Phytoplankton–bacterioplankton interactions and carbon fluxes through microbial communities in a microtidal lagoon

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Introduction

Across-ecosystem analyses of the relationships between bacterial carbon production (BCP) or bacterial carbon demand (BCD) and primary production (PP) have supported the general conceptual model that the bacterioplankton–phytoplankton coupling varies as a function of ecosystem characteristics. In particular, a trophic dependence of bacteria on algae, through the photosynthetic production of dissolved organic carbon [dissolved primary production (DPP)], would be characteristics of aquatic ecosystems where bacteria are carbon limited. There is a general trend towards a tight linkage in open oceans and clear water lakes and of loose coupling in coastal or eutrophic sites. In open seas and clear water lakes, the general picture is one of a nutrient-controlled energy flow, where phytoplankton particulate organic matter is the main energy source for grazers and dissolved organic carbon (DOC) derived from phytoplankton (e.g. direct exudation through DPP, lyses) represents the main energy source for bacteria, supporting all the BCD, so that the bacteria–phytoplankton coupling is tight. In contrast, in estuaries and coastal lagoons, the plankton system might show a net heterotrophic metabolism (respiration > gross primary production) or a seasonal shift from net autotrophy to net heterotrophy that may be due to turbidity and nutrient variations and due to inputs of organic matter allochthonous to the planktonic system (Revilla et al., 2002; Hopkinson & Smith, 2005). As a consequence, in transitional ecosystems, BCD may be met not only by DPP but also by nonphytoplanktonic (leachates from plants and exudates from macrophytes, pore water from
sediments) or by allochthonous sources (runoff, riverine inputs) of organic carbon, leading to a loose coupling between bacteria and phytoplankton. High bacteria biomass and BCP may therefore occur in transitional ecosystems, where bacteria and phytoplankton communities are often uncoupled, plankton community respiration (CR) may exceed phytoplankton photosynthesis and secondary production may be based, at least during some seasons, on bacterial mobilization of preformed organic matter, rather than on contemporaneous phytoplankton photosynthesis (Gaedke & Kamjunke, 2006; Berglund et al., 2007).

The pattern of strength variation of the bacterioplankton–phytoplankton coupling appears evident from across-ecosystem studies, but it does not necessarily hold for smaller spatial and temporal scales. In some coastal ecosystems, indeed, the reverse pattern has been observed, and a tight coupling between DPP and BCD is recorded at the most eutrophic sites and a loose linkage at the most oligotrophic ones (Gonzalez-Benitez & Gattuso, 2003; Rochelle-Newall et al., 2008).

The Lagoon of Venice (northern Adriatic Sea) is the largest Italian lagoon and one of the largest in the Mediterranean. The lagoon is intrinsically characterized by a high heterogeneity in morphological structures and in the spatial distribution of physical, biogeochemical and biological parameters (Bianchi et al., 2000; Ravera, 2000; Acri et al., 2004; Solidoro et al., 2004). The plankton communities in this environment undergo complex spatial and temporal dynamics. Exchanges with the sea, benthic–pelagic coupling and river inflows appear to be important in defining the spatial distribution of the plankton communities, even though seasonality is the main factor leading succession (Bernardi Aubry & Acri, 2004; Bandelj et al., 2008). Phytoplankton community production has been traditionally considered the main process sustaining the whole planktonic food web in the lagoon (Bianchi et al., 2000; Acri et al., 2004), while bacterial production and biomass have been largely neglected, being studied only sporadically (Sorokin et al., 1996, 2002). No information on the bacteria–phytoplankton coupling and on the carbon fluxes through the microbial communities in Lagoon of Venice is available, whereas long-term studies on the biomass and taxonomic composition of phytoplankton and macrophytes are well documented for the last 30 years (Acri et al., 2004; Sfriso et al., 2005). The net ecosystem metabolic balance for the whole lagoon has been calculated on the basis of the nutrient budget, in the framework of the Land-Ocean Interactions in the Coastal Zone activities (Giordani et al., 2005), and it showed a state of near-balance between autotrophic and heterotrophic processes, on a yearly base, with net autotrophy prevailing only in summer. A prevalent heterotrophic metabolism of the lagoon has been evidenced through dissolved oxygen mass-balance models applied to time series of high-frequency oxygen data collected in situ (Ciavatta et al., 2008).

