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1 **Abstract**

2 A survey of a Ligurian tourist harbour was carried out during winter 2006 and summer 2007 in  
3 order to study the organic matter (OM) turnover through extracellular enzymatic activity. Seawater  
4 and sediments were sampled at six stations, three inside the port boundaries, one outside the port  
5 and two in an area influenced by the outflow of a minor river (Boate). The seawater showed OM  
6 turnover times similar to other oligo-mesotrophic coastal areas, and low concentrations of  
7 chlorophyll-a and inorganic nutrients. The sediments, instead, revealed high OM loads and a  
8 predominance of proteolysis. A significant reduction of the OM loads was observed in the outside  
9 station, indicating that the OM accumulation was due to the structures and activities of the harbour  
10 and to the Boate influence. The OM biotic recycling via enzymatic activity was enhanced especially  
11 during summer. Although the carbohydrates were probably highly refractory, their turnover was  
12 notably faster, due to glycolytic enzymatic activity that was enhanced more than the proteolytic in  
13 both the sediment and in the seawater. This suggested that the removal and recycling of OM were  
14 potentially efficient, and prevented the shift to eutrophication of the Rapallo harbour area.

15

16 **Key words:** organic matter, hydrolytic enzymatic activity, turnover times, harbour, NW  
17 Mediterranean.

## 1 **1. Introduction**

2 The efficiency of organic matter (OM) recycling is crucial at harbour sites because the sediment and  
3 seawater are nearly always characterised by extremely high organic matter contents, depending on  
4 several factors such as eutrophication, sedimentary texture, allochthonous inputs, low  
5 hydrodynamism (Estacio et al., 1997; Danulat et al., 2002; Guerra-García and García-Gómez,  
6 2005). Biotic consumption and degradation, that should be the main processes of OM mobilisation,  
7 are not often efficient enough to maintain the ecosystem balance, due to the excess of detrital OM,  
8 increasing anoxic conditions and, often, the presence of significant levels of contamination by  
9 heavy metals and other toxic components (Cornelissen et al., 2008; Kaufman et al., 2004). In  
10 addition, the occurrence of biochemical interactions between labile OM and recalcitrant OM  
11 molecules (humic acids for instance, Hubberten et al., 1994) originating from allochthonous  
12 continental inputs (rivers, sewage) may modify the turnover times and pathways of organic matter  
13 recycling.

14 In some cases mechanical removal via dredging seems to be the only way to bypass the problems  
15 arising from OM loads, although chemical and biological techniques have been proposed to  
16 mitigate the deleterious effects of OM excess (Murphy et al., 1999). In order to improve the natural  
17 self-repair and/or to choose a suitable, eco-sustainable intervention, knowledge of the peculiar  
18 pathways of OM recycling in a harbour environment is fundamental to gathering significant and  
19 low-cost results (Fabiano et al., 2003).

20 The biological recycling of OM starts with the consumption and degradation of detrital OM by  
21 microbes, carried out especially (but not exclusively) by bacteria. Due to their small size, bacteria  
22 have firstly to cut the polymeric compounds into smaller units by means of extracellular enzymatic  
23 activities, whose features have been considered as proxies of OM turnover and bioavailability and,  
24 in turn, of potential diagenetic fate (Manini et al., 2003; Caruso et al., 2005; Danovaro et al., 2005).

25 The present study aims to investigate the main pathways of OM degradation in Rapallo Harbour,  
26 one of the main tourist harbours of Liguria (NW Mediterranean). OM turnover through extracellular

1 enzymatic activities is determined to highlight the potential responses of the ecosystem to sediment  
2 mobilisation and OM loads, with special emphasis on the quality of the OM (available for or  
3 refractory to consumption).

4

## 5 **2. Material and Methods**

### 6 *2.1 Study site*

7 Rapallo Harbour is the largest tourist harbour in the Marconi Gulf, and is divided into two basins:  
8 the public port and the private Carlo Riva International Tourist Port (Fig. 1). The dominant coastal  
9 current enters from the east, generating feeble internal circulation from the public (maximum 4 m  
10 deep) to the private sector (9 m deep), which was built to facilitate the movement and mooring of  
11 large recreational vessels. The small river Boate (generally carrying coarse organic and inorganic  
12 debris, and used seasonally for mooring small recreational boats) empties into the northern part of  
13 the harbour, which is protected from a massive flow of freshwater from the Boate by a concrete  
14 pier. The harbour and the Boate receive surface runoff and uncontrolled wastewaters, both  
15 municipal and from small factories and farms situated along the 1.9 km course of the small river.

16

### 17 *2.2 Sampling*

18 Sampling was carried out during December 2006 and July 2007 at 6 stations (Fig. 1). Stations 1 and  
19 2 were placed in the private sector of the harbour, station 4 was placed in the public sector, stations  
20 5 and 6 at the mouth of the Boate. Station 3, was situated immediately outside the harbour. At each  
21 station temperature (°C) and salinity profiles were acquired using a Sea Bird probe (SBE 19).  
22 Turbidity was measured with a Hach 2100P turbidimeter, calibrated for turbidity using the  
23 formazine mixtures, and expressed as formazine turbidity units (ftu).

24 Seawater was collected from the water layer next to the sediment (1 m above the bottom) using a  
25 Niskin bottle. Sediment samples were collected using a modified Van Veen grab (surface area 216  
26 cm<sup>2</sup>). The surface layer (0-2 cm depth) was collected.

1

2 *2.3 Sample treatment*

3 For the dissolved oxygen determination, subsamples of seawater were carefully collected from the  
4 bottle, avoiding air bubbles, fixed and analysed following Carpenter (1965). Seawater and sediment  
5 subsamples were immediately processed for the enzymatic analyses as described below.  
6 Subsamples for bacterial counts (both seawater and sediment) were stored with buffered formalin (2  
7 % final concentration). Acetone was immediately added to the sediment subsamples for the  
8 phytopigment extraction. The remaining sediment was stored at -20°C until analysed for the OM  
9 content.

10 Seawater was transferred to the laboratory within two hours of sampling. Seawater subsamples were  
11 filtered through 0.45 µm pore-size membranes and the filtered seawater was analysed for dissolved  
12 chromophoric organic compounds and inorganic nutrients. Seawater subsamples (500-1000 ml)  
13 were filtered through Whatman GF/F filters in order to retain the particulate suspended fraction.  
14 The filters for the phytopigment extraction were immediately placed in acetone. The other filters  
15 were stored at -20°C for the organic matter analyses.

16

17 *2.4 Analytical procedures*

18 Inorganic nutrient (nitrite+nitrate and orthophosphate) concentrations were determined following  
19 Hansen and Grasshoff (1983) with a Nutrient Probe Analyser SYSTEA.

