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remineralsation at two coastal sites in the Ligurian Sea
(NW Mediterranean) differently influenced by
anthropogenic forcing**

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1 **Organic matter features, degradation and remineralisation at two coastal sites in the Ligurian**
2 **Sea (NW Mediterranean) differently influenced by anthropogenic forcing**

3

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1 **Abstract.** Organic matter (OM) features, degradation and remineralisation were studied in the
2 seawater of a natural coastal site (Marine Protected Area of Portofino) and at a urbanised coastal
3 site (Quarto, Genoa city) of the Ligurian Sea. The accumulation of low trophic value OM and the
4 persistence of phytoplanktonic biomass throughout the year were observed only at the urbanised
5 site. Efficient OM degradation and remineralisation via high-activity (two times higher than at the
6 Portofino site) hydrolytic enzymatic activities (alkaline phosphatase and leucine aminopeptidase)
7 were recorded. This active degradation indicated a generally good resistance of the Quarto system
8 to environmental pressures (coastal inputs and anthropogenic influence). However, the high
9 potential release of recycled inorganic nutrients and the favourable environmental conditions (i.e.
10 higher seawater temperature at the Quarto site and allochthonous inputs), could encourage
11 unpredictable development of the autotrophic fraction, including an already observed dystrophic
12 blooming of toxic microalgae.

13

14 **Key words:** organic matter, enzymatic activity, autotrophic biomass, degradation, remineralisation,
15 coastal waters, urbanisation, Ligurian Sea, NW Mediterranean

1 **1. Introduction**

2 At coastal sites and in natural conditions the allochthonous substances entering the seawater
3 (particulate and dissolved matter such as lithogenic particles, organic detritus, inorganic nutrients)
4 may be delivered by riverine inflow, derive from atmospheric fallout, as well as marine-derived
5 organic matter (OM) from adjacent coastal waters (Graham et al., 2001; Mfilinge et al., 2005).
6 These substances have the potential to modify and drive the ecosystems, giving them the additional
7 energy that makes estuaries and, in general, coastal sites ecologically rich areas (McLusky and
8 Elliott, 2004). However, profound anthropogenic alteration of coastal areas, a common problem in
9 the Mediterranean Sea in over recent decades due to growing urbanisation (Airoldi and Beck,
10 2007), has modified the natural hydrodynamic processes (through defence structures and ports, for
11 instance, Varela and Prego, 2003) and has led to an increase in pollutants and human-derived OM in
12 marine coastal waters via the effects of urbanisation, industrialisation and regional development
13 (Galois et al., 2000; Yamamuro, 2000; Medeiros et al., 2005; Cornelissen et al., 2008). There is
14 great interest in identifying the efficiency of the degradation and remineralisation pathways and the
15 features of the OM, because they drive oxygen demand, nutrient regeneration and energy supply
16 within shallow coastal systems (Goñi et al., 2003; Dunn et al., 2008), and are involved in the
17 regulation of dystrophic events such as eutrophication and changes in autotrophic species
18 composition (Boyes and Elliott, 2006).

19 The degradation and remineralisation of OM are mediated by hydrolytic enzymatic activity (EA)
20 (Manini et al., 2003) and occur in different stages, starting with the transformation of polymers to
21 oligomers or monomers and being completed by the release of inorganic nutrients. Several studies
22 have focused on the induction and/or suppression of EA by its organic substrates (Huston and
23 Deming, 2002; Hoch and Bronk, 2007), indicating as relevant regulating factors the OM origin and
24 lability (Hoppe and Ullrich, 1999; Taylor et al., 2003). Especially in coastal areas, the labile OM
25 (such as proteins and sugars) might become recalcitrant to degradation by complex interactions with

1 mineral particles and/or highly refractory organic compounds (Keil et al. 1994, Dell'Anno et al.,
2 2000), often deriving from anthropogenic activities and allochthonous inputs (Goñi et al., 2003).

3 The OM degradation in the coastal Ligurian Sea has been studied in relation with the energy and
4 material transfer to higher trophic levels and in relation to local and global environmental forcing
5 (Fabiano et al., 1995; Danovaro and Fabiano, 1997; Misic and Covazzi Harriague, 2008). The
6 coastal area of the Ligurian Sea is generally oligotrophic, although signals of dystrophic events
7 have been recorded (Mangialajo et al., 2008). Urbanisation has occurred along almost the entire
8 coast and the areas where human influence is negligible are very few. One of these zones is the
9 Marine Protected Area of Portofino, just to the east of Genoa, the main city of Liguria, and
10 protected from urban influence by a coastal current flowing towards the west. In this area (hereafter
11 called Portofino) and in a urbanised site (Quarto) placed within the Genoa city area, an almost-year-
12 long survey of the quantitative and qualitative features of the OM and of the EA in the coastal
13 surface waters was carried out in 2007.

14 This study aims at identifying the main differences of OM features and degradation and
15 remineralisation processes at the human-forced and natural coastal sites, highlighting the role that
16 these features could have on potential dystrophic events in the human-influenced zone.

