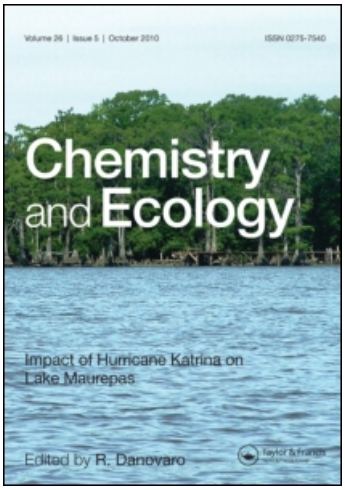


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# Microbial respiratory and ectoenzymatic activities in the Northern Adriatic Sea (Mediterranean Sea)

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# MICROBIAL RESPIRATORY AND ECTOENZYMATIC ACTIVITIES IN THE NORTHERN ADRIATIC SEA (MEDITERRANEAN SEA)

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The carbon transfer through the microbial community in two areas of the Northern Adriatic Sea was estimated by proteolytic and respiratory activities during four oceanographic surveys carried out in June, 1996, 1997 and February, 1997, 1998.

In front of the Po Delta (area A), the mean rates of proteolytic activity range from 4.9 to 9.9 $\mu g\,C\,h^{-1}\,l^{-1}$; near Ancona (area B), they range from 3.1 to 7.6 $\mu g\,C\,h^{-1}\,l^{-1}$. Respiratory rates vary between 0.19 and 2.29 and between 0.24 and 1.40 $\mu g\,C\,h^{-1}\,l^{-1}$ in areas A and B, respectively. In general, high rates occur in the surface layers, within the first 10 m of depth. In area A, proteolytic and respiratory rates undergo seasonal course, with high activity in warm periods. In area B, respiration and bacterioplankton abundance increase from the first to the second year, whilst proteolytic activity decreases.

The sequence of metabolic steps in the carbon transfer within the bacteria, from the biotic *vs.* the abiotic compartment, was drawn in order to define the actual role of bacterial biomass in the biogeochemical fluxes in an ecosystem which often suffers distrophic crises. Respiratory turnover rates, in the upper 10 m depth, reach low values in cold periods and high values in June, 1997. The carbon transfer versus mineralization flows better in the summer period, in particular in June, 1997. However, the bacterial growth efficiency ranges from 17 to 38% in area A and from 13 to 44% in area B with highest values in February, 1997, when bacteria contribute in a relevant way to the overall respiration.

*Keywords*: Microbial communities; Adriatic Sea; respiration

## 1. INTRODUCTION

Bacteria, as an important part of the planktonic community, contribute significantly to regulate the flux of organic matter in the aquatic ecosystem. The carbon transfer through the biotic and abiotic compartments takes place through a sequence of metabolic steps. The initial breakdown, occurring through extracellular hydrolytic enzymes produced by bacteria, transforms biological polymers or macromolecular

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organic materials, part of particulate organic carbon (POC), into soluble products. The resulting dissolved organic carbon (DOC) may be consumed by bacteria and incorporated as newly produced biomass (BCP, bacterial carbon production). Consequently, respiration (R) by means of the metabolic oxidation of organic matter liberates carbon dioxide newly available for the autotrophic production (Heip *et al.*, 1995; Azam *et al.*, 1993; Del Giorgio *et al.*, 1997).

In the framework of the CNR-MURST Prisma II project (subproject: Biogeochemical Cycles) a study on bacterioplankton abundance, ecto-enzymatic and respiratory activities over the organic matter has been carried out in order to quantify the carbon transfer through the microbial community in two areas of the Adriatic Sea. The peculiar hydrographic features of this environment are the low depth, the strong fluvial inputs in the Northern basin with the related occurrence of frontal systems between the saltier and more diluted waters, and finally the alternation of winter remixing and summer stratification, all strongly influencing the microbial activities (Artegiani *et al.*, 1997; Puddu *et al.*, 1998).