20 Dissolved chromophoric compounds were determined using synchronous fluorescence  
21 spectroscopy, an instrumental technique that involves the simultaneous scanning of excitation and  
22 emission monochromators, keeping a constant difference between them. Generally, the lower the  
23 difference, the higher the resolution. We utilised a difference of 25 nm, as suggested by Ferrari and  
24 Mingazzini (1995). The 2D-spectra, obtained with a Jasco FP50 spectrofluorometer, were corrected  
25 by comparison with the spectrum of ultrapure water (Coble, 1996). We focused on two categories  
26 of dissolved organic matter, defined by an excitation wavelength between 275 and 300 nm (protein-

1 like signal) and between 325 and 400 nm (humic-like signal) (De Sousa Sierra et al., 1994). Due to  
2 the semi-quantitative nature of the analysis, the results were expressed as fluorescence units (fu).  
3 Seawater and sedimentary chlorophyll-a were extracted according to Holm-Hansen et al. (1965).  
4 The Jasco FP50 spectrofluorometer was calibrated with a solution of chlorophyll-a from *Anacystis*  
5 *nidulans*. Autotrophic biomass was calculated by converting the chlorophyll-a concentration to  
6 carbon content, using a conversion factor of 40 (de Jonge, 1980).  
7 For the determination of OM, the seawater and sedimentary proteins and the carbohydrates were  
8 estimated according to Hartree (1972) and Dubois et al. (1956), respectively. A Jasco V-500  
9 spectrophotometer was calibrated with bovine serum albumin and glucose solutions. Proteins and  
10 carbohydrates were converted into carbon equivalents using a factor of 0.49 and 0.40 for proteins  
11 and carbohydrates, respectively (Fabiano et al., 1995).  
12 Bacteria counts were performed with epi-fluorescence microscopy (Zeiss Universal Microscope)  
13 using 0.2  $\mu\text{m}$  black Nuclepore filters after acridine orange staining (Hobbie et al. 1977). The  
14 contribution of different size classes to the total bacterial biomass was evaluated by assigning  
15 bacteria to different size classes, small-size bacteria (diameter  $< 0.5 \mu\text{m}$ , volume =  $0.065 \mu\text{m}^3$ )  
16 medium-size bacteria (diameter from 0.5 to 1  $\mu\text{m}$ , volume =  $0.320 \mu\text{m}^3$ ), large-size bacteria  
17 (diameter from 1 to 2  $\mu\text{m}$ , volume =  $0.574 \mu\text{m}^3$ ), and by converting the biovolume into carbon  
18 biomass content, assuming  $310 \text{ fg C } \mu\text{m}^{-3}$  as the conversion factor (Fry 1990).  
19 The ectoenzymatic kinetic parameters ( $V_{\text{max}}$  and  $K_{\text{m}}$ ) of  $\beta$  glucosidase (BG) and leucine  
20 aminopeptidase (LA) were determined using gradual concentrations (62.5, 125, 250, 500 and 1000  
21  $\mu\text{M}$  final concentration) of 4-methylumbelliferyl glucoside (excitation at 365 nm and emission at  
22 460 nm) and of L-leucine 7-amido-4-methylcoumarin hydrochloride (excitation at 380 nm and  
23 emission at 440 nm) respectively (Hoppe, 1983). The samples and controls (sample water and  
24 sediment boiled as a blank for the accidental contamination due to handling and for abiotic cleavage  
25 of the artificial substrates) were incubated in duplicate with 0.5 ml of substrate solution for 5 h.  
26 Incubations in the dark respected in-situ temperatures. Fluorescence was measured with a Jasco



1 FP50 spectrofluorometer previously calibrated with 4-methylumbelliferone and 7-amino-4-  
2 methylcoumarin solutions. The kinetic parameters were determined from a Michaelis-Menten  
3 model.

4 LA and BG activities were converted into equivalents of C mobilised assuming that 1 nmol of  
5 substrate hydrolysed enzymatically corresponded to 72 ng of mobilised C (Caruso et al., 2005).

6 The OM turnover times (days) were calculated converting proteins and carbohydrates into C  
7 equivalents and then dividing by the the LA and BG activities transformed in equivalent of C  
8 mobilised.

9

## 10 *2.5 Statistical analyses*

11 We tested the differences between the results of a single variable over the different sampling dates  
12 with one way ANOVA analysis. To test the relationships between the various variables, a  
13 Spearman-rank correlation analysis was performed. We used the PRIMER 6 $\beta$  programme package  
14 to perform cluster analyses of the 4th-root-transformed data to reveal similarities between sampling  
15 dates. The considered variables were: enzymatic activities ( $V_{\max}$  and  $K_m$ ), organic matter, bacterial  
16 and autotrophic biomass. Similarities were calculated using Euclidean distances. The complete  
17 group linkage method was used to calculate dendrograms. Similarity percentage analysis (SIMPER)  
18 was used to analyse variable dominance. LINKTREE analysis was applied to the seawater matrix to  
19 test which environmental variable (salinity, turbidity, dissolved oxygen, inorganic nutrient  
20 concentrations, dissolved OM) determined the main differences between stations.

21

## 22 **3. Results**

### 23 *3.1 Environmental variables and dissolved organic matter*

24 Table 1 reports the results related to the environmental variables. Significant increases ( $p < 0.01$ )  
25 were observed from December to July for temperature (from  $16.4 \pm 0.3$  to  $23.9 \pm 1.2$  °C), salinity  
26 (from  $36.3 \pm 1.0$  to  $38.4 \pm 0.3$ ) and turbidity (from  $2.73 \pm 1.23$  to  $5.08 \pm 1.16$  ftu). In particular, the

1 increase of turbidity was highly significant for the harbour-influenced stations (1, 2 and 4)  
2 ( $p < 0.001$ ). Dissolved oxygen (DO) and the nutrient concentrations, instead, decreased significantly  
3 (from  $8.8 \pm 0.9$  to  $7.7 \pm 0.4$  mg l<sup>-1</sup> for DO,  $p < 0.05$ ; from  $2.4 \pm 0.6$  to  $0.8 \pm 0.3$   $\mu$ M for nitrite+nitrate and  
4 from  $0.24 \pm 0.02$  to  $0.15 \pm 0.03$   $\mu$ M for phosphate,  $p < 0.001$ ).

5 Comparing the outside station 3 with the others, we observed significantly lower turbidity values  
6 ( $p < 0.001$  for December,  $p < 0.05$  for July). Considering the nutrient concentrations, only  
7 nitrite+nitrate at the July sampling were lower at station 3 compared to the others ( $p < 0.05$ ). No  
8 differences were observed for the DO.

9 December showed turbidity values significantly lower ( $p < 0.05$ ) at the harbour stations ( $2.37 \pm 0.91$   
10 ftu) compared to the Boate-influenced ones ( $3.71 \pm 1.15$  ftu), where significantly higher DO values  
11 ( $p < 0.001$ ) were observed, probably due to the shallower depth. The stations influenced by the Boate  
12 (especially station 6) showed significantly higher phosphate concentrations during July ( $p < 0.05$ )  
13 compared to the harbour ones.

14 Table 1 reports the results related to the fluorescent dissolved organic matter. While no differences  
15 were observed comparing the December and the July samplings for the Boate stations ( $22.0 \pm 2.9$  and  
16  $21.5 \pm 3.1$  fu for protein-like and  $26.6 \pm 13.8$  and  $16.4 \pm 7.5$  fu for humic-like), the harbour ones  
17 showed significant increases of both protein-like (from  $12.1 \pm 2.9$  to  $59.7 \pm 16.9$  fu) and humic-like  
18 signals (from  $7.6 \pm 5.4$  to  $38.7 \pm 34.5$  fu) ( $p < 0.001$ ). The outside station 3 showed values  
19 significantly lower than the other stations for the July sampling ( $p < 0.001$ ).