17

18 **2. Material and Methods**

19 *2.1 Study site and sampling*

20 Seawater was collected at two sites, characterised by rocky shores: Portofino, inside a marine
21 protected area and protected from direct urban effluents; Quarto, inside the Genoa urban area, near
22 the mouth of a small torrent (300 m eastward) (Fig. 1), used by bathers from spring to autumn and
23 immediately adjacent to recreational facilities.

24 Sampling was carried out monthly from February to November 2007. Seawater for organic matter
25 and enzymatic activity determinations was collected from the surface water-layer (0.5 m depth).

1 A Seabird SBE 25 probe was used to measure temperature, salinity and fluorescence (transformed
2 into chlorophyll-a equivalents). The fluorometer, calibrated by the manufacturer just before the
3 sampling start, was further calibrated against field samples (n=205) collected at different water
4 depths (from surface to 80 m) and immediately processed following Holm-Hansen et al. (1965)
5 during the sampling period at the Portofino site. The chlorophyll-a values were converted to C
6 equivalents following DeJonge (1980).

7

8 *2.2 Analytical procedures*

9 Dissolved organic matter (DOM): We focused on two categories of dissolved organic matter, the
10 protein-like and the humic-like, which are defined by their excitation-emission wavelengths when
11 analysed via synchronous fluorescence spectroscopy. Samples were collected in triplicate by
12 filtering the seawater through a 0.4- μm pore-size membrane and processed immediately.
13 Traditionally, marine organic matter has been divided into DOM and POM on the basis of its
14 behaviour during filtration. Organic matter retained by filters with pores varying between 0.2 and
15 1.0 μm is considered POM, whereas the filterable material is considered DOM (Verdugo et al.,
16 2004). Nevertheless, it must be kept in mind that the 0.4 μm cut-off we chose allowed a fraction of
17 the colloids and small bacteria to pass through the filter and thus be incorporated in the DOM.
18 Synchronous fluorescence spectroscopy is an instrumental technique that involves the simultaneous
19 scanning of excitation and emission monochromators, keeping a constant difference between them.
20 We utilised a difference of 25 nm, as suggested by Ferrari and Mingazzini (1995). An excitation-
21 emission wavelength of 275–300 nm was selected for the protein-like signal, while excitation-
22 emission wavelengths of 395–425 and 420–450 nm were selected for the humic-like fraction (De
23 Sousa Sierra et al., 1994). The 2-D spectra, obtained with a Jasco FP50 spectrofluorometer, were
24 corrected by comparison with the spectrum of ultrapure water (Coble, 1996). Due to the semi-
25 quantitative nature of the analysis, the results were expressed as fluorescence units (fu).

1 Particulate organic carbon and nitrogen (POC and PON): For the particulate organic matter (POM)
2 determinations 1000 ml of seawater were filtered through Whatman GFF glass-fibre filters (nominal
3 pore size 0.4 μm), in triplicate for each sampling. The filters were analysed with a Carlo Erba Mod.
4 1110 CHN Elemental Analyser after acidification with hydrochloric acid fumes to remove the
5 inorganic carbonate fraction, following Hedges and Stern (1984). Cyclohexanone-2,4-dinitrophenyl
6 hydrazone was chosen as standard.

7 Bacterial biomass (BBM) and bacterial specific biomass: Bacterial counts (in triplicate) were
8 performed with epi-fluorescence microscopy (Zeiss Universal Microscope) using 0.2 μm black
9 Nuclepore filters after acridine orange staining (Hobbie et al. 1977). The contribution of different
10 size classes to the total bacterial density was evaluated by assigning bacteria to different size classes
11 according to Palumbo et al. (1984), and by converting the biovolume into BBM carbon content,
12 assuming 310 fg C μm^{-3} as the conversion factor (Fry 1990). We chose this conversion factor in
13 order to compare the results with previous studies in the Ligurian coastal area; nonetheless, very
14 different coefficients may be found in the literature. The specific biomass was obtained by dividing
15 the biomass by the abundance, and expressed as fgC cell⁻¹.

16 Enzymatic activity: The ectoenzymatic kinetic parameters (V_{max} and K_m) of alkaline phosphatase
17 (AP) and leucine aminopeptidase (LA) were determined using gradual concentrations (12.5, 25, 50,
18 100 and 200 μM final concentration) of 4-methylumbelliferyl phosphate (excitation at 365 nm and
19 emission at 460 nm) and of L-leucine 7-amido-4-methylcoumarin hydrochloride (excitation at 380
20 nm and emission at 440 nm), respectively (Hoppe, 1983). The samples and controls (sample water
21 boiled as a blank for accidental contamination due to handling and for abiotic cleavage of the
22 artificial substrates) were incubated in duplicate with 0.5 ml of substrate solution for 3 h in the dark
23 and at in-situ temperatures. Fluorescence was measured with a Jasco FP50 spectrofluorometer
24 previously calibrated with 4-methylumbelliferone and 7-amino-4-methylcoumarin solutions. The
25 kinetic parameters were determined using a Michaelis–Menten model. The Lineweaver–Burke,
26 double-reciprocal, plot was used.

