress increased. Likewise, the significant negative correlation between TEP and chl *a* (Fig. 3A) may imply that an increasingly stressed, low biomass of phytoplankton released greater amounts of TEP.

In contrast, during the mixed period when the annual phytoplankton bloom occurred, upwelling of nutrient-rich, deep water resulted in a sharp drop of APA rates, indicating a relaxation in P stress. With sufficient nutrients and adequate irradiance, a tight coupling between photosynthetic carbon uptake and incorporation into biomass would limit the amount of TEP produced as excess carbon (Berman-Frank & Dubinsky 1999). Thus, during the mixed season, no significant correlations were found between TEP and chl *a* or between TEP and APA.

Spatial TEP distribution during the stratified season

Our measurements covering a transect of the pelagic Levantine basin during 2 stratified summer periods (SESAME and ISRLEV) demonstrated that TEP concentrations in the euphotic zone increased from west to east (Table 1), as contrasted with declining chl *a* (Fig. 4). Nutrient (N, P) concentrations also indicated increasing oligotrophy from west to east in the Mediterranean and the Levantine basin (Harris et al. 2001, Tanaka et al. 2007, Rahav pers. comm.). The inverse relationship between chl *a* and TEP observed during the stratified season across the Levantine Basin (Fig. 2) is consistent with the idea that nutrient limitation enhanced TEP release from phytoplankton (Fig. 3).

Increased release and subsequent sedimentation of TEP and larger TEP-mediated aggregates in the euphotic zone of the Levantine basin during summer stratification when nutrient limitation is maximal could provide an important source of POC and other material that would be available for microorganisms in deeper waters (Engel 2004, Beauvais et al. 2006, Patara et al. 2009).

Depth distribution of TEP: particle formation, utilization, and carbon cycling in the Levantine basin

To obtain an overview of the depth distribution of TEP, chl *a*, and BP, we averaged the values of these parameters measured at each depth (normalized to the 5 m sample taken as 100%) for all pelagic stations of the SESAME and ISRALEV cruises (Fig. 5). We used

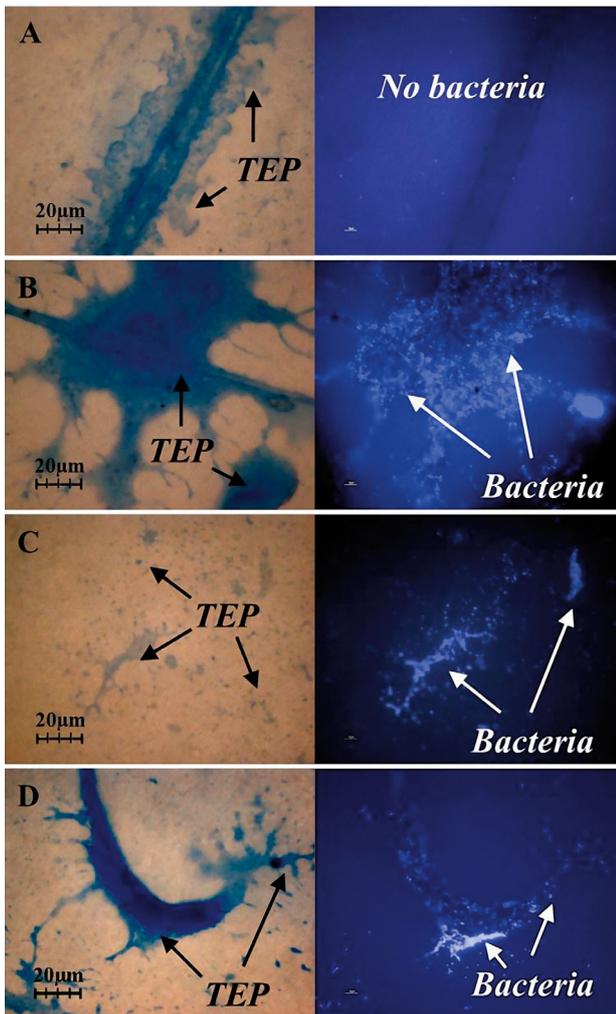


Fig. 6. Microscopic visualization of transparent exopolymer particles (TEP; stained with Alcian Blue, using bright field microscopy) and bacteria (stained with DAPI, using epifluorescent microscopy). Samples from the ISRLEV cruise: (A & B) from 5 m, (C) from the deep chlorophyll maximum, and (D) from 1000 m