### 21 3.2 Autotrophic and bacterial biomasses

22 Comparing the autotrophic biomass of the seawater of December and July, we observed similar  
23 values for the harbour stations ( $19.3 \pm 2.3$  and  $11.8 \pm 11.7$   $\mu$ g l<sup>-1</sup>), but a significant increase for the  
24 Boate stations (from  $16.2 \pm 1.6$  and  $102.8 \pm 12.9$   $\mu$ g l<sup>-1</sup>) ( $p < 0.001$ ). The autotrophic biomass (Fig. 2A)  
25 of the outside station 3 showed values significantly higher ( $p < 0.001$ ) in the seawater of December  
26 compared to the other stations, while they were significantly lower ( $p < 0.05$ ) for July. The

1 autotrophic biomass of the sediment (Fig. 2C) showed a significant ( $p<0.001$ ) decrease from  
2 December ( $113.5\pm 67.9$  ng C  $g^{-1}$ ) to July ( $9.2\pm 5.5$  ng C  $g^{-1}$ ). While in December, the outside station  
3 3 showed a significantly lower content of sedimentary autotrophic biomass ( $p<0.05$ ), a more  
4 homogeneous distribution was recorded for July.

5 Considering the bacterial biomass, the comparison of the seawater data of December and July (Fig.  
6 2B) highlighted a slight increase in July (from  $8.8\pm 1.7$  to  $9.7\pm 2.8$   $\mu\text{gC l}^{-1}$ ). Instead a significant  
7 ( $p<0.05$ ) decrease for the sedimentary bacterial biomass (from  $25.7\pm 8.9$  to  $16.9\pm 9.1$   $\mu\text{gC l}^{-1}$ ) (Fig.  
8 2D) was observed. In July the bacterial biomass of the sediment of station 3 was significantly lower  
9 ( $p<0.01$ ) than in the other stations.

10 Both the seawater and the sediment showed a significant increase ( $p<0.05$ ) from December to July  
11 of the bacterial: autotrophic biomass ratio values (for the seawater:  $0.5\pm 0.1$  vs.  $1.4\pm 1.2$ ; for the  
12 sediment:  $0.3\pm 0.1$  vs.  $2.4\pm 1.7$ ).

13

### 14 3.3 Enzymatic activity

15 Significant increases of enzymatic activity from December to July were observed in the seawater  
16 (Fig. 3A and B) (LA: from  $119.3\pm 25.9$  to  $703.0\pm 282.8$   $\text{nmol l}^{-1}\text{h}^{-1}$ ; BG: from  $0.6\pm 0.2$  to  $10.1\pm 4.4$  n  
17  $\text{mol l}^{-1}\text{h}^{-1}$ ,  $p<0.001$ ), and in the sediment (Fig. 3C and D) (LA: from  $38.0\pm 11.8$  to  $125.3\pm 77.6$  n mol  
18  $g^{-1}\text{h}^{-1}$ ; BG: from  $1.9\pm 1.7$  to  $7.3\pm 3.1$  n mol  $g^{-1}\text{h}^{-1}$ ,  $p<0.01$ ). The BG activity generally had higher  
19 increases (7-fold in the sediment and 18-fold in the seawater) compared with LA (4 and 6 times in  
20 the sediment and seawater, respectively) from December to July.

21 Comparing the different zones of the sampling area, in the seawater the outside station 3 showed  
22 generally significantly lower enzymatic activity ( $p<0.05$ ) compared to the other stations. In the  
23 sediment the BG activity was significantly lower at station 3 ( $p<0.05$ ), while no differences were  
24 observed for LA activity.

1 The enzymatic activity was significantly related to the turbidity. The correlations were significant  
2 for the seawater ( $r=0.68$  for LA and  $r=0.66$  for BG,  $p<0.05$ ,  $n=12$ ), and for the sediment (LA:  
3  $r=0.72$ ,  $n=10$ ,  $p<0.05$ , BG:  $r=0.88$ ,  $n=11$ ,  $p<0.001$ ).

4 The LA:BG ratio values showed the predominance of proteolysis (on average  $138.1\pm 86.3$  in the  
5 seawater and  $29.3\pm 27.2$  in the sediment).

6 Enzyme affinity for substrates (Table 2) generally showed no differences between December and  
7 July. The only significant difference was recorded for the seawater  $LA_{Km}$ , which showed higher  
8 values during July ( $p<0.01$ ).

9

#### 10 *3.4 Organic matter*

11 The seawater particulate protein and carbohydrate concentrations (Fig. 4A and B) were significantly  
12 higher ( $p<0.01$ ) in July (protein: from  $126.2\pm 27.4$  to  $319.4\pm 59.9 \mu\text{g l}^{-1}$ ; carbohydrate: from  
13  $68.4\pm 16.0$  to  $145.0\pm 37.3 \mu\text{g l}^{-1}$ ). The particulate protein concentrations were lower at the outside  
14 station 3, while the particulate carbohydrates showed no differences. In the sediment (Fig. 4C and  
15 D) we observed a significant decrease ( $p<0.05$ ) for the carbohydrate content at July (from  
16  $5711.2\pm 4294.9$  to  $2858.5\pm 1570.3 \mu\text{g g}^{-1}$ ). The decrease of carbohydrates was observed at stations 2,  
17 4 and 5, while proteins decreased significantly ( $p<0.05$ ) at station 5. Station 3, placed outside the  
18 harbour, had significantly lower carbohydrate and protein content ( $p<0.001$ ) in the sediment for  
19 both samplings. Significant correlations were observed in the seawater between LA and particulate  
20 proteins ( $r=0.89$ ,  $n=12$ ,  $p<0.001$ ) and BG and particulate carbohydrates ( $r=0.79$ ,  $n=12$ ,  $p<0.01$ ). In  
21 the sediment a significant correlation between BG activity and carbohydrates ( $r=0.89$ ,  $n=6$ ,  $p<0.05$ )  
22 was observed for December.

23 The turnover times of particulate proteins and carbohydrates in the seawater (Fig. 5A and B) were  
24 significantly lower during July ( $p<0.01$ ). The turnover times of proteins in the sediment (Fig. 5C)  
25 were similar for the different sampling dates ( $p>0.05$ ), also due to the high variability observed at  
26 the stations. The turnover times of sedimentary carbohydrate (Fig. 5D) were, instead, significantly

1 lower during July ( $p < 0.001$ ). The protein turnover rate was 48 times faster than the carbohydrate  
2 rate in the sediments and 71 times faster in the seawater.

3

#### 4 *3.5 Cluster analysis*

5 The multivariate analysis performed on the seawater data (Fig. 6A) confirmed the strong  
6 differentiation between December and July samplings. The enzymatic activity and the particulate  
7 protein concentrations explained up to 70% of this grouping (SIMPER analysis). The LINKTREE  
8 analysis indicates that the changes of salinity (significantly higher at July) and of the inorganic  
9 nutrient supply (significantly lower) exerted a strong influence on the OM production and on its  
10 degradation via enzymatic activity. The December group was further divided into two parts, mainly  
11 due to LA activity, particulate protein and carbohydrate concentrations (explaining 71% of the  
12 differentiation, SIMPER analysis). Salinity, turbidity and nitrite+nitrate concentration were related  
13 to this grouping (LINKTREE analysis). The July sampling showed a division driven by  
14 chlorophyll-a (autotrophic biomass) and by enzymatic activities (78%, SIMPER analysis). The  
15 significantly higher phosphate concentrations observed in the Boate stations, linked to this grouping  
16 (LINKTREE analysis), should have enhanced the primary biomass development and the related  
17 autotrophic and bacterial enzyme activity.