1 Qualitative and functional ratios to determine trophic value: The contributions of the autotrophic
2 and some of the heterotrophic (i.e. bacterial) microbial components to the POC were determined
3 using the percentage contribution of phytoplanktonic biomass (phytoBM) to POC for the former
4 and the percentage contribution of bacterial C to POC for the latter. The qualitative features of the
5 POM were studied utilising the ratio between POC and PON. The POC:PON ratio is based on the
6 assumption that an N-rich POM is more useful to consumers than a C-rich one, therefore the lower
7 the ratio value the higher the potential gain for the consumers.

8

9 *2.3 Statistics*

10 We tested the differences of the same variable between different samplings with one-way ANOVA
11 followed by Newman-Kneuls post-hoc test. To test the relationships between the various
12 parameters, a Spearman-rank correlation analysis was performed. We used the PRIMER 6 β
13 program package to perform cluster analysis of the fourth root transformed data on the organic
14 matter and enzymatic activity to reveal similarities between samplings. Similarities were calculated
15 using Euclidean distances. The complete group linkage method was used to calculate the
16 dendrogram.

17

18 **3. Results**

19 The atmospheric pressure (data provided by www.meteoliguria.it/difi/difi-frame.html) during the
20 summer showed lower values, associated with higher rainfall, than the early spring, which was
21 characterised by generally good weather and low or no rainfall (Table 1). The Quarto site showed
22 slightly higher seawater temperature values (on average 20.3 ± 4.2 °C) than Portofino (19.4 ± 4.2 °C)
23 but, from March to June, a lower salinity (on average 0.2 lower) and the salinity fluctuations were
24 generally wider at Quarto than at Portofino (Table 1).

25 The chlorophyll-a concentrations (Table 1) were significantly lower at Portofino ($p<0.001$) than at
26 Quarto (on average 0.17 ± 0.11 and 0.40 ± 0.14 $\mu\text{g l}^{-1}$, respectively). Portofino followed a temperate-

1 latitude trend, with the highest value in February (spring phytoplanktonic bloom) and then showing
2 a notable decrease (values $\leq 0.21 \mu\text{g l}^{-1}$). At Quarto, instead, high chlorophyll-a concentrations
3 persisted also during summer (values between 0.24 and $0.54 \mu\text{g l}^{-1}$) and, irregularly, in autumn
4 ($0.18\text{-}0.54 \mu\text{g/l}$).

5 The protein-like, fluorescent dissolved organic matter (protein-like DOM) (Fig 2A) at Portofino
6 showed an irregular trend, up to 39.7 ± 2.8 fu in October but down to 4.2 ± 3.2 and 7.4 ± 2.2 fu in
7 September and November, respectively. At Quarto, instead, the summer-autumn values were quite
8 low (below 10 fu) especially during summer and autumn. The humic-like DOM (Fig. 2B) at
9 Portofino showed the highest values on February and June (higher than 10 fu), at a time of high
10 rainfall, but, surprisingly, the November rainfall did not influence the concentrations (2.7 ± 0.5 fu).
11 At Quarto the values were significantly ($p < 0.05$) higher during spring-early summer (from February
12 to June, ranging from 5.1 ± 0.7 to 16.3 ± 1.8 fu) than during late summer-autumn (from July to
13 November, from 2.5 ± 0.3 to 6.0 ± 1.7 fu).

14 The particulate organic carbon (POC) and nitrogen (PON) showed similar trends (Fig. 2C and 2D).
15 Portofino showed an increase during late spring (POC: $222.2\pm 0.9 \mu\text{g l}^{-1}$, PON: $42.1\pm 0.1 \mu\text{g l}^{-1}$) and
16 then a decrease (POC below $170 \mu\text{g l}^{-1}$ and PON below $30 \mu\text{g l}^{-1}$), while Quarto showed the
17 opposite trend, with the highest values on October (POC: $480.9\pm 82.3 \mu\text{g l}^{-1}$, PON: $78.3\pm 14.5 \mu\text{g l}^{-1}$).
18 The summer-autumn POM values of Quarto were significantly higher than those recorded at
19 Portofino ($p < 0.05$).

20 The bacterial biomass (Fig. 3A) generally did not show significant differences between the two sites
21 (on average 8.5 ± 4.5 and $7.4\pm 4.0 \mu\text{gC l}^{-1}$ for Portofino and Quarto, respectively). The trends were
22 also similar, showing increases starting from May-June. At Quarto the bacterial biomass was
23 significantly correlated with the POC (Table 2). A significantly higher bacterial specific biomass
24 was observed from May (Fig. 3B) ($p < 0.05$ for Portofino and $p < 0.01$ for Quarto). The mean values
25 during the whole year were similar for the two sites (on average 59.9 ± 9.4 and $56.2\pm 9.3 \text{fgC cell}^{-1}$
26 for Portofino and Quarto, respectively).