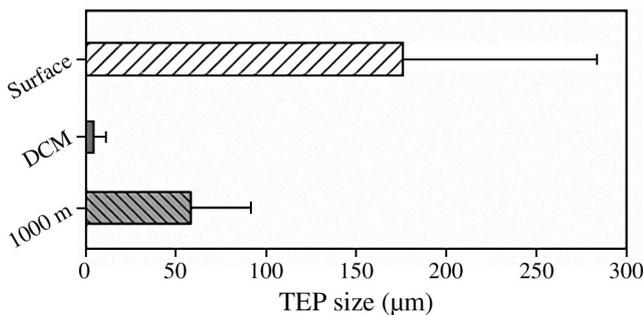


Fig. 7. Size distribution of transparent exopolymer particles (TEP; determined according to axis of maximum length) at different depths from all pelagic stations. Surface: n = 35; deep chlorophyll maximum (DCM): n = 196; and 1000 m: n = 22. Means ± SD

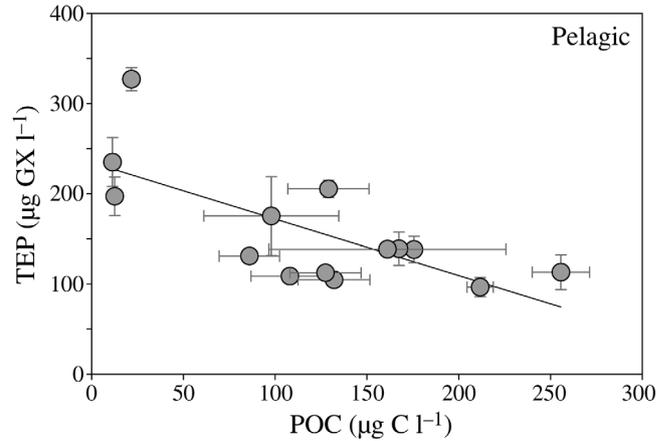


Fig. 8. Relationship between transparent exopolymer particles (TEP) and particulate organic carbon (POC) ($r^2 = 0.5$, $p = 0.003$, $n=14$; Pearson correlation test). Means ± SD

these profiles together with the microscopic images of TEP to infer a generalized account of the role of these particles in organic carbon cycling in the Levantine Basin during the stratified period (summarized in Fig. 9).

Euphotic zone (0 to 120 m)

Maximum concentrations of TEP in the water column were consistently observed in the upper euphotic zone. TEP images from these depths showed mostly large (mean ± SD : $176 \pm 105 \mu\text{m}$) sheet-like AB-stained particles that frequently appeared to be sloughing off from different micro-phytoplankton cells (Fig. 6A,B). Bacteria and bacterial colonies were associated with these large TEP in >50% of the microscopically visualized images from these depths. The high BP rates measured at these depths may have resulted both from bacterial utilization of readily available DOC directly released by phytoplankton and from bacterial growth on TEP substrates (Azam & Malfatti 2007). We deduced from our data that most of the TEP in the upper photic zone was derived from direct release by phytoplankton and bacteria, from cellular mucoid envelopes or other detrital material, rather than by abiotic self-assembly from colloid precursors (Verdugo & Santschi 2010). These TEP may then have formed 'hot spots' of microbial metabolic activity fueling the microbial loop, as well as aggregating further to produce marine snow (Verdugo et al. 2004, Azam & Malfatti 2007, Verdugo & Santschi 2010).

Intense bacterial activity, combined with microplankton grazing, may have promoted breakdown of large TEP as they sedimented down through the euphotic water column and could explain the decrease

Table 3. Concentrations of transparent exopolymer particles (TEP) reported from different pelagic environments. n: no. of samples