18 The Fig. 6B reports the results of the multivariate analysis performed on sediment data. The  
19 grouping of December and July sampling was visible, although not so clear as for the seawater. In  
20 particular, the dendrogram indicated that in December the outside station 3 showed the lowest OM  
21 content (carbohydrate and proteins explained 72% of the difference, SIMPER analysis), but a  
22 higher autotrophic biomass (explaining 10% of the difference, SIMPER analysis). The Boate  
23 stations in December were different compared to the harbour ones, mainly due to the higher OM  
24 and autotrophic biomass content (explaining 79% of the difference, SIMPER analysis). The harbour  
25 stations in December were more similar to the July sampling, although showing higher  
26 carbohydrate and autotrophic biomass contents, that explained 47% of the difference (SIMPER

1 analysis). The higher LA activity in July explained 16% of the difference (SIMPER analysis). The  
2 July stations showed a homogenisation of the characteristics in the whole sampled area.

3

#### 4 **4. Discussion**

5 Previous studies of Rapallo Harbour defined this site as an OM-rich area, although only moderately  
6 impacted by contaminants such as heavy metals (Covazzi Harriague et al., 2007). The present study  
7 confirms that after 10 years the sediments in Rapallo Harbour were characterised by a high OM  
8 content, similar to those found in eutrophic coastal areas (Fabiano et al., 2001). The significantly  
9 lower values of the outside area (namely station 3) indicated the limited spatial influence of the  
10 enrichment processes depending on the harbour structures and activities. In particular, protein and  
11 carbohydrate sediment content were much higher than those reported for the adjacent coastal areas  
12 of the Ligurian Sea (Danovaro and Fabiano, 1995; Misic and Covazzi Harriague, 2008), but they  
13 were one order of magnitude lower than those found in the larger harbour of the neighbouring city  
14 of Genoa (Fabiano et al., 2003). In the seawater the chlorophyll-a and inorganic nutrient  
15 concentrations were lower than those recorded in the waters of the eutrophic harbour of A Coruña,  
16 Spain (Varela and Prego, 2003). The seawater chlorophyll-a concentrations and the particulate OM  
17 were quite similar or only slightly higher than those recorded in the neighbouring coastal Ligurian  
18 Sea (Danovaro and Fabiano, 1997). These observations have led us to the conclusion that seawater  
19 and sediment may follow separate trophic pathways in Rapallo Harbour: the former towards  
20 mesotrophy and oligotrophy, the latter towards eutrophy and, possibly, eutrophication. The different  
21 trends of the seawater and sediments were due to the small size of the harbour and to the good  
22 internal water circulation, as revealed by the high dissolved oxygen concentrations. Furthermore,  
23 the irregular influence of the Boate did not allow permanent gradients for the studied variables.  
24 Similarly, studies carried out in larger harbours (Moreno et al., 2008) have highlighted that  
25 environmental sedimentary variables change significantly over a hundred-metre scale and do not  
26 follow a distribution gradient from the inner to the outer harbour.

1 The OM recycling via biotic processes was performed by enzymatic activities. The origin of these  
2 enzymes was not clear. In fact we observed a low bacterial contribution (Fig. 2), significantly  
3 decreasing from the December sampling to the July one in the sediment, and the uncoupling of  
4 enzymatic activity and bacterial biomass in the seawater and sediment. Several studies have  
5 demonstrated that enzymatic activity is not only a feature of bacteria and heterotrophic organisms,  
6 but also of vegetals such as unicellular algae (Dyrhman, 2005 and references therein). In our study  
7 the bacterial:autotrophic biomass ratio values in the sediment and in the seawater were significantly  
8 lower at December compared to July, indicating a higher potential relevance of the autotrophic  
9 compartment in remineralisation processes especially during the former sampling. The values of the  
10 autotrophic biomass of the harbour and Boate-influenced stations with the outside station 3, were  
11 very variable both in the seawater and in the sediment, suggested that the Rapallo harbour area  
12 should follow peculiar autotrophic development trends, as observed by Varela and Prego (2003) for  
13 the A Coruña Harbour (NW Spain).

14 In our study we observed significant increases in enzymatic activity in both the seawater and the  
15 sediment at nearly all the stations during July (Fig. 3), and these variations were the main factors  
16 responsible for the grouping of the seawater stations provided by the multivariate analysis (Fig.  
17 6A). Previous studies in the Rapallo harbour reported an increase of protein and carbohydrate flux  
18 to the sediment during summer, with a strong reduction during winter (Covazzi-Harriague et al.,  
19 2007). An increase of organic matter should naturally stimulate a higher expression of enzymatic  
20 activity (Danovaro et al., 2005), as confirmed by the significant correlations found between the  
21 enzymatic activity and their relative organic substrates in the seawater during our study. In addition,  
22 other research performed in the adjacent coastal Ligurian Sea reported irregular increases of the  
23 enzymatic activity during summer and autumn (Misic and Covazzi-Harriague, 2008) and Cividanes  
24 et al. (2002) observed at coastal sites an increase of degradation processes of OM during summer.  
25 Furthermore, in our study the mixing of the surface layer of the sediment with oxygenated seawater,  
26 characteristic of the summer season when movements of the touristic boats increase (Covazzi

1 Harriague et al., 2007), could have changed the chemical medium, thus facilitating the natural  
2 development of oxidising activities in the water column and in the sediment.

3 Although a low temperature could be a limiting factor on enzymatic activity in seawater and  
4 sediment during winter (Patel et al., 2000), the quantitative and qualitative features of the OM  
5 should influence enzyme expression. The faster turnover of proteins in both the seawater and  
6 sediment (Fig. 5) showed that they were the preferred substrate for degradation. The relevance of  
7 proteins depends on their value both as energy and amino acid source, avoiding energy-expensive,  
8 ex-novo synthesis. Especially in the sediment, the unchanged turnover time values between  
9 December and July indicated that proteins were exploited at quite a constant rate in the two  
10 samplings, although in the seawater the different LA  $K_m$  observed between December and July  
11 suggested a change of the enzyme characteristics.

12 The features of the protein degradation processes highlighted the difference between the seawater  
13 and sediment. In the seawater, the comparison of our data with that collected in other areas with  
14 different trophic states, showed that the LA activity ( $75.2 \text{ g C m}^{-3} \text{ year}^{-1}$  during December and  $443.3$   
15  $\text{g C m}^{-3} \text{ year}^{-1}$  during July) was similar to coastal lagoons ( $96.2\text{-}474.5 \text{ g C m}^{-3} \text{ year}^{-1}$ ) (Caruso et al.,  
16 2005) characterised by oligo-mesotrophy. In contrast in the sediment, the predominance of  
17 proteolysis observed during our study would indicate a tendency to eutrophication (Meyer-Reil and  
18 Köster, 2000). However, the turnover times in the sediment in our sampling sites were similar to  
19 those found by Manini et al. (2003) (on average about 5 days) in coastal lagoons characterised by a  
20 large and efficient channelling of OM through the microbial food web to higher trophic levels.

21 The mechanical mobilisation of sediments could have influenced the protein dynamics. The  
22 increased tourist activity in the harbour during summer (Covazzi Harriague et al., 2007) allowed the  
23 resuspension of the sediment and exposed the organic materials embedded in the inorganic matrix  
24 during winter and spring. The significantly higher turbidity values in July compared to December  
25 (Table 1) especially at the harbour stations, confirmed that such resuspension processes occurred  
26 and, in addition, the increases of protein-like signals, observed again at stations 1, 2 and 4, but not



1 at the outside station 3, suggested that the higher dynamism of the proteic fraction was confined to  
2 the harbour area. The significant correlations between turbidity and enzymatic activity in the  
3 seawater and in the sediment supported the hypothesis of stimulation of enzymatic activity by water  
4 column-sediment mixing. The mechanical mobilisation of sedimentary proteins could explain the  
5 decrease of the station 5 protein content. In fact, station 5 was placed at the end of a concrete pier  
6 influenced by both the Boate and harbour loads, but not protected like the inner stations and  
7 probably subjected to a higher exchange of seawater with the outside area. At the same time, the  
8 protein resuspended in the water had likely stimulated microbial activities and might have settled on  
9 the sediment of the other stations, generating allochthonous increases of both the detrital and labile  
10 protein contents.