1 The enzymatic activity of alkaline phosphatase (AP Vmax) (Fig. 3C) was significantly higher
2 ($p < 0.01$) at Quarto ($27.1 \pm 11.7 \text{ nM h}^{-1}$) than at Portofino ($13.9 \pm 6.8 \text{ nM h}^{-1}$). The trends at both sites
3 showed increases during March (up to 19.1 ± 0.3 and $29.1 \pm 0.9 \text{ nM h}^{-1}$ at Portofino and Quarto,
4 respectively) and during summer (up to 29.4 ± 0.4 and $50.8 \pm 0.9 \text{ nM h}^{-1}$ during July at Portofino and
5 during August at Quarto), although the Quarto activity was prolonged to autumn ($23.4 \pm 0.6 \text{ nM h}^{-1}$
6 during October). A significant correlation was found at Quarto between AP Vmax and temperature
7 (Table 2). The AP Km (Fig. 3D) showed initially high values during spring ($17.3 \pm 1.2 \text{ }\mu\text{M}$ at
8 Portofino and $27.2 \pm 3.1 \text{ }\mu\text{M}$ at Quarto), but quickly decreased to irregularly lower values for both
9 the sites (from 3.3 ± 0.1 to $13.9 \pm 0.4 \text{ }\mu\text{M}$ at Portofino and from 5.5 ± 0.2 to $16.1 \pm 0.3 \text{ }\mu\text{M}$ at Quarto).

10 The enzymatic activity of leucine aminopeptidase (LA Vmax) (Fig. 3E) was significantly ($p < 0.01$)
11 higher at Quarto ($200.7 \pm 93.3 \text{ nM h}^{-1}$) than at Portofino ($110.1 \pm 32.3 \text{ nM h}^{-1}$). It showed increases
12 during the summer (from values below 100 nM h^{-1} during spring to values up to $165.6 \pm 3.5 \text{ nM h}^{-1}$
13 during July at Portofino and $326.8 \pm 9.4 \text{ nM h}^{-1}$ during August at Quarto), accordingly to increased
14 seawater temperature (Table 2). At Quarto the LA Vmax showed a significant correlation with the
15 PON (Table 2). The LA Km (Fig. 3F) showed at Quarto during summer and autumn (from August
16 to November) values significantly lower than those of Portofino ($p < 0.05$). The specific enzymatic
17 activity per biomass unit of AP (data not shown) was higher at Quarto than at Portofino (AP
18 Vmax:BBM ratio: 5.8 ± 4.7 vs. $2.5 \pm 2.3 \text{ nmol }\mu\text{gC}^{-1}\text{h}^{-1}$, respectively). Similarly, the LA Vmax:BBM
19 ratio (data not shown) was also higher at Quarto than at Portofino (41.6 ± 38.5 vs. $18.5 \pm 14.9 \text{ nmol}$
20 $\mu\text{gC}^{-1}\text{h}^{-1}$, respectively).

21 The analysis of the contribution of the autotrophic fraction to the POC (phytoBM:POC ratio, Fig.
22 4A) highlighted a low phytoplanktonic contribution at Portofino, with only the February value
23 (spring bloom) higher than 20%, while the other samplings showed values $\leq 5\%$. Quarto also
24 showed rather high values, higher than 20%, during February and April, but autotrophic biomass
25 contribution higher than 5% was observed until July. The contribution of the bacterial biomass to
26 the POC (BBM:POC ratio, Fig. 4B) was very similar at the two sites until May, with February

1 values up to 10% and then decreasing to values below 3%. While at Portofino the bacterial
2 contribution showed irregular increases during summer and autumn, reaching the maximum during
3 September (10.6%), at Quarto it remained below 4% throughout these seasons. The quality of the
4 POM (POC:PON ratio) (Fig. 4C) showed, on average, a significantly higher trophic value at
5 Portofino ($p < 0.01$), where it ranged between 4.9 and 8.0. At Quarto, instead, the values were
6 higher, especially during spring and early summer, when they reached a maximum of 9.5 during
7 May.

8
9 The multivariate analysis (Fig. 5) performed on all the organic matter and enzymatic data showed a
10 first division (group A) and a second one (groups B and C). The spring-early summer samplings of
11 Portofino (February) and Quarto (February-May) (group A) were mainly characterised by their high
12 humic-like DOM signals and chlorophyll-a concentrations (Table 3). The summer-early autumn
13 samplings at Quarto (July-October, group B) were mainly characterised by the highest enzymatic
14 activities and POM quantities (Table 3). All the Portofino samplings, except February, and the
15 November sampling of Quarto were instead clustered into group C, showing low or intermediate
16 values (Table 3).

17 18 **4. Discussion**

19 *4.1 Organic matter (OM) features, degradation and remineralisation at the human-forced and at* 20 *the natural sites*

21 The first difference we observed between Portofino and Quarto was the particulate OM (POM)
22 accumulation at the human-forced site, especially during the summer-autumn period. The origins of
23 the increased POM concentrations observed at Quarto could be several (Goñi et al., 2003).
24 Autochthonous accumulation depends on accumulation of autotrophic and heterotrophic biomass
25 produced in-situ. Anyway, the low contribution of both biomasses to the total POM bulk indicated

1 that generally the POM accumulation relied on autochthonous processes only for short time lags
2 and for a minor extent.