In this work, we aim at evaluating the strength of the bacteria–phytoplankton coupling and at quantifying the importance and the significance of the microbially mediated carbon fluxes in the Lagoon of Venice and their seasonal and spatial variations, with an emphasis on trophic state variability. Our work focuses on the water column, which, in this shallow environment, is the result of the continuous water–sediment interactions. We considered PP (total, particulate and dissolved), BCP and CR as the main indicators of the planktonic system functioning, responding to environmental variables such as light, nutrients and organic matter availability that are commonly influenced by the catchment’s features and disturbance due both to anthropic and to natural changes. We wish to make a contribution to the debate about the environmental factors that influence the bacteria–phytoplankton linkage and the microbially mediated C fluxes from a within-system viewpoint. In particular, we aimed at assessing, in an area characterized by different trophic conditions: (1) the strength of the coupling between phytoplankton and bacterial communities (relationships between DPP and BCD), (2) the prevalent base of the planktonic food web (relationships between BCP and PP) and (3) the net autotrophy vs. the net heterotrophy nature of the planktonic system (relationships between CR and PP).

Materials and methods

Study area

The Lagoon of Venice (Fig. 1) is a large Mediterranean lagoon of ~550 km², located in the northern Adriatic Sea. It is surrounded by densely inhabited shores and industrial plants and it hosts ports, shipyards, marinas, fisheries, aquaculture and recreational activities. The lagoon has an average depth of ~1 m and it is morphologically characterized by the presence of large shallow areas and by a network of deeper (5–10 m) channels. It is connected to the Adriatic Sea by three inlets, through which tidal currents drive water exchanges. The tidal amplitude is ~100 cm, with maxima up to 150 cm. The lagoon can be classified as polyhaline. Twelve main tributaries annually carry about 35 m⁻³ s⁻¹ of fresh-water in the lagoon (Zuliani et al., 2005); nitrogen and phosphorus load are in the order of 4000 t N year⁻¹ and 230 t P year⁻¹ (Collavini et al., 2005).

The Lagoon of Venice presents a high variability in most environmental parameters and a high habitat heterogeneity. Within this ecosystem, we studied three shallow water (2–3 m deep) sites (Fig. 1) located in plankton-dominated areas of the lagoon. In these areas, the presence of macrophytes is negligible and most of the PP is by the phytoplankton (Acri et al., 2004; Sfriso et al., 2005).
The three stations represent different environments typical of the central and northern lagoon. St. 1 is influenced by urban wastewaters from the town of Mestre; st. 2 is a marshy area and it represents a typical lagoon environment; st. 3 is close to the inlet of Lido that allows the communication of the lagoon with the Adriatic Sea, but it is also influenced by the inputs of freshwater channels.

**Sampling**

Transparency (Secchi disk), photosynthetically active radiation (PAR; LiCor Li-192), temperature, salinity, dissolved oxygen and pH (Idronaut Ocean Seven 316 multiprobe) were measured throughout the water column. Samples for dissolved inorganic macronutrients (Grasshoff et al., 1999), phytoplankton and bacterial communities, respiration, particulate organic carbon (POC) and DOC were collected at the surface water layer, representative of the whole water column. The water column was assumed to be well mixed because the differences in surface and bottom salinity and temperature were minimal. Hydrological parameters, nutrients and chlorophyll a (chl) were determined monthly, and the microbial carbon parameters and fluxes seasonally (January, April, July and October 2005). Samplings were always performed at neap tide, in order to minimize the effects of tidal currents.

**Phytoplankton production and CR**

Phytoplankton chl was determined spectrofluorometrically according to Holm-Hansen et al. (1965). Samples for total primary production (TPP) ($^{14}$C method) were incubated under simulated in situ conditions (natural sunlight and maintaining in situ temperature) for 2 h around noon. Subsamples (250 mL) and a dark bottle were inoculated with 148 kBq of NaH$^{14}$CO$_3$. TPP was determined on duplicate subsamples (5 mL) that were acidified and stirred for one hour, before being radioassayed. Particulate primary production (PPP) and DPP was determined after filtrations of 10 mL of samples on 0.2 μm polycarbonate filters at a low (< 10 mmHg) vacuum pressure. The DPP was always significantly different from the corresponding dark bottle values. In order to also take into account the potential rapid bacterial uptake of labelled dissolved organic carbon (DO$^{14}$C) during incubations, we measured the $^{14}$C-labelled 0.2–2-μm size fraction as well, by an additional filtration on 2-μm pore-size polycarbonate filters. Because the 0.2–2-μm size fraction (PP$^{pico}$ as the difference between the 0.2 and the 2 μm filters) also includes the $^{14}$C incorporated by autotrophic picoplankton, the sum of DPP and PP$^{pico}$ could be taken to represent the maximum potential carbon exudation rates.
The daily TPP was estimated considering the ratio between the duration of the incubation (around 2 h) and the total day length that ranged between 9 h (in winter) and 15 h (in summer). Considering the inherent daily variability of the phytoplankton community, related to the tidal hydrodynamics – not assessed in the present work – the daily TPP extrapolated from the hourly data must be considered as a potential value.