11 Referring to the statistical multivariate analysis, we observed that the sedimentary carbohydrates of  
12 the Rapallo Harbour contributed to the station grouping from 22 to 46% (Fig. 6B), showing a  
13 notable relevance among the other variables and, therefore, probably having a major role in the  
14 harbour trophic dynamics. They showed turnover times similar to those found for coastal lagoons,  
15 where a lower efficiency in OM mobilisation was observed with consequent higher retention and  
16 possible burial (Manini et al., 2003). This indicated that the carbohydrate pool of the Rapallo  
17 Harbour sediments should actually have been refractory, and explained the high sedimentary  
18 content we found, significantly higher than the content of station 3. Nevertheless, the significant  
19 correlation between BG activity and carbohydrates in December and the significantly lower  
20 turnover times of carbohydrate of July indicated that, despite their refractivity, the carbohydrates  
21 might contribute to the supply of energy to microbes. No significant changes in  $BG_{Km}$  were  
22 observed between the different sampling dates, indicating that the glycosidic enzyme did not change  
23 its characteristics.

24

## 25 **5. Conclusions**

1 The main weakness of the Rapallo Harbour environment, and of harbour areas in general, is the  
2 high OM content of the sediment, especially the carbohydrate content. This feature characterised  
3 the area as eutrophic, where the shift to eutrophication might be quite easy. On the contrary, the  
4 seawater showed oligo-mesotrophic conditions and had good oxygenation. The sediment and water  
5 compartments were able to express strong enzymatic activity during summer, as a result of the  
6 seasonal, natural increase of degradation activities but, partially, also due to the sediment  
7 mobilisation related to the increased recreational activities, as previously observed in the same area.  
8 The general reduction of the turnover times of the OM stimulated the removal and recycling of OM  
9 and prevented the shift to eutrophication.

10

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3

ACCEPTED MANUSCRIPT

1 Table 1. Water column depth of the sampling stations and values of the physical and chemical variables of the bottom water layer (1 m above the  
 2 bottom).  
 3  
 4

Month	Station	Depth (m)	Temperature		Salinity		Turbidity		Oxygen		Nitrite+Nitrate		Phosphate		Protein-like		Humic-like	
			°C	sd	sd	ftu	sd	mg l <sup>-1</sup>	sd	μM	sd	μM	sd	fu	sd	fu	sd	
December	1	9	17.00	0.18	37.08	0.12	2.46	0.86	6.96	0.64	1.73	0.66	0.26	0.02	15.5	0.6	6.4	1.2
	2	5	16.12	0.07	37.02	0.02	1.56	0.28	9.44	0.35	2.02	0.22	0.21	0.01	10.5	1.7	3.0	1.9
	3	5	16.03	0.02	37.12	0.03	1.46	0.23	9.11	0.33	2.45	0.78	0.23	0.00	14.5	1.4	4.0	0.7
	4	3	16.31	0.13	34.95	0.40	3.42	0.10	8.79	0.95	2.59	0.68	0.24	0.01	10.5	0.3	13.5	1.1
	5	4	16.39	0.12	36.22	0.33	2.68	0.29	9.36	0.01	2.42	0.73	0.23	0.01	26.4	9.5	36.4	2.6
	6	2	16.31	0.20	35.15	1.16	4.74	0.12	9.02	0.03	3.46	0.51	0.24	0.01	17.5	1.5	16.8	7.3
July	1	9	22.10	0.18	38.38	0.06	5.41	0.32	7.23	0.09	0.96	0.02	0.16	0.02	46.4	3.5	18.7	1.5
	2	5	23.64	0.24	38.20	0.05	5.63	0.08	7.88	0.04	0.58	0.19	0.15	0.01	54.1	8.0	18.8	2.2
	3	5	23.25	0.06	38.14	0.07	3.65	0.46	8.33	0.42	0.52	0.02	0.13	0.01	14.3	0.3	13.3	2.0
	4	3	24.34	0.34	38.69	0.12	6.26	0.97	7.59	0.02	0.66	0.17	0.10	0.00	78.8	6.0	78.5	5.1
	5	4	25.14	0.15	38.34	0.04	5.92	0.32	7.72	0.06	0.66	0.22	0.18	0.01	19.3	1.8	11.1	1.9
	6	2	25.01	0.55	38.93	0.32	3.61	0.29	7.65	0.12	1.29	0.18	0.17	0.02	23.8	7.0	21.7	15.2

5  
6

Table 2. Estimated  $K_m$  values ( $\mu\text{M}$ ) of leucine aminopeptidase (LA) and  $\beta$  glucosidase (BG) in seawater and sediment. nd: not determined.

sampling station	seawater			sediment			
	LA $K_m$	sd	BG $K_m$	LA $K_m$	sd	BG $K_m$	sd
December							
1	0.04	0.01	0.06	0.04	0.04	0.29	0.09
2	0.04	0.00	0.09	0.04	0.04	0.04	0.06
3	0.05	0.00	0.09	0.01	0.01	0.05	0.02
4	0.06	0.00	0.05	0.01	0.01	0.01	0.04
5	0.04	0.00	0.03	0.00	0.00	0.14	0.04
6	0.05	0.00	0.07	0.01	0.01	0.10	0.03
average	0.05		0.06			0.42	0.05
sd	0.01		0.02			0.23	0.02
July							
1	0.08	0.01	0.09	0.03	0.03	0.03	0.00
2	0.08	0.01	0.07	0.01	0.01	0.00	0.13
3	0.10	0.01	0.04	0.00	0.00	0.02	0.07
4	0.08	0.00	0.04	0.00	0.00	0.55	0.60
5	0.07	0.00	0.03	0.00	0.00	0.15	0.15
6	0.06	0.00	0.02	0.00	0.00	nd	nd
average	0.08		0.05			0.32	0.20
sd	0.01		0.02			0.16	0.23

1

2

3



## 1 **Figure captions**

2 Fig. 1. Sampling sites in Rapallo Harbour (Ligurian Sea).

3 Fig. 2. Autotrophic and bacterial biomass in the water ( $\mu\text{g C l}^{-1}$ , A) and in the sediment ( $\mu\text{g C g}^{-1}$ ,  
4 B). Bars denote standard deviation. Asterisks denote significant differences ( $p < 0.05$ ) between the  
5 December and July values of the same station. nd: not determined.

6 Fig. 3. Enzymatic activity ( $V_{\text{max}}$ ) in the seawater ( $\text{nmol l}^{-1} \text{h}^{-1}$ , above) and sediment ( $\text{nmol g}^{-1} \text{h}^{-1}$ ,  
7 below) at the six stations. LA: leucine aminopeptidase (A, C), BG:  $\beta$  glucosidase (B, D). Bars  
8 denote standard deviation. Asterisks denote significant differences ( $p < 0.05$ ) between the December  
9 and July values of the same station. nd: not determined.