3 For what concerns the autotrophic organisms role in the POM accumulation, the multivariate
4 analysis highlighted the grouping of the Portofino February and the Quarto February-to-May
5 samplings (group A of Fig. 5). The statistical analyses indicated that the phytoplanktonic
6 contribution to POC was, during these samplings, the highest (on average $18.8 \pm 5.9\%$) and, at
7 Portofino, the autotrophic biomass values were significantly higher than during the rest of the
8 sampling time. This indicated that the autotrophic fraction had a relevant role during February at
9 Portofino and during the early spring at Quarto, due to the spring bloom (Danovaro and Fabiano,
10 1997). However, the importance of autotrophic-derived OM was short, quickly replaced by detrital
11 OM accumulation. To explain the reason of this short autotrophic relevance, we considered the
12 allochthonous inputs to the coastal waters, that have been classified as oligotrophic (Vidussi et al.,
13 2000; Misic and Fabiano, 2006). The influence of rainfall for both sites and of the torrent for the
14 Quarto site was indicated by the humic-like DOM, whose signal has been related to continental
15 inputs (Vignudelli et al., 2004). The significantly higher humic-like DOM values for the samplings
16 belonging to the group A indicated that the allochthonous inputs could have favoured the
17 production and accumulation of autotrophic biomass adding inorganic nutrients to the coastal
18 seawater. The different trends of the humic-like DOM at the two sites indicated a relevant influence
19 of allochthonous inputs for Portofino only during February, when rainfall occurred. At Quarto,
20 instead, the humic-like DOM indicated allochthonous inputs also during the dry March-May period.
21 Therefore, we observed that the autotrophic biomass accumulation at Quarto was regulated by the
22 Priaruggia torrent inflow. The torrent input was shown also by the salinity values, that during this
23 period were lower at Quarto than at Portofino, despite the absence of rainfall in the March-May
24 period.

25 The autotrophic biomass showed similar values also in the group B of Fig. 5, clustering the Quarto
26 sampling from June to October, although the phytoplankton biomass contribution to POC was low

1 due to the detrital POM accumulation during summer. We hypothesise that, although the
2 continental inputs were lower than during spring (as indicated by the irregularly similar salinity
3 values of the two sites and by the quite low humic-like DOM signal), in-situ OM degradation and
4 remineralisation processes (due to the high EAs) sustained the autotrophic activity also during a
5 season when natural autotrophic processes are generally highly reduced (Danovaro and Fabiano,
6 1997), as actually happened at the Portofino natural site. Anyway, enhanced microzooplankton
7 grazing (not determined) could be considered as a further pressure, down-regulating the
8 phytoplanktonic biomass of Portofino, as often observed in other areas during summer (Huang et
9 al., 2011).

10 The relevance of bacterial biomass on the POM bulk at Portofino was on average slightly lower
11 than previous observations (performed with the same analytical procedures and conversion factors)
12 carried out along the Ligurian coast (8.8 % Danovaro and Fabiano, 1997). It was even lower at
13 Quarto, indicating that at this site BBM was even less important for POM accumulation, likely as a
14 result of a strong top-down pressure (Pagano et al., 2006). Anyway, the significantly higher EA
15 activities found for the group B indicated that bacteria were potentially highly active, but also
16 organisms other than bacteria produce enzymes (Tamburini et al., 2000) and, therefore, we could
17 have overestimated the bacterial activity.

18 The significant increase of the POM bulk at Quarto highlighted by all the samplings belonging to
19 group B was therefore mainly due to accumulation of detrital POM (Taylor et al., 2003), although
20 the increase of POM could be also related to an increase of micro-organisms other than bacteria.

21 The particulate fraction of OM showed a lower trophic quality compared to the natural site (high
22 POC:PON ratio values and low contribution of the labile components such as chlorophyll-a), in
23 accordance with its allochthonous origin (Pusceddu et al., 2009). Detritus could be drained by rain
24 flowing above the soil (Macko et al., 1993) and enter the coastal seawater. During dry periods,
25 instead, this accumulation could be due mainly to the potentially uncontrolled inputs of wastewater
26 from the small torrent and from the neighbouring coastal area, and in general to all the effects

1 largely due to a high population density and to the intense recreational use of the shore especially
2 during summer and early autumn, as in other coastal zones (Castro et al., 2003).

3 *4.2 Role of the OM recycling in potential dystrophic events*

4 The OM degradation and remineralisation processes have been considered within those that are
5 predictive of the changing trophic status of the marine environments (Meyer-Reil and Köster,
6 2000). The loosening of direct relationships between microbial activities (e.g. hydrolytic enzymatic
7 activities) and detrital organic substrates have been indicated as a shift to eutrophication and, more
8 generally, to dystrophic conditions. Following these assumptions, we observed that our human-
9 forced study site (Quarto) showed no relevant signs of alteration. The significantly higher OM
10 concentrations at Quarto than at Portofino were naturally balanced by increased enzymatic activities
11 (both AP and LA), also favoured by the slightly higher seawater temperature of Quarto (Patel et al.,
12 2000). In addition, the significantly lower LA Km values of summer-autumn at Quarto suggested a
13 higher affinity for substrate of this EA. The significant correlation between the PON and LA Vmax
14 observed at Quarto suggested a direct induction of LA synthesis by its particulate substrate, not
15 observed at the other sampling site (Portofino), and the positive correlations of the BBM with the
16 POM at Quarto indicated that bacteria took advantage of the particulate fraction.