| Sample area | TEP ($\mu\text{g GX l}^{-1}$) | Source |
|---|---------------------------------|-------------------------------|
| Pacific | | |
| Monterey Bay | 50–310, n = 18 | Passow & Alldredge (1995) |
| Great Barrier Reef | 23–791, n = 14 | Wild (2000) |
| East Sound | 73–159, n = 50 | Kiørboe et al. (1996) |
| Atlantic | | |
| NE Atlantic, 47° N | 27–279 | Engel (2004) |
| South Sea | | |
| Antarctic Peninsula | 15.4 (0–48.9) | Ortega-Retuerta et al. (2009) |
| Ross Sea | 308–2800 | Hong et al. (1997) |
| Red Sea | | |
| Gulf of Aqaba | 25–230, n = 16 | Bar Zeev et al. (2009) |
| Mediterranean Sea | | |
| Gulf of Cadiz | 100–600 | García et al. (2002) |
| Straits of Gibraltar | 41–296 | Prieto et al. (2006) |
| Alboran Sea | <25–539, n = 13 | Prieto et al. (2006) |
| Northern Adriatic (mucus event) | 4–14800 | Radić et al. (2005) |
| Mediterranean Sea (without Levantine basin) | 4.5–94.3, n = 123 | Ortega-Retuerta et al. (2010) |
| Levantine basin | 19–600, n = 72 | Present study |

of TEP concentrations with depth towards the DCM observed at all SESAME and ISRLEV stations (Fig. 5). Ortega-Retuerta et al. (2010) reported a similar pattern of TEP depth distribution in the upper 200 m of nearby regions in the eastern Mediterranean.

DCM (110 to 130 m)

Within the DCM slightly higher ambient DIP and nitrate + nitrite concentrations (~ 0.25 and $\sim 3.75 \mu\text{M}$, respectively) facilitated the growth of a low-light (0.1 to 1% surface irradiance) adapted pico-planktonic, autotrophic community dominated mainly by *Synechococcus* spp. and *Prochlorococcus* spp. (data not shown). Across the Levantine basin, TEP concentrations were minimal at the DCM (Fig. 5) and were comprised of mostly small (0.5 to