Previous researches in the Northern Adriatic Sea have shown that the bacterial abundance undergoes small variations during the seasonal course, whilst different cell size and morphotypes, genera composition and activity succeed during the year (La Ferla *et al.*, 1998a; Puddu *et al.*, 1998; Zaccone and Caruso, 1996; Zaccone *et al.*, 1998a).



## 2. MATERIALS AND METHODS

During 1996–1998, four oceanographic cruises were performed in summer (June, 1996 and 1997) and in winter (February 1997 and 1998) by R/V "Urania"(CNR) in two areas of the Northern Adriatic Sea: the first one, A area, in front of the Po Delta and the second one, B area, near the Ancona coast (La Ferla *et al.*, 1998b). A total of 120 (area A) and 116 (area B) sea water samples were collected at different depths at stations chosen according to the frontal system position.

Extracellular enzymatic activity (EEA) was estimated according to the Hoppe method (1983), using a specific fluorogenic substrate. We used L-leucine-7-amido-4-methyl-coumarin hydrochloride (Leu-MCA) at saturating concentration (200 μM) to estimate leucine aminopeptidase activity as an indicator of protein hydrolysis.

The respiration rates and the consequent metabolic production of carbon dioxide (R) were measured by the study of the Electron Transport System activity (ETS); the assay is based on the conversion of tetrazolium salt in formazan (Packard, 1971), the results are reported as V *max* and have been converted into carbon by using a respiratory quotient of 1.

Bacterial abundance was determined by DAPI staining technique (Porter and Feig, 1980). Bacterial abundance data of A area have kindly been provided by Del Negro (LBM, Trieste). Cell numbers were converted in carbon bacterial biomass using the conversion factor of 20 fg C/cell (Ducklow and Carlson, 1992).

In the B area, bacterioplankton cell volumes and morphotypes were determined by the microphotographic technique using Acridine Orange (AO) dyeing, according to La Ferla *et al.* (1998a).

POC content has been provided by Rabitti (IBM-CNR, Venice); DOC by Pettine and bacterial carbon production (BCP), by using the thymidine incorporation method, by Puddu (IRSA-CNR, Rome).

Pearson correlation coefficients were computed for describing the associations between pairs of variables.

## 3. RESULTS

The areas examined exhibited quantitative differences in the distribution of the microbial activities and biomass (Tab. I). In the A area, ecto-enzymatic activity showed a seasonal course with a peak in June, 1996 and minimum in February, 1998. Respiratory rates showed a similar trend, however the minimum occurred in February, 1997 and the maximum in summer 1997. Bacterial biomass (BB) showed unusual variations, with increasing densities during the years, particularly in February, 1998. In the B area, all the parameters showed lower values with respect to the A area and a more irregular distribution without any relationship with sampling periods. Interannual differences between the two years were observed, with a decrease in average values of EEA and an increase in respiratory activity and bacterial biomass.

Cell abundance resulted quite uniform along the water column. The cell volume and morphology changed between warm and cold periods, as studied in area B. The mean cell volume, evaluated with AO staining, ranged from 0.155 to 0.138 $\mu m^3$ in warm periods (June, 1996 and 1997) and from 0.048 to 0.021 $\mu m^3$ in cold periods (February, 1997 and 1998), confirming previous research carried out in the Adriatic Sea (La Ferla *et al.*, 1998a; Puddu *et al.*, 1998).

Since the abundance did not show great variations during the years, the mean biovolume (cell volume $ml^{-1}$) varied according to cell volumes (Tab. II). The structure of bacterial population also changed in the different seasons. In warm periods, bacilli and vibrios, together with spirilli, constituted the main morphotypes, while during the cold periods, cocci prevailed. These observations were in agreement with variations in composition genera with a prevalence of *Vibrionaceae* reported by Zaccone *et al.* (1998a) in the Northern Adriatic Sea in summer, suggesting the occurrence of bacterial succession.