17 Although the POM at Quarto was less labile than at Portofino, as indicated by the significantly
18 lower trophic value of the POM of the former site, we observed a qualitative adaptation of the
19 heterotrophic microbial organisms to the available resources. We observed that the LA and AP
20 activities scaled to BBM were higher at Quarto than at Portofino, indicating that Quarto bacteria
21 were potentially more closely adapted to high molecular weight polymer hydrolysis than Portofino
22 bacteria (Tamburini et al., 2002). Anyway, these results have to be considered with caution and
23 only as a potential comparative tool for the two sites, because they imply that, for instance, all
24 bacteria in the assemblage are producing EA at the same rate and that EA is primarily of bacterial
25 origin. Even if entirely of bacterial origin, some enzymes included in EA measurements may have
26 been released from intracellular pools, following cellular damage due to grazing activities.

1 The characteristics of the Quarto OM related processes could have strong influence on the
2 ecological features. The slightly higher seawater temperature of Quarto and the inorganic nutrients,
3 potentially provided by the continental and human-derived inputs and by the relevant processes of
4 degradation and remineralisation via EA, favoured the development and persistence of quite
5 constant chlorophyll-a concentrations throughout the year, resembling the trends previously
6 observed in a neighbouring site in Rapallo harbour, severely forced by anthropogenic activity
7 (Mistic and Covazzi Harriague, 2009). The chlorophyll-a values were never above $1 \mu\text{g l}^{-1}$ and thus
8 quite far from the values typical of eutrophicated areas (Lawrie et al., 2010), similar to those
9 observed in the Ligurian coastal area (Mistic and Covazzi Harriague, 2008). Anyway, dystrophic
10 conditions in estuarine and coastal marine ecosystems may be indicated by the change in the
11 phytoplankton species composition to taxa that may be toxic or inedible (e.g., bloom-forming
12 dinoflagellates) (Boyes and Elliott 2006; McQuatters-Gollop et al., 2009). This effect has already
13 been observed in previous years at Quarto as dystrophic blooming of toxic microalgae (Mangialajo
14 et al., 2008). This allow us to conclude that the same heterotrophic processes that positively
15 enhance the resistance of Quarto to POM accumulation may play a pivotal role at providing the
16 favourable conditions to potential autotrophic-related dystrophic events. A more relevant microbial
17 activity in OM recycling may channel the resources mainly to the microbial food web and to
18 unsuitable autotrophic micro-organisms, thus avoiding the transfer of energy and materials to the
19 higher trophic levels and affecting in an underhanded way the integrity of the urbanised coastal
20 ecosystem of Quarto.

21

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- 9

1 **Figure Captions**

2 Fig. 1. Position of study sites along the Ligurian coast: Quarto (left panel) and Portofino (right
3 panel).

4 Fig. 2. Organic matter features. A: protein-like, fluorescent dissolved organic matter (protein-like
5 DOM) (fu). B: humic-like, fluorescent dissolved organic matter (humic-like DOM) (fu). C:
6 particulate organic carbon (POC, $\mu\text{g l}^{-1}$). D: particulate organic nitrogen (PON, $\mu\text{g l}^{-1}$). Standard
7 deviations are reported; where reported standard deviations are not visible they are included in the
8 marker size.

9 Fig. 3. Bacterial variables (A: bacterial biomass - $\mu\text{gC l}^{-1}$ - B: specific bacterial biomass - fgC cell^{-1}
10 $^{-1}$), alkaline phosphatase (C: AP V_{max} , nM h^{-1} , D: AP K_{m} , μM) and leucine aminopeptidase (E: LA
11 V_{max} , nM h^{-1} , F: LA K_{m} , μM). Standard deviations are reported; where reported standard
12 deviations are not visible they are included in the marker size.

13 Fig. 4. Qualitative ratios of the particulate organic matter. A: contribution of the autotrophic
14 component (phytoBM:POC, %), B: contribution of the bacterial component (BBM:POC, %), C:
15 qualitative features of the particulate organic matter (POC:PON ratio).

16 Fig. 5. Cluster analysis of the organic matter and enzymatic activity variables for the samplings
17 carried out in the different months and sites (P: Portofino, Q: Quarto).