CR was estimated as the difference in dissolved oxygen at the beginning and after a 24-h incubation of triplicate 250-mL water samples in the dark and at in situ temperature. Dissolved oxygen was assayed by potentiometric Winkler titrations (794 Basic Titrino, Metrohm). The oxygen uptake rates were transformed into inorganic carbon production assuming, in the absence of detailed information about the composition of the substrate used, a respiratory quotient of 1.

Bacterial abundance and ectoenzymatic activities

Heterotrophic bacterial abundances were estimated on samples fixed with 2% final concentration borate-buffered prefiltered formalin, following a modification of the method of Porter & Feig (1980). Subsamples (10 mL) were stained with 4’,6-diamidino-2-phenylindole (Sigma; 1 μg mL⁻¹ final concentration) and enumerated by an Olympus BX 60 F5 epifluorescence microscope at 1000 using a UV filter set (BP 330–385 nm). Bacterial abundance was converted into carbon equivalents (heterotrophic bacterial biomass, HBB) using the conversion factor of 20 fg C per cell (Lee & Fuhrman, 1987).

BCP was estimated by the incorporation of ³H-leucine (Leu-BCP) (Kirchman et al., 1985; Smith & Azam, 1992). Triplicate 1.7-mL aliquots and two killed controls [90 μL, 100% trichloroacetic acid (TCA)] were amended with 20 nM radiotracer and incubated at 100% TCA] were amended with 20 nM (Leu-BCP) (Kirchman et al., 1985; Smith & Azam, 1992). After the addition of 1 mL of the samples was determined by a liquid scintillation counter (Packard Tri-Carb 300) after the addition of 1 mL of the system blanks (8.7 ⁰C/h) and dividing by the slope of the calibration curve (Thomas et al., 1995). Incorporation of ³H-leucine was converted into carbon produced using bacterial protein production according to Simon & Azam (1989), assuming a twofold isotope dilution for Leu.

BCD was estimated as Leu-BCP/BGE, with BGE values calculated from the del Giorgio & Cole (1998) empirical model. The daily rates of bacteria activity were estimated assuming the activity rates to be constant throughout the day. Hydrolytic ectoenzyme activities were measured using fluorogenic substrates (Hoppe, 1993) derived from 7-amino-methyl-coumarin (AMC) and 4-methyl-umbelliferone (MUF). Aminopeptidase activity was assayed as the hydrolysis rate of L-leucine-AMC, β-D-Glucosidase, β-D-galactosidase, β-D-galactosidase, lipase, N-acetyl glucosaminidase and alkaline phosphatase were assayed using MUF derivatives. Enzyme activities were expressed in terms of the rate of MUF or AMC production. After evaluation of the saturating concentration, hydrolysis was measured by incubating 2.5-mL subsamples with 50 μM MUF phosphate and 200 μM leucine-AMC and other MUF substrates for 1 h at in situ temperature in the dark. All samples were run in triplicate with 0.2 μm filtered and boiled seawater as controls. The fluorescence released by enzymatic cleavage of the artificial substrates was measured fluorometrically at 380/ 365 nm excitation and 440/455 nm emission for AMC/MUF substrates using a Shimadzu RF 1501 fluorometer. Standard solutions of MUF and AMC were used to perform calibration curves. The velocity of hydrolysis (μM h⁻¹) of β-D-glucosidase, β-D-glucosidase, β-D-galactosidase, β-D-galactosidase, lipase, N-acetyl glucosaminidase and leucine-aminopeptidase was converted into the C content of the organic component hydrolyzed from the model substrate using the conversion factor of 72 in cases where the organic component is glucose or a 6-C monosaccharide or a 6-C amino acid (Hoppe, 1993) and of 216 where the organic component is oleic acid. The sum of the enzymatic activities, expressed as μg C L⁻¹ h⁻¹, represents the rate of C potentially mobilized from the macromolecules by hydrolytic enzymes.