TABLE I Minima, maxima and mean values with standard deviation (s.d.) of the parameters studied

| | | *A area* | | | | *B area* | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | *n* | *range* | *mean* | *s.d.* | *n* | *range* | *mean* | *s.d.* |
| EEA | Jun '96 | 30 | 0.85–44.25 | 9.97 | 10.72 | 32 | 0.14–27.27 | 7.60 | 9.06 |
| ($\mu g\, Cl^{-1}\, h^{-1}$) | Feb '97 | 30 | 0.24–37.59 | 6.66 | 8.85 | 23 | 0.03–28.82 | 6.00 | 9.01 |
| | Jun '97 | 30 | 0.61–59.45 | 8.29 | 14.59 | 30 | 0.49–8.02 | 3.09 | 2.64 |
| | Feb '98 | 30 | 0.51–31.08 | 4.86 | 8.60 | 27 | 0.35–11.16 | 3.66 | 4.09 |
| R | Jun '96 | 30 | 0.13–3.82 | 0.99 | 0.84 | 32 | 0.09–1.01 | 0.37 | 0.25 |
| ($\mu g\, Cl^{-1}\, h^{-1}$) | Feb '97 | 30 | 0.03–0.44 | 0.19 | 0.11 | 23 | 0.06–0.63 | 0.24 | 0.15 |
| | Jun '97 | 30 | 0.19–17.40 | 2.29 | 3.20 | 30 | 0.20–4.03 | 1.40 | 1.03 |
| | Feb '98 | 30 | 0.01–1.33 | 0.38 | 0.28 | 27 | 0.08–1.28 | 0.45 | 0.32 |
| BB | Jun '96 | 30 | 2.93–21.30 | 9.22 | 5.22 | 32 | 1.04–15.50 | 7.97 | 3.04 |
| ($\mu g\, Cl^{-1}$) | Feb '97 | 30 | 6.83–37.40 | 14.80 | 6.05 | 23 | 2.20–41.20 | 7.72 | 8.55 |
| | Jun '97 | 28 | 9.76–37.60 | 22.30 | 7.69 | 30 | 3.00–18.40 | 9.32 | 4.16 |
| | Feb '98 | 30 | 23.80–279 | 43.90 | 45.60 | 27 | 4.60–15.50 | 9.74 | 2.69 |

TABLE II Bacterioplankton cell volumes, biovolumes and morphotypes

| | *June '96* | *Feb '97* | *June '97* | *Feb '98* |
|---|---|---|---|---|
| Cell volumes ($\mu m^3$) | 0.155 | 0.048 | 0.138 | 0.021 |
| Biovolume/ml | 0.165 | 0.070 | 0.234 | 0.091 |
| Morphotypes (%) | | | | |
| Cocci | 37.54 | 60.07 | 25.91 | 79.38 |
| Bacilli | 46.84 | 35.91 | 47.87 | 15.98 |
| Vibrios | 9.97 | 3.02 | 21.04 | 4.12 |
| Spirillae | 5.65 | 1.01 | 5.18 | 0.52 |

Distribution of respiratory and ecto-enzymatic rates in the study areas during the years have been previously referenced by Zaccone *et al.* (1998b) and La Ferla *et al.* (1998b). They showed that most microbial activity was confined to the more surface layers from 0–10 m depth. In areas A and B, the EEA values generally showed a negative gradient from the nearshore stations to offshore ones, even though some intermediate stations had high values, sometimes due to fresh water input. Respiratory activity in area A showed a less evident coast-offshore gradient than proteolytic activity, while in B area a gradient only in the summer months occurred, particularly in June, 1997. Below 10 m, microbial protease was notably reduced; an increase near the bottom was observed during February, 1997 probably caused by the breakdown of the thermocline.



Pearson correlation coefficients were computed among the different examined parameters (Tab. III). In general, the salinity negatively correlated with respiration and proteolysis, indicated that the supply of freshwater was the predominant factor influencing microbial activity, even more so than the temperature, as although observed in both areas (Zaccone *et al.*, 1998b). R and EEA activities often resulted to be well correlated, showing a tightly coupling between the processes within the bacterial cells. In area A, during February, 1997 only, R correlations with other parameters were strangely lacking. The significance of coefficients calculated between R and EEA generally increased from the first to second year in both the study areas. Bacterial carbon production (BCP) always appeared strongly correlated with protease activity since the amino acids released by enzymatic hydrolysis may be readily taken up by bacteria cells for the new production.