Highlights of the manuscript:

“Organic matter features and recycling in the seawater of two coastal sites in the Ligurian Sea (NW Mediterranean) differently influenced by anthropogenic forcing”

by C. Mistic, M. Castellano, A. Covazzi Harriague

- seawater organic matter (OM) dynamics were studied at two coastal sites (natural and urbanised)
- allochthonous inputs and slightly higher temperature were observed at the urbanised site
- low trophic value OM accumulated at the urbanised site in summer-autumn
- efficient degradation and remineralisation activity coped with OM accumulation
- allochthonous inputs and efficient OM recycling sustain microautotrophs also in summer-autumn
- these autotrophs (toxic dinoflagellates in previous years) may lead to severe dystrophic conditions

	atmospheric pressure (hPa)	rainfall (mm)	temperature (°C)		salinità		chlorophyll-a ($\mu\text{g l}^{-1}$)	
			Portofino	Quarto	Portofino	Quarto	Portofino	Quarto
February	1014	1.5	13.6	14.8	37.3	37.9	0.46	0.54
March	1026	0.0	14.8	15.6	37.9	37.8	0.21	0.25
April	1021	0.0	15.1	16.2	38.1	37.9	0.07	0.46
May	1012	0.0	20.0	21.3	37.7	37.4	0.16	0.45
June	1013	5.1	21.9	22.1	37.4	37.1	0.13	0.45
July	1016	1.1	25.2	25.6	37.6	37.8	0.14	0.54
August	1015	1.0	23.7	25.3	38.1	37.9	0.14	0.24
September	1013	0.0	22.1	23.3	38.1	38.2	0.08	0.37
October	1022	3.7	21.5	23.1	38.2	38.2	0.15	0.54
November	1012	9.3	15.8	16.0	37.1	37.2	0.19	0.18

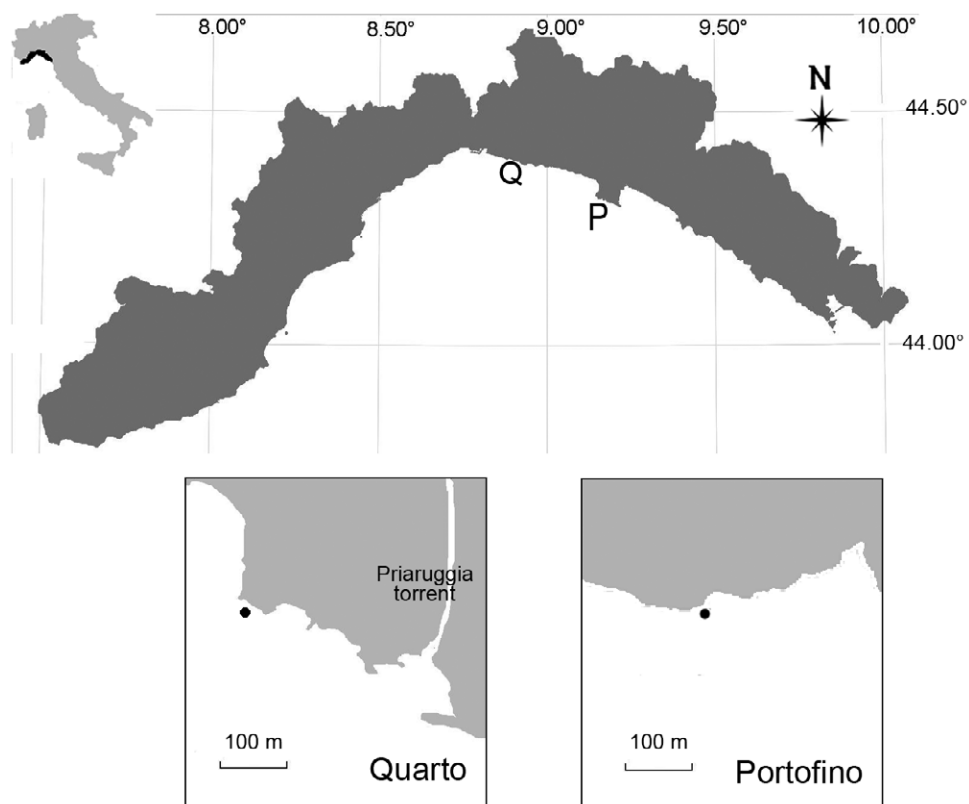
Table 1. Meteorological (atmospheric pressure and rainfall), seawater physical variables (temperature and salinity) and autotrophic biomass (chlorophyll-a) at the two sites during 2007.

Variable 1	Variable 2	r	p<
POC (Quarto)	BBM	0.70	0.05
AP Vmax (Quarto)	temperature	0.74	0.05
LA Vmax (Portofino)	temperature	0.77	0.05
LA Vmax (Quarto)	temperature	0.92	0.001
LA Vmax (Quarto)	PON	0.66	0.05

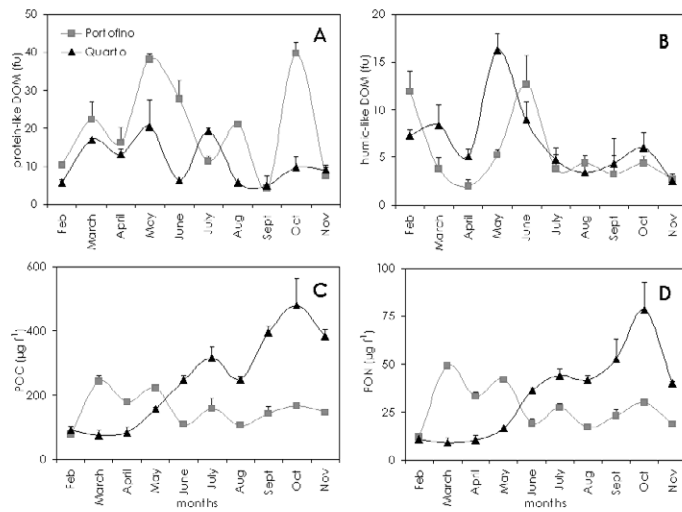
Table 2. Significant correlations between different variables found at the two sites. The number of observations is always 10.