Organic carbon

Water samples for DOC, chromophoric dissolved organic material (CDOM) and POC were analyzed after filtration through precombusted (4 h, 500 °C) Whatman GF/F filters. DOC concentrations were measured using a Shimadzu TOC 5000 Analyzer with a 1.2% Pt on silica as a catalyst at 680 °C (Cauwet, 1994). Samples were acidified (pH = 2) with HCl and purged with pure air for 10 min immediately before analysis. DOC concentrations were calculated by subtracting the system blanks (8.7 ± 0.9 μmol L⁻¹) and dividing by the slope of the calibration curve (Thomas et al., 1995). The fluorescence released by enzymatic cleavage of the artificial substrates was measured fluorometrically at 380/365 nm excitation and 440/455 nm emission for AMC/MUF substrates using a Shimadzu RF 1501 fluorometer. Standard solutions of MUF and AMC were used to perform calibration curves. The velocity of hydrolysis (μM h⁻¹) of β-D-glucosidase, β-D-glucosidase, β-D-galactosidase, β-D-galactosidase, lipase, N-acetyl glucosaminidase and leucine-aminopeptidase was converted into the C content of the organic component hydrolyzed from the model substrate using the conversion factor of 72 in cases where the organic component is glucose or a 6-C monosaccharide or a 6-C amino acid (Hoppe, 1993) and of 216 where the organic component is oleic acid. The sum of the enzymatic activities, expressed as μg C L⁻¹ h⁻¹, represents the rate of C potentially mobilized from the macromolecules by hydrolytic enzymes.

The UV-Vis spectra of the filtered lagoon water were performed by a UV2 spectrophotometer ATI Unicam from 270 to 800 nm with a 5-cm quartz optical cell using Milli-Q
water as the blank, as reported by Vodacek et al. (1997). To quantify the CDOM in each sample, we used the absorption coefficient (aCDOM, in m$^{-1}$) at 355 nm as a proxy, calculated from the sample absorbance (A) in nm$^{-1}$ and path length (L) in meters as follows:

$$a_{\text{CDOM}}(355) = 2.303A_{\text{CDOM}(355)}/L$$

**Results**

### Trophic characteristics and climatology

At the three selected stations, pluriannual studies for hydrology, nutrients and plankton have been carried out (Bianchi et al., 2003; Acri et al., 2004). These stations are reference ones for long-term ecological research studies (LTER) in the Lagoon of Venice, one of the LTER national and international sites. The monthly variations of the main hydrological and trophic parameters, averaged for the last three years (2006–2008) and compared (median comparison, Mann–Whitney test) with those observed in 2005, did not show statistically significant differences: the year 2005 therefore represents fairly well the most recent conditions of the Lagoon of Venice.

The main hydrological and trophic variables at the three stations are reported, as averages for each season from the monthly samplings, in Table 1. Temperature showed the typical seasonal pattern, with the lowest values in winter and the highest in summer at all the three stations. The lowest salinity was measured at st. 1, from winter to summer, and at st. 3 in autumn. The reversed pattern was recorded for nutrients, which showed the highest concentrations at st. 1, from winter to summer, and at st. 3 in autumn. At all the stations, the dominant form of DIN was nitrate and the N/P ratio was always much higher than 16, pointing out that inorganic P could be a potential limiting macronutrient, even though P was never exhausted. Chl peaked at every station in summer; the maxima were recorded at st. 1 from spring to autumn. St. 2 exhibited the highest salinity and the lowest nutrient and chl concentrations.

From the seasonal samplings, it appears that the whole water column (maximum depth: 2 m) was always euphotic at every station, even though PAR was rapidly attenuated and only 5–20% of the surface irradiance reached the bottom.

According to the $K_d$ (Table 2), PAR attenuation in the water column was the highest at st. 1 and the lowest at st. 3.