Different correlations were observed between microbial activities and the dissolved and particulate organic matter. The presence and the different degrees of significance between the microbial activities and the organic matter pool seem mainly related with the weak or refractory nature of the carbon compounds more than with quantity (Pettine *et al.*, in press).

Bacterial abundance determined with DAPI staining showed no correlations with other parameters in area A, whilst it resulted well correlated with bacterial activities in area B.

## 4. DISCUSSION

The high levels of microbial activity recorded in the upper layers are consistent with Middelboe *et al.* (1995) findings, suggesting that autochthonous (phytoplankton ex-

TABLE III Pearson correlation coefficients between R, EEA and DAPI *vs.* other parameters

| | *A AREA* | | | | *B AREA* | | | |
|---|---|---|---|---|---|---|---|---|
| | *June '96* | *Feb '97* | *June '97* | *Feb '98* | *June '96* | *Feb '97* | *June '97* | *Feb '98* |
| **R** *vs.* | | | | | | | | |
| DOC | 0.39* | | | 0.66** | | | 0.69** | 0.78** |
| BCP | | | 0.53** | 0.73** | 0.77** | 0.54** | | 0.79** |
| EEA | 0.39* | | 0.55** | 0.67** | 0.37* | 0.62** | 0.80** | 0.79** |
| T | 0.44* | | 0.47** | −0.52** | | −0.51* | | −0.74** |
| S | −0.58* | | −0.65** | −0.69** | −0.62** | | −0.78** | −0.61** |
| POC | | | 0.74** | n.d. | | 0.61** | 0.86** | n.d. |
| MA | | | 0.61** | | 0.79** | 0.60** | | 0.61** |
| DAPI | | | | | 0.35* | 0.54** | 0.72** | 0.50** |
| CHL | | | 0.83** | 0.67** | 0.49** | 0.50* | 0.79** | 0.78** |
| **EEA** *vs.* | | | | | | | | |
| DOC | 0.47** | 0.81** | 0.67** | 0.73** | | | 0.56** | 0.87** |
| BCP | 0.75** | 0.47** | 0.99** | 0.98** | 0.51** | 0.98** | | 0.98** |
| R | 0.39** | | 0.55** | 0.67** | 0.37* | 0.62** | 0.80** | 0.79** |
| T | 0.49** | | 0.61** | −0.65** | | −0.90** | | −0.93** |
| S | −0.75** | −0.42** | −0.94** | −0.95** | −0.48** | −0.85** | −0.56** | −0.89** |
| POC | | 0.55** | 0.94** | n.d | | 0.93** | 0.91** | n.d. |
| MA | | | 0.63** | 0.49** | 0.48** | 0.90** | | 0.71** |
| DAPI | | | | | | 0.57** | 0.76** | 0.70** |
| CHL | 0.49** | 0.68** | 0.83** | 0.87** | 0.51** | 0.71** | 0.84** | 0.99** |
| **DAPI** *vs.* | | | | | | | | |
| DOC | | | | | | | | 0.74** |
| BCP | | 0.42* | | | 0.41** | 0.52* | | 0.67** |
| R | | | | | 0.35** | 0.54** | 0.72** | 0.50** |
| EEA | | | | | | 0.57** | 0.76** | 0.70** |
| T | | | | −0.53** | | −0.56** | −0.57** | −0.76** |
| S | | | | −0.71** | | −0.46* | | −0.68** |
| POC | | | | n.d | | | 0.73** | n.d |
| MA | | | | | | 0.56** | | 0.53** |
| CHL | | | | | | 0.79** | 0.62** | 0.72** |

*$P < 0.05$; **$P < 0.01$.

cretion) and allochthonous (riverine inputs) organic matter loading are a significant source of dissolved organic carbon for the bacteria.