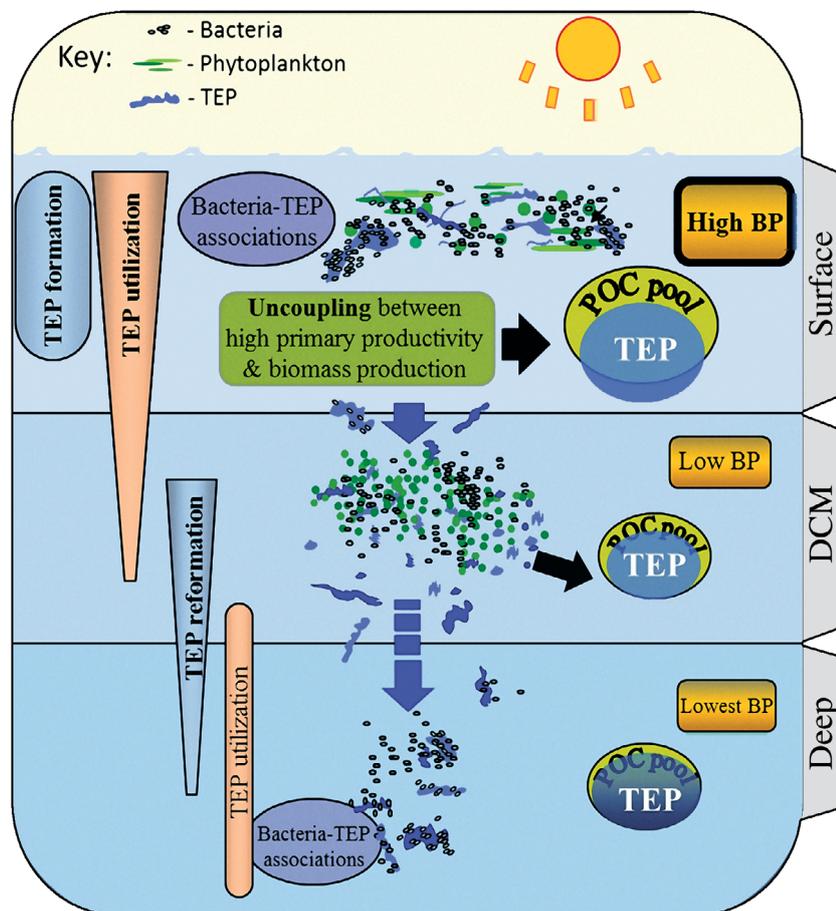


Fig. 9. Schematic model of transparent exopolymer particles (TEP) formation and utilization in the oligotrophic open ocean under stratified conditions. In the upper photic zone, nutrient-stressed phytoplankton release large amounts of TEP in addition to dissolved organic carbon (DOC). Bacteria exploit TEP-C and extracellularly released DOC effectively; thus bacterial productivity (BP) is also high. As TEP settles to the deep chlorophyll maximum (DCM), they are degraded by bacterial utilization and micro-zooplankton grazing. At the DCM, TEP concentrations are minimal and BP is also much lower than in the upper euphotic zone. TEP (perhaps derived from pico-phytoplankton and bacteria) are very small and with few associated bacteria. With increasing depth below the DCM, aggregation of colloidal precursors and previously formed TEP lead to greater concentrations of these particles. Below 300 m, TEP appear as large amorphous particles, some with associated bacteria. At these depths BP is very low, presumably due to a lack of readily available substrates. Size of arrows, squares, and ellipses are proportional to their relative importance, with TEP contribution to the particulate organic carbon (POC) pools at the different depths designated by ellipses

4 μm) sized TEP, as visualized and measured microscopically (Figs. 6C & 7).

The TEP minimum at the DCM may have resulted from a shift in the amount of extracellular TEP released from larger (~2 to ~40 μm) near-surface phytoplankton to that produced by predominantly small (1 to 2 μm) pico-phytoplankton growing under conditions of tight coupling between photosynthesis and assimilation of carbon into biomass (Tanaka et al. 2007, Rahav pers. comm.). We suggest much of the TEP in the DCM was formed from abiotic self-assembly of smaller precursors (Verdugo & Santschi 2010). Because we measured only the concentrations of TEP retained on 0.4 μm filters (see 'Materials and methods'), we were unable to determine the concentrations of any precursor pool of smaller (0.05 to 0.4 μm), colloidal-sized TEP (Villacorte et al. 2010) that may have been significant at these depths.

Very few bacteria were associated with the small TEP at the DCM (Fig. 6), indicating that these particles did not offer a suitable substrate for bacterial colonization (Passow 2002) and possibly explaining the low measured BP rates (Fig. 5). Additionally, heterotrophic bacteria may have been outcompeted for P and N by the dominant pico-autotrophic populations growing at the DCM (Engel 2004, Engel et al. 2004, Tanaka et al. 2007). The low levels of BP in the DCM and deeper waters may have enabled most of the newly formed small TEP precursors to coagulate without degrading and to aggregate to larger TEP. These larger TEP particles were then able to sink down to deeper waters as hypothesized by Beauvais et al. (2003), Burd & Jackson (2009) and Verdugo & Santschi (2010).

Deep water column (300 to 1000 m)

Although TEP were determined only at 300 and 1000 m, we surmise that the concentrations measured at these depths were representative for the mesopelagic water column from ~300 to 1000 m, due to the relatively uniform characteristics of this water mass during the stratified season (Krom et al. 2005). At these depths TEP and TEP-mediated aggregates (with amorphous shapes) were significantly larger than those found within the DCM (Fig. 6D). The observed increase from the DCM to 300 m in the concentration of larger TEP (>4 μm) (Fig. 5) may have been due mostly to abiotic TEP self-assembly (Verdugo et al. 2004, Verdugo & Santschi 2010). The low rates of BP measured at these depths may have limited disintegration of these TEP as they sank to deeper waters. Low sedimentation rates of TEP and marine snow would be expected below the thermocline within the mesopelagic zone (Beauvais et al. 2003, Fischer & Karakas 2009). Moreover, the general absence of upwelling

events in the Mediterranean during this period (Krom et al. 2005, Tanaka et al. 2007, Patara et al. 2009) would tend to inhibit any upward flux of TEP (Azetsu-Scott & Passow 2004). In general, the sedimentation of TEP and marine snow may be enhanced by the incorporation of inorganic 'ballast' material into these particles (Armstrong et al. 2001).

Most images of TEP from 1000 m showed relatively large ($60 \pm 33 \mu\text{m}$) amorphous particles, usually with associated bacteria (Fig. 6D). BP measurements indicated that bacterial metabolism on these particles was low (Fig. 5). Nevertheless, the frequent presence of bacteria associated with significant concentrations of TEP (83 to 386 $\mu\text{g GX l}^{-1}$) in 1000 m samples suggests that these particles may serve as an important source of available organic carbon for slow-growing bacteria at depth.