### Phytoplankton production, bacterial production and community respiration

The remarkable degree of trophic variability among the three stations was also reflected in the wide seasonal and spatial range of chl and TPP (Table 2).
TPP increased markedly at all stations from winter to summer, when the maxima were attained, then decreasing in autumn, in accordance with temperature and phytoplankton biomass variations. Indeed, considering the whole data set, TPP was strongly correlated with both temperature ($r = 0.82, P < 0.05, n = 12$) and chl ($r = 0.86, P < 0.05, n = 12$). The highest TPP was measured at st. 1 from spring to autumn.

HBB and Leu-BCP showed a seasonal variation qualitatively similar to that of phytoplankton biomass, increasing at all the stations from winter to summer and then decreasing in autumn (Table 2). The highest HBB and BCP were prevalently recorded at st. 1 (Table 2). The heterotrophic metabolism appeared to be much less variable with respect to the widely ranging phytoplankton production, in particular at st. 1. Leu-BCP was correlated, although weakly, with TPP ($r = 0.58, P < 0.05, n = 12$).

Hourly phytoplankton production efficiency ($P_b$) followed the pattern of TPP and chl at st. 1 and st. 2, while at st. 3, it increased also from summer to autumn, when the maximum value was attained (Table 2). The hourly BCP efficiency (ratio between BCP and HBB, Table 2) varied much more irregularly than $P_b$, without showing a clearly identifiable pattern among stations and through seasons.

The daily values of BCP and TPP are given in Table 3. On a daily basis, Leu-BCP$_d$ prevailed over TPP$_d$ only in winter and summer at st. 3, in winter and spring at st. 1 and in winter, spring and autumn at st. 2. The daily respiration rates (CR$_d$, Table 3) showed the same seasonal pattern as TPP at every station, with the maximum attained at st. 1 in summer. CR$_d$ was indeed correlated both with temperature ($r = 0.67, P < 0.05, n = 12$) and with TPP ($r = 0.91, P < 0.05, n = 12$), while the correlation with HBB was not significant. Daily CR prevailed over TPP$_d$ always at st. 3, in winter and spring at st. 2 and only in winter at st. 1.

### Table 2. Selected hydrological and biological parameters and activities at the three stations, in the four sampling periods