With the aim of estimating the microbial processes coupling, we examine the EEA/BCP ratio calculated on average values (Fig. 1). High ratios indicate an overproduction of enzymes with the incorporation rates (area A in June, 1996, February, 1997, and in February, 1998), while a low ratio value indicates a greater coupling of processes, with a higher possibility of incorporation in new biomass of the carbon released by enzymatic hydrolysis (June, 1997, in both areas). The EEA/BCP ratio was higher in the particle-attached bacteria in rolling tanks experiments than in the free-living bacteria (Unanue *et al.*, 1998). A controversial question still remains about a possible release of free aminoacids and monosaccharides in the environment deriving from this uncoupling between hydrolysis and uptake and the possible advantage for free-living bacteria. Smith *et al.* (1992), Smith *et al.* (1995), Middelboe *et al.* (1995) report that particle-attached bacteria exhibit high hydrolytic activities but do not take advantage of monomeric compounds produced. The polymer hydrolysis can exceed the carbon demand in attached bacteria and may provide a carbon and nitrogen source for



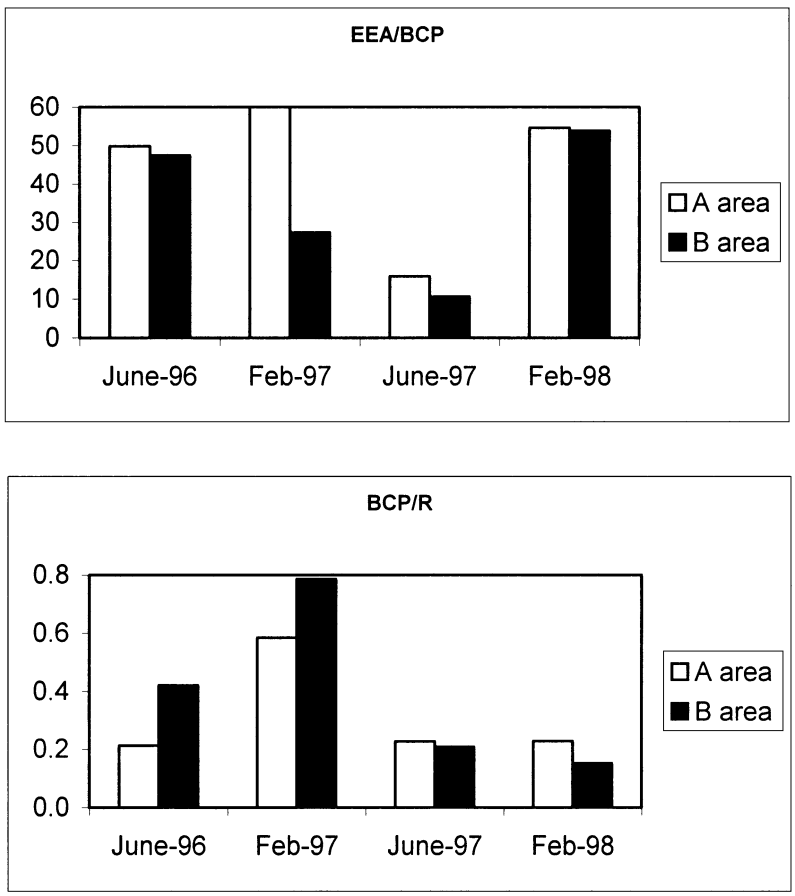


FIGURE 1 EEA/BCP and BCP/R ratios calculated in the A and B areas.

free living bacteria (Karner and Herndl, 1992; Middelboe *et al.*, 1995). The prevalence of particle attached bacteria is hypothesised, because of the presence of mucilage aggregates visible in the sea during all the cruises.