TEP as a part of the POC pool

Expressing TEP concentrations in terms of carbon is crucial in determining the role of TEP as a constituent of the POC pool and in organic carbon cycling. Only a few studies have attempted to quantitatively evaluate the carbon content of TEP (Mari 1999, Engel & Passow 2001). Using various cultures or natural populations of algae to generate TEP, Engel & Passow (2001) derived a series of empirical conversion factors from micrograms of gum xanthan per liter to micrograms of carbon. We used the lowest conversion factor reported by Engel & Passow (2001) to calculate TEP-C concentrations in relation to POC measured during the ISRALEV and SESAME cruises. This factor ostensibly yielded realistic results, indicating that TEP-C constituted from ~60 to ~100% of total POC in samples from the euphotic zone. However, the same conversion factor gave unreasonably high values (>>100%) for TEP-C as a percentage of total POC when applied to 1000 m samples. As noted by Beauvais et al. (2003), TEP may be somewhat overestimated relative to POC, because the former was collected on 0.4 μm polycarbonate filters, whereas the latter was collected on ~0.7 μm GF/F filters; thus, an unmeasured fraction of POC was not included. Nevertheless, it would appear that even the low conversion factor that we used is unsuitable for ultra-oligotrophic regions such as the Levantine basin, and our numerical values for TEP carbon as a percentage of total POC must be viewed with caution.

Despite the uncertainty of these absolute values, our measurements support previous reports (Azetsu-Scott & Passow 2004, De Vicente et al. 2009), indicating that TEP-C constitutes a significant portion of the POC pool in oligotrophic seas, with an apparent tendency to increase with depth (Fig. 5). Additionally, the signifi-

cant negative correlation of TEP (as $\mu\text{g GX l}^{-1}$) with POC (as $\mu\text{g C l}^{-1}$) indicated that when POC concentrations were highest, the TEP carbon contribution to the total POC pool was lowest (Fig. 8). With increasing evidence that TEP-C constitutes a non-trivial proportion of POC in many aquatic environments (De Vicente et al. 2009, Ortega-Retuerta et al. 2010), our results highlight the need for more accurate conversion factors or other direct methods of determining TEP concentrations in terms of carbon.

CONCLUSIONS

The present study provides the first description of the seasonal, spatial, and depth dynamics of TEP in the ultra-oligotrophic Levantine basin. Based on our data and published literature (Beauvais et al. 2003, Azam & Malfatti 2007, Burd & Jackson 2009, Patara et al. 2009, Verdugo & Santschi 2010), we suggest the following, possible scenario for the role of TEP in organic matter cycling and flux in these waters during the stratified summer to fall season as shown schematically in Fig. 9.

In the upper euphotic zone (0 to 50 m) relatively large ($>10 \mu\text{m}$) TEP, mostly released by or derived from phytoplankton, bacteria, and other plankton, provide a carbon-rich, sticky substrate for bacteria and other microorganisms and serve as a 'microbial shuttle' that transports them to deeper water. This dynamic pool of TEP is continuously formed by biotic and abiotic processes (Verdugo & Santschi 2010), and is further modified by grazing and microbial utilization as it settles through the euphotic water column. Extracellular bacterial hydrolytic enzymes facilitate the utilization of TEP as a renewable source of available organic carbon and other nutrients that fuels the microbial pump (Verdugo et al. 2004, Azam & Malfatti 2007). Most of this TEP is depleted before reaching the DCM, where minimal TEP concentrations and size occur. Below the DCM, TEP concentrations increase, perhaps mainly via the self-assembly aggregation pathway (Verdugo & Santschi 2010). Sedimentation of these particles may transport significant amounts of organic matter and associated microorganisms to abyssal depths.

With depth, the proportion of the TEP-C in the pool of total POC rises and probably becomes increasingly recalcitrant (Fig. 9). In an oligotrophic region, such as the Levantine basin, the TEP fraction of the POC pool may be particularly important for carbon and nutrient cycling and distribution in an otherwise impoverished system.

Better understanding of the role of TEP in organic carbon cycling and transportation in the oceans remains a challenge. As we have shown, existing TEP carbon conversion factors are uncertain. We have no good measure of the relative amounts of TEP or TEP

precursors that are formed via the aggregation pathway (Verdugo & Santschi 2010) or are derived directly from algal or bacterial extracellular release or detrital material. Moreover, no data on the sedimentation and disintegration rates of TEP through the water column are presently available. To be able to construct more detailed oceanic carbon cycling models, quantification of these processes in terms of carbon should be the aim of future studies.

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