Variable	group A		group B		group C	
	average	sd	average	sd	average	sd
chlorophyll-a ($\mu\text{g l}^{-1}$)	0.43 (C***)	0.11	0.43 (C***)	0.13	0.14 (A***,B***)	0.04
POC ($\mu\text{g l}^{-1}$)	96.8 (B***)	34.5	338.5 (A***,C**)	100.0	186.2 (B**)	82.3
PON ($\mu\text{g l}^{-1}$)	11.9 (B***,C*)	2.8	50.6 (A***,C**)	16.6	30.0 (A*,B**)	11.0
BBM ($\mu\text{gC l}^{-1}$)	4.6	3.4	9.1	1.7	9.1	4.7
specific BBM (fgC cell^{-1})	48.1 (B*,C**)	8.0	61.4 (A*)	2.9	61.3 (A**)	8.8
protein-like (fu)	13.4	5.7	9.3	5.9	19.7	12.5
humic-like (fu)	9.8 (B*,C*)	4.4	5.5 (A*)	2.1	4.5 (A*)	3.0
AP Vmax (nM h^{-1})	19.5 (B**)	7.6	34.6 (A**,C***)	10.5	13.9 (B***)	6.8
AP Km (μM)	12.1	9.6	7.8	1.8	8.0	4.5
LA Vmax (nM h^{-1})	122.4 (B**)	47.3	270.0 (A**,C***)	76.6	114.6 (B***)	31.3
LA Km (μM)	12.8	3.7	11.1	2.2	15.9	6.4

Table 3. Mean values and standard deviations of the involved variables for the three sampling-date groups identified by the multivariate analysis (see Fig. 5). The letters in brackets indicate if the related value is statistically different from the other group ones (one-way ANOVA and Newman-Keuls post-hoc test. *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$)

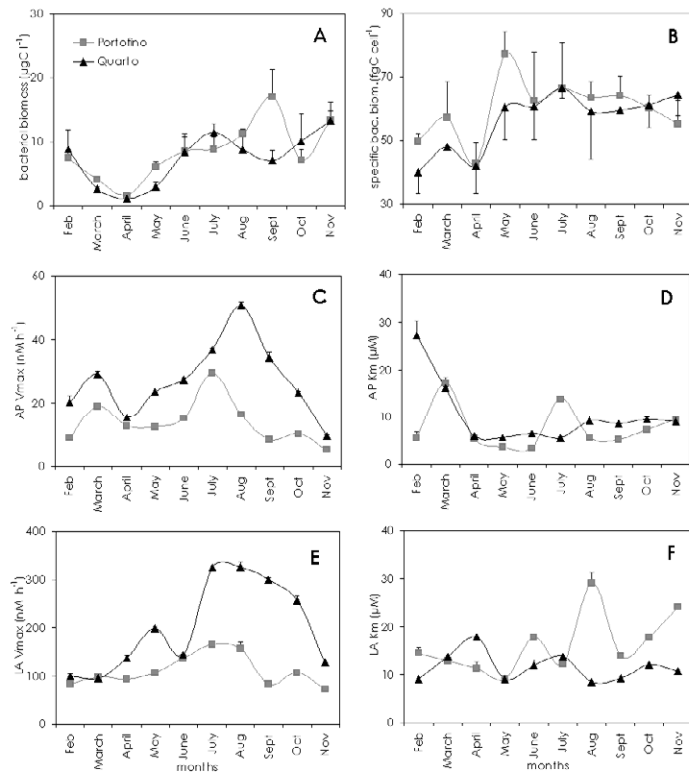


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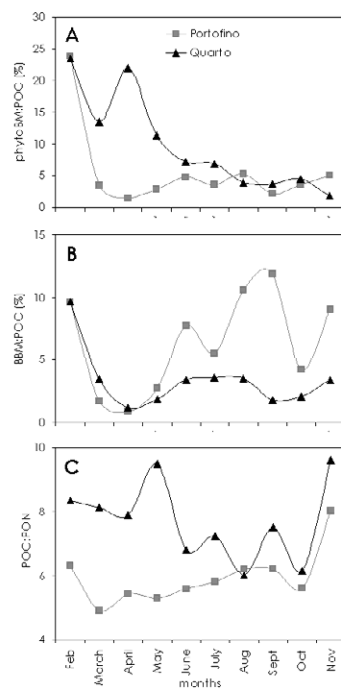
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