Quantifying carbon flux into bacteria largely depends on the accuracy of the estimates of the BCP/R ratio that indicates whether a balance exists between production of new bacterial biomass and consumption by means of oxidative metabolism. Our estimates result lower than 1, indicating that oxidative processes exceeded the productive ones. The ratios range from 0.2 to 0.6 in the A area and from 0.15 to 0.78 in the B area (Fig. 2). BCP/R maxima, occurring in February, 1997 in both areas, indicate that most respiratory activity may be sustained by bacterial production. In sea water cultures collected from the Northern Baltic Sea, Zweifel *et al.* (1995) determined a BCP/R ratio of 0.36 while, in samples collected in the Mississippi river plume, Amon and Benner (1998) estimated the ratios of 0.6–1.7 and 2.5–3.7 from bacterial cell production and bacterial leucine incorporation.



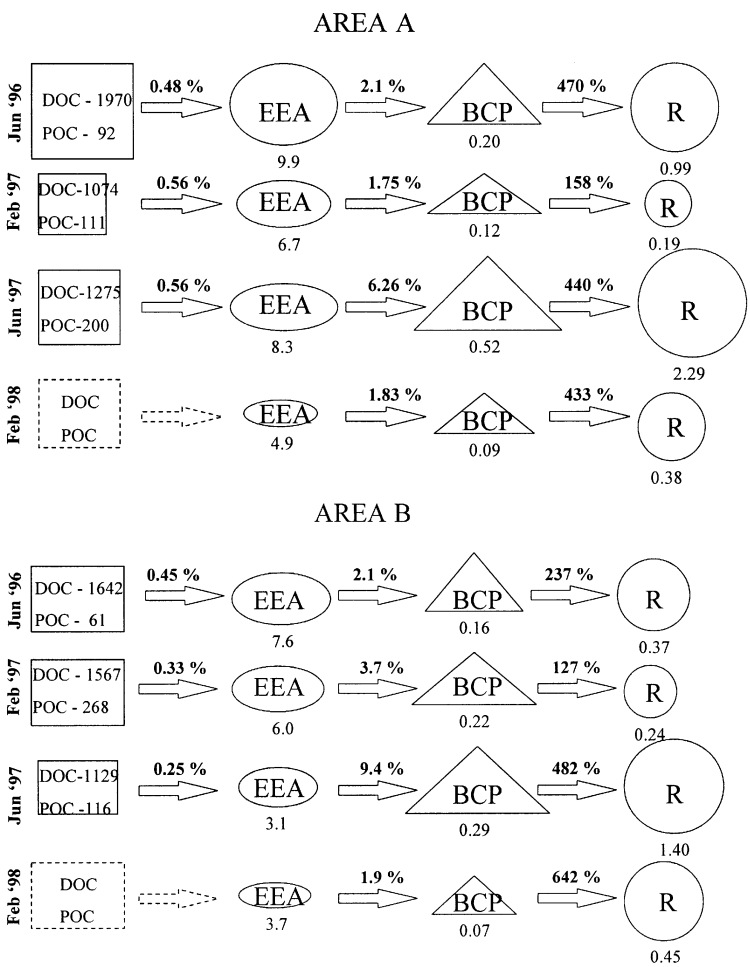


FIGURE 2 Scheme of a theoretical organic carbon flux through the bacterial cells. DOC and POC: dissolved and particulate organic carbon (μg $C^{-1}l$); EEA: ecto-enzymatic activity (μg $Cl^{-1}h^{-1}$); BCP: bacterial carbon production (μg $Cl^{-1}h^{-1}$); R: respitatory activity as $CO_2$ (μg $Cl^{-1}h^{-1}$)

TABLE IV Bacterial growth efficiency

| | *A Area %* | *B Area %* |
|---|---|---|
| Jun '96 | 17 | 29 |
| Feb '97 | 38 | 44 |
| Jun '97 | 18 | 17 |
| Feb' 98 | 18 | 13 |

The bacterial growth efficiency (BGE), as the percentage ratio between bacterial production (BCP) and consumption processes (R + BCP), ranges from 17 to 38% in area A and from 13 to 44% in area B with highest values in February, 1997, in each site (Tab. IV). Del Giorgio *et al*. (1995) reported that BGE ranges from less than 10 to 25% in most freshwater and marine systems, while in estuaries it ranges from 5 to 60%. The increase observed in both A and B areas, during February, 1997, may be linked to changes in both the rate of supply and nutritional quality of organic carbon substrates available for bacterial biomass as well as the occurrence of other specific growth factors (*i.e*., iron or phosphorus). According to Middleboe *et al*. (1995), the attached bacteria seem to have a lower BGE (10%) due to the energy consumption for attachment with respect to a BGE (30%) for the free-living bacteria.