10 Fig 4. Organic matter. Particulate protein (A) and carbohydrate (B) concentrations in the seawater  
11 ( $\mu\text{g l}^{-1}$ ) and protein (C) and carbohydrate (D) contents ( $\mu\text{g g}^{-1}$ ) in the sediment. Bars denote standard  
12 deviation. Asterisks denote significant differences ( $p < 0.05$ ) between the December and July values  
13 of the same station. nd: not determined.

14 Fig. 5. Potential turnover time (days) for proteins (A, C) and carbohydrates (B, D) in the seawater  
15 (upper) and the sediment (lower). nd: not determined.

16 Fig. 6. Cluster analysis performed on seawater (A) and sediment (B) variables (see text for  
17 explanations). At each significant grouping, the dominant variables (SIMPER analysis) are reported  
18 with their contribution (%). Plus and minus indicate the group showing, respectively, the highest  
19 and lowest values of the dominant variables.

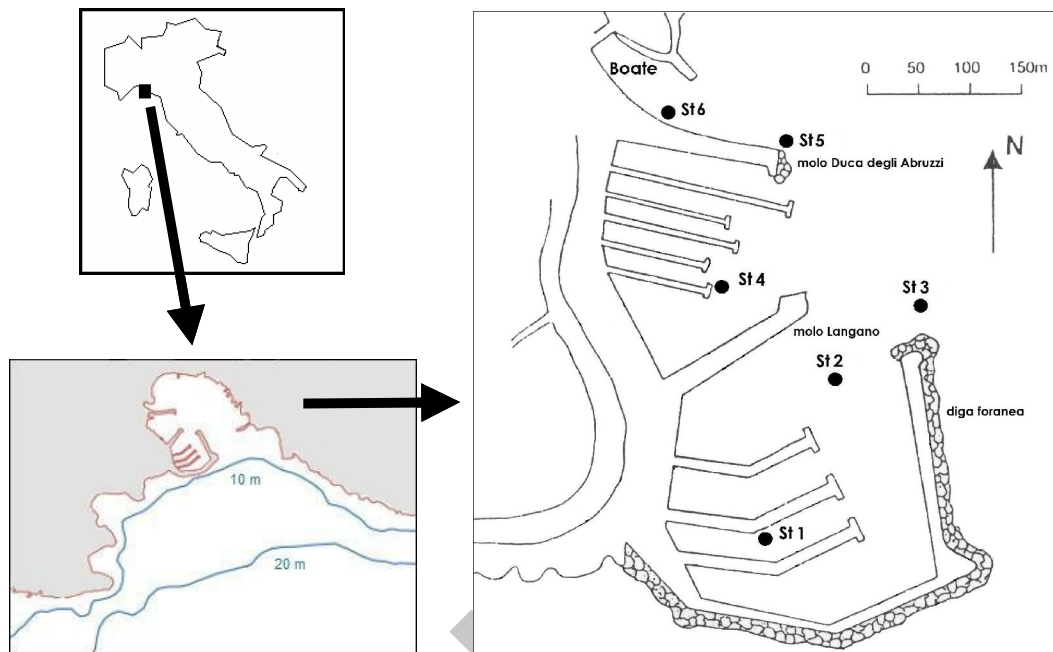


Figure 1

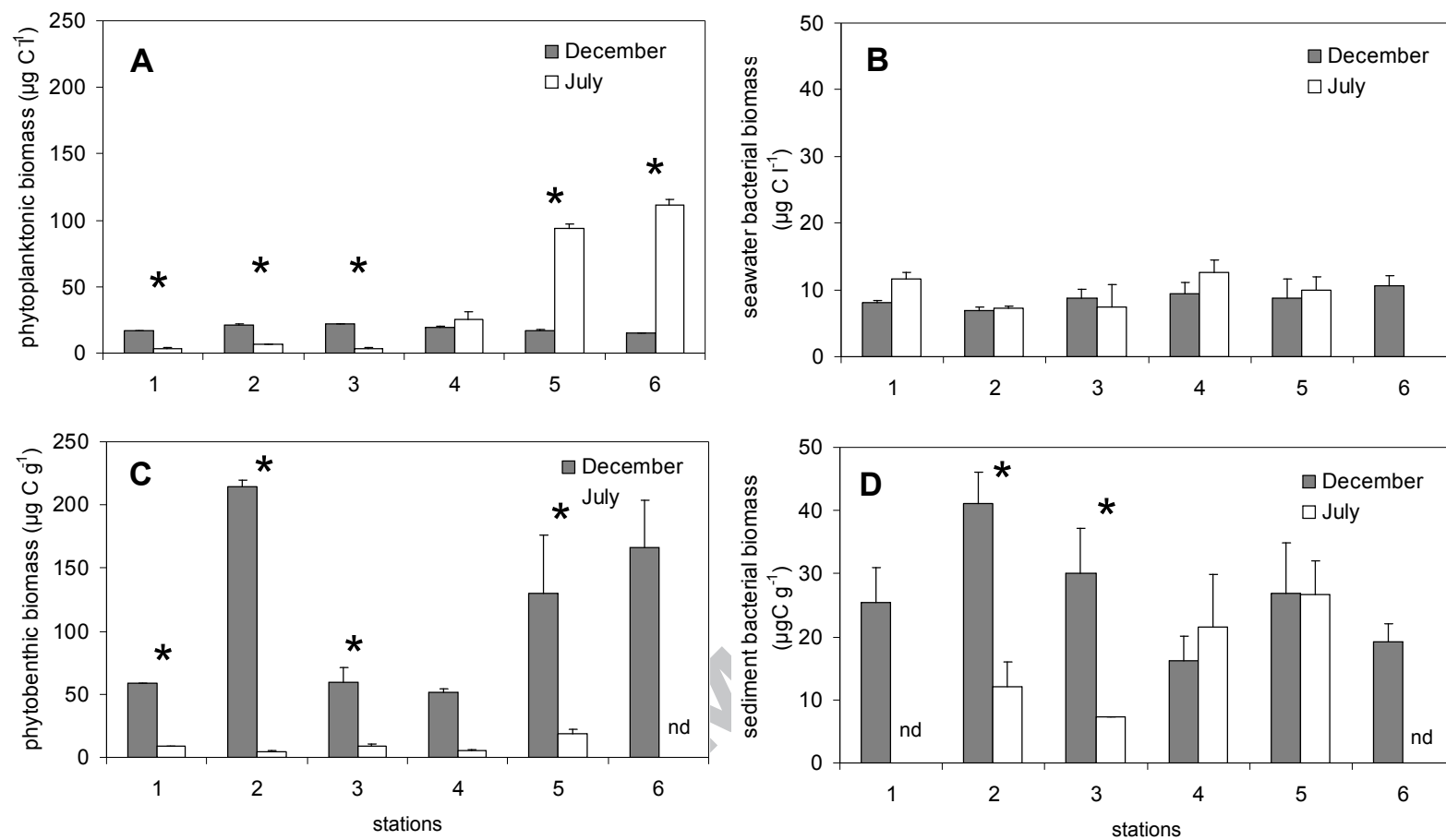


Figure 2

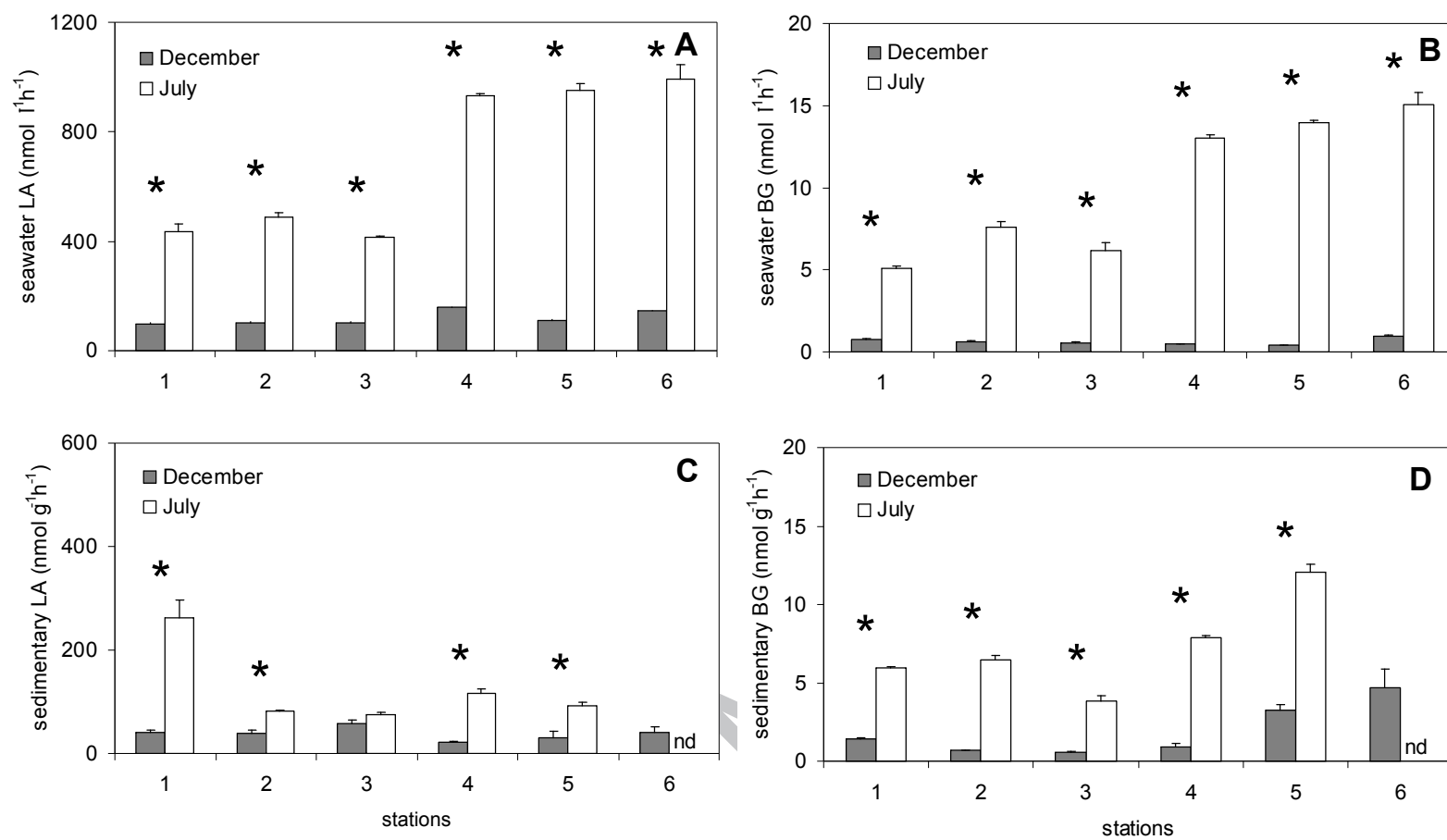


Figure 3

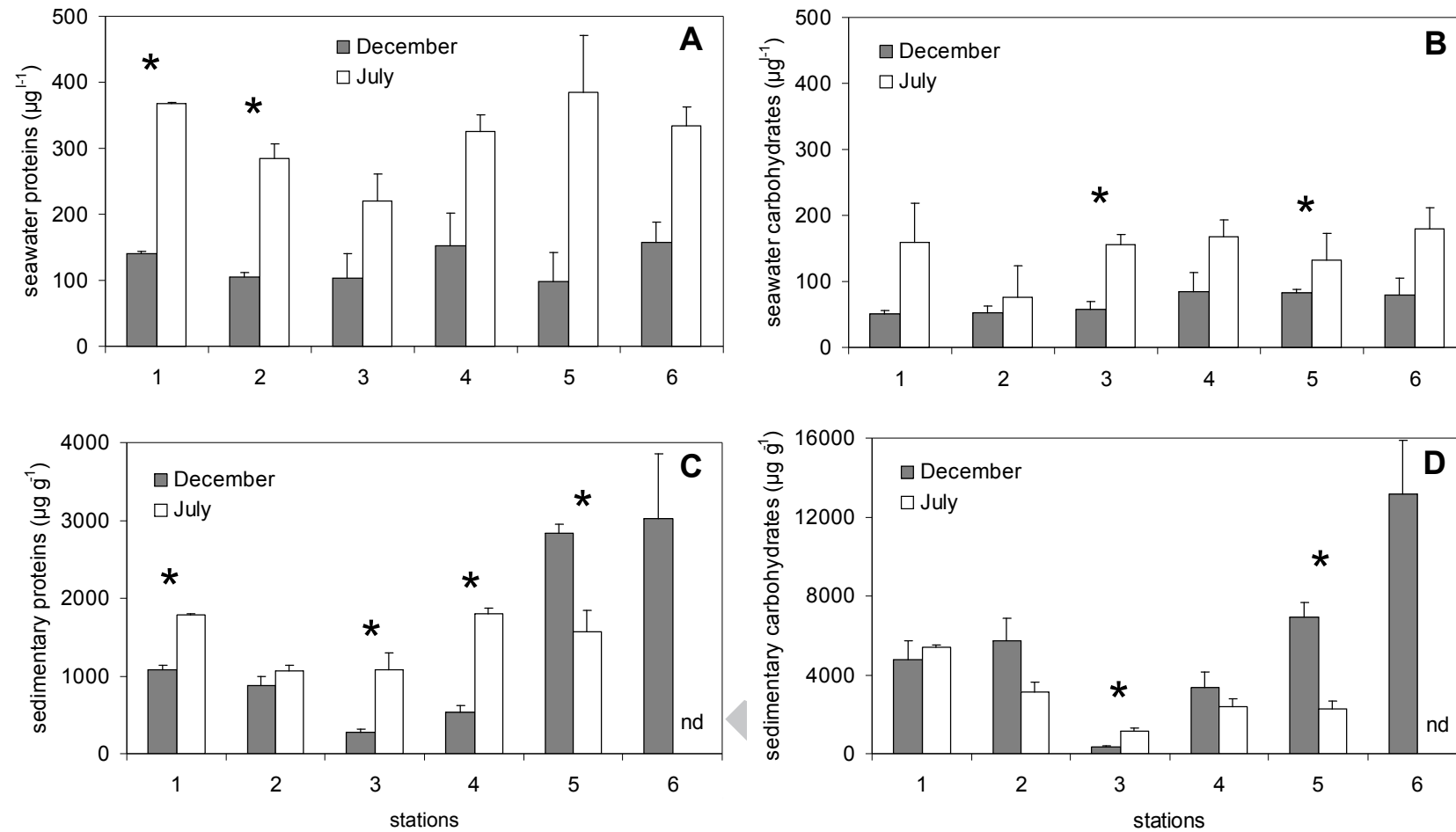


Figure 4

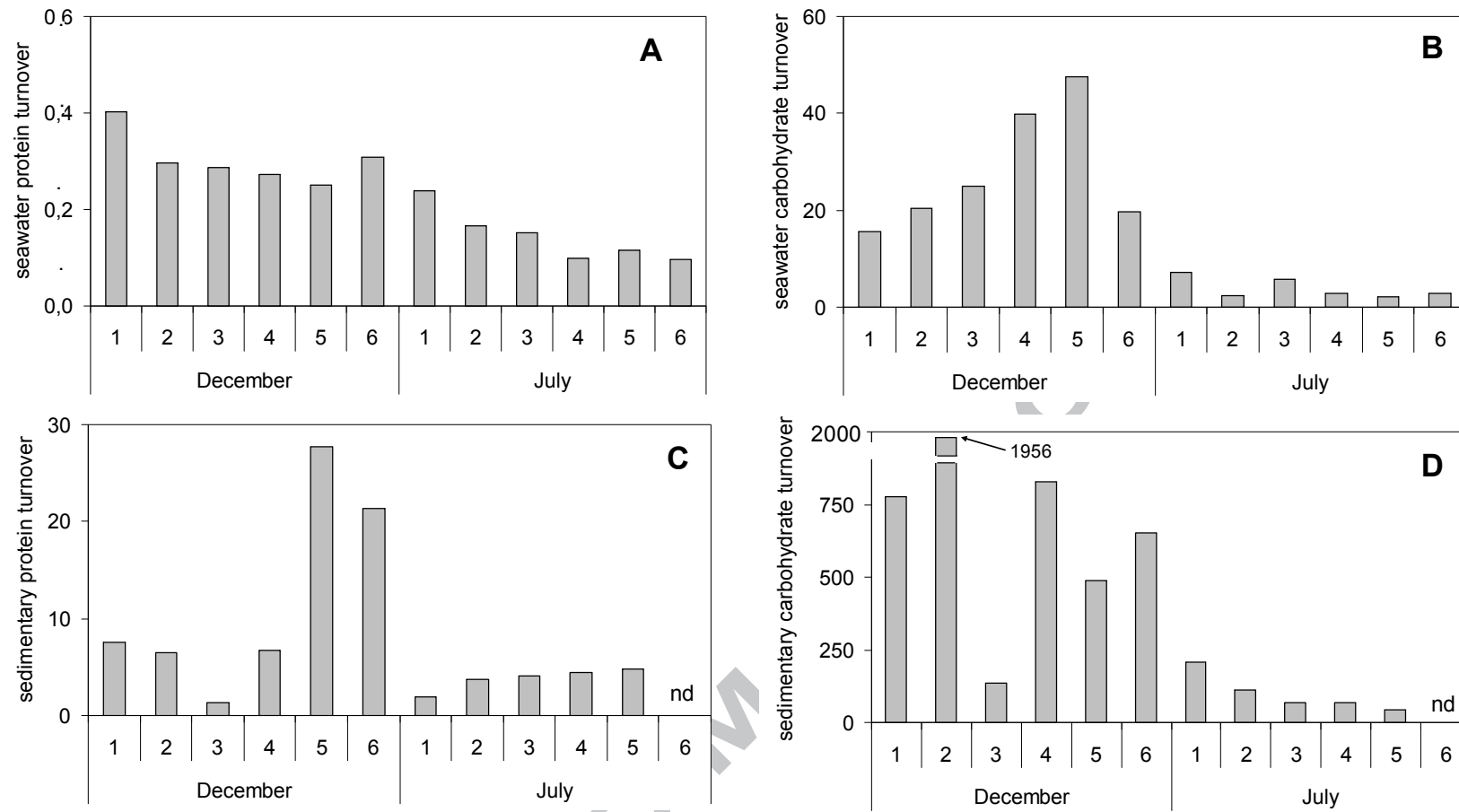


Figure 5

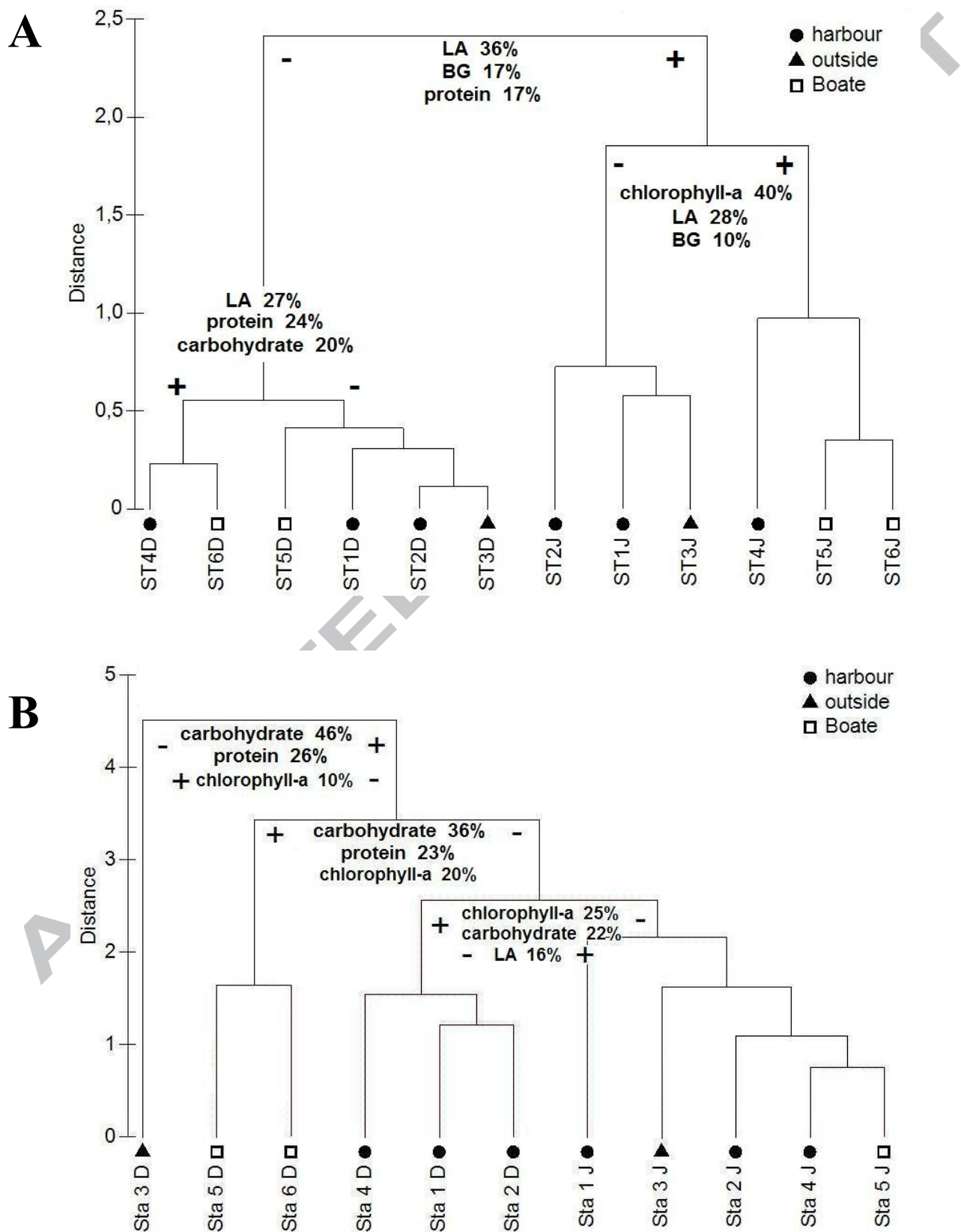


Figure 6