In the Prisma II Project, sediment trap studies (Rabitti, personal communications) demonstrated that only 10% of the particulated organic carbon trapped within the first 10 m of the water column reaches sediment. The percentage ratio between organic matter content (as DOC + POC) and respiration shows that the respiratory turnover rate (RTR) *per* day, in the layer between 0 and 10 m, varies between 0.34–4.15% in A area and 0.32–2.93% in B area (Tab. V). In warm seasons, the carbon flux was highly efficient in the oxidative steps. In particular, in area A and in June, 1997 the highest respiratory turnover rates occurred. In the same 0–10 m layer, the enzymatic activity mobilizes a percentage variable between 14.47 and 19.65% (A area) and 5.59 and 10.11% (B area) of the total organic matter *per* day.

The role of the bacterial population in the biogeochemical carbon cycle in the Northern Adriatic Sea is schematized in Figure 2 where some steps of the carbon flux are described. In the A area the proteolitic turnover rates (EEA/POC + DOC, as percentage) remain quite constant with time (range 0.48–0.56%), even though the average protease activity slightly decrease from the 1st to 2nd year and from summer to winter (range 4.9–9.9 $\mu g\,C\,h^{-1}\,l^{-1}$). The hydrolysed matter may then be uptaken by bacterial production. BCP decreases in winter (range 0.09–0.52 $\mu g\,C\,h^{-1}\,l^{-1}$), but the BCP/EEA (as percentage) increases from the 1st to 2nd year in summer (range 1.75–

TABLE V Respiratory turnover rates per day in the layers between 0 and 10 m

| | *RTR %* | |
|---|---|---|
| | *A area* | *B area* |
| Jun '96 | 1.47 | 0.50 |
| Feb '97 | 0.34 | 0.32 |
| Jun '97 | 4.15 | 2.93 |
| Feb '98 | 0.91* | 1.26* |

*RTR calculated only using DOC values.

6.26%). The final step of the carbon flux towards mineralisation shows lower values in winter ($R = 0.19$ and $0.38\,\mu g\,C\,h^{-1}\,l^{-1}$). Since respiration also includes other, but strictly bacterial, microbial fraction, the BCP/R (%) ratios exceed the bacterial production (range 158–470%). In February 1997, bacteria may contribute in a relevant way to respiration, while in the other periods a greater contribution by other non-bacteria components may occur.

In the B area, the enzymatic turnover rate of the total organic matter (%) decreases with time (range 0.25–0.45%), as well as the EEA value (range $3.1–7.6\,\mu g\,C\,h^{-1}\,l^{-1}$). In contrast the ratio BCP/EEA as a percentage, indicating the carbon available for new production, increases from 2.1 to 9.4%. The respiration rates, ranging from 0.24 to $1.40\,\mu g\,C\,h^{-1}\,l^{-1}$, show a similar trend as observed in the A area, with a strong reduction in February, 1997.

## 5. CONCLUSIONS

Quantitative differences in activities occur between both the areas and the seasons examined. The A area is characterised by a marked seasonality in the values of all the parameters, with the highest activity in the summer period.

Interannual variations are mainly recorded in the B area both for the respiratory and enzymatic activity; respiration increases from the first to the second year, whilst proteolytic activity decreases.

The nature of the substrates present in the environment seems to influence the course of the microbial processes in a different way in the various seasonal periods; determine the actual lability of organic carbon is really difficult.

The carbon transfer from the biotic to the abiotic compartment through the microbial community seems to flow better in the summer period, particularly in the A area in June, 1997.



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