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Winter</th>
<th>Spring</th>
<th>Summer</th>
<th>Autumn</th>
</tr>
</thead>
<tbody>
<tr>
<td>$K_d$ (m$^{-1}$)</td>
<td>3.8 1.5 0.1</td>
<td>1.7 1.2 0.8</td>
<td>2.5 1.6 0.6</td>
<td>3.5 1.3 1.4</td>
</tr>
<tr>
<td>Chlorophyll a ($\mu$g L$^{-1}$)</td>
<td>1.3 2.1 0.3</td>
<td>3.8 1.2 1.6</td>
<td>19.9 4.9 1.8</td>
<td>9.7 1.0 0.9</td>
</tr>
<tr>
<td>HBB ($\mu$g C L$^{-1}$)</td>
<td>66.5 23.0 21.8</td>
<td>68.5 63.2 31.9</td>
<td>212.3 109.3 119.1</td>
<td>40.8 53.4 31.5</td>
</tr>
<tr>
<td>POC (µM)</td>
<td>63.0 62.0 41.0</td>
<td>149.9 27.9 47.6</td>
<td>149.9 67.8 71.8</td>
<td>86.9 27.2 30.4</td>
</tr>
<tr>
<td>DOC (µM)</td>
<td>173.0 160.0 181.0</td>
<td>271.1 186.2 182.3</td>
<td>250.0 266.0 180.0</td>
<td>271.5 158.5 202.8</td>
</tr>
<tr>
<td>DOC/TOC (%)</td>
<td>73 72 82</td>
<td>64 87 79</td>
<td>63 80 71</td>
<td>76 85 87</td>
</tr>
<tr>
<td>aCDOM$_{355}$ (nm$^{-1}$)</td>
<td>0.3 0.8 0.6</td>
<td>3.9 1.0 –</td>
<td>3.1 1.1 0.4</td>
<td>3.7 1.7 0.5</td>
</tr>
<tr>
<td>TPP ($\mu$g C L$^{-1}$ h$^{-1}$)</td>
<td>0.02 1.4 0.9</td>
<td>15.3 5.0 11.5</td>
<td>300.4 71.9 16.1</td>
<td>92.8 5.4 12.1</td>
</tr>
<tr>
<td>DPP ($\mu$g C L$^{-1}$ h$^{-1}$)</td>
<td>0.0 0.2 0.1</td>
<td>1.1 1.5 0.2</td>
<td>3.1 0.6 0.2</td>
<td>4.2 0.0 0.6</td>
</tr>
<tr>
<td>PPP ($\mu$g C L$^{-1}$ h$^{-1}$)</td>
<td>0.1 1.2 0.9</td>
<td>14.3 3.5 11.3</td>
<td>297.3 71.3 15.9</td>
<td>88.6 5.4 11.5</td>
</tr>
<tr>
<td>PER (%)</td>
<td>1.0 14.3 5.6</td>
<td>6.9 30.0 1.7</td>
<td>1.0 0.8 1.1</td>
<td>4.5 0.2 5.0</td>
</tr>
<tr>
<td>$P_c$ (µg C µg Chl L$^{-1}$ h$^{-1}$)</td>
<td>0.1 0.7 2.9</td>
<td>4.0 4.2 7.2</td>
<td>15.1 14.6 8.8</td>
<td>9.6 5.6 14.2</td>
</tr>
<tr>
<td>Leu-BCP ($\mu$g C L$^{-1}$ h$^{-1}$)</td>
<td>2.3 3.3 2.9</td>
<td>9.7 4.7 3.0</td>
<td>11.5 6.6 5.6</td>
<td>5.0 2.7 0.6</td>
</tr>
<tr>
<td>Leu-BCP/TPP</td>
<td>116.45 2.38 3.18</td>
<td>0.63 0.94 0.26</td>
<td>0.04 0.09 0.35</td>
<td>0.05 0.50 0.05</td>
</tr>
<tr>
<td>BCD ($\mu$g C L$^{-1}$ h$^{-1}$)</td>
<td>6.2 7.8 7.0</td>
<td>17.6 9.9 7.2</td>
<td>20.4 12.8 11.3</td>
<td>10.3 6.7 3.3</td>
</tr>
<tr>
<td>BCD/TPP</td>
<td>&gt; 100 38.8 &gt; 100</td>
<td>16.8 6.6 36.2</td>
<td>6.6 21.4 66.3</td>
<td>2.5 &gt; 100 5.5</td>
</tr>
<tr>
<td>Leu-BCP/HBB (h$^{-1}$)</td>
<td>0.04 0.15 0.13</td>
<td>0.14 0.07 0.09</td>
<td>0.05 0.06 0.05</td>
<td>0.12 0.05 0.02</td>
</tr>
<tr>
<td>CR ($\mu$g C L$^{-1}$ h$^{-1}$)</td>
<td>0.1 1.1 2.1</td>
<td>4.2 3.2 3.6</td>
<td>24.2 7.3 7.3</td>
<td>7.2 0.9 4.5</td>
</tr>
<tr>
<td>AMA (nM h$^{-1}$)</td>
<td>549.7 407.1 255.0</td>
<td>1351.7 556.3 490.0</td>
<td>3155.0 796.0 475.0</td>
<td>753.2 181.0 231.6</td>
</tr>
<tr>
<td>APA (nM h$^{-1}$)</td>
<td>11.3 19.6 4.0</td>
<td>24.6 20.0 31.8</td>
<td>39.7 90.9 161.6</td>
<td>81.8 19.2 51.4</td>
</tr>
<tr>
<td>LA (nM$_{258}$)</td>
<td>1.7 90.8 25.8</td>
<td>24.0 15.4 24.8</td>
<td>12.4 8.0 2.7</td>
<td>840.2 878.7 55.5</td>
</tr>
<tr>
<td>α-glu (nM h$^{-1}$)</td>
<td>2.0 7.5 3.3</td>
<td>4.7 4.5 6.8</td>
<td>9.5 7.2 5.7</td>
<td>15.1 2.7 2.8</td>
</tr>
<tr>
<td>β-glu (nM h$^{-1}$)</td>
<td>3.5 1.8 0.5</td>
<td>5.1 3.0 6.0</td>
<td>11.8 15.0 18.5</td>
<td>22.6 4.7 3.7</td>
</tr>
<tr>
<td>α-gal (nM h$^{-1}$)</td>
<td>1.1 2.3 1.6</td>
<td>3.6 3.3 7.8</td>
<td>7.7 6.0 0.0</td>
<td>116.2 6.4 7.6</td>
</tr>
<tr>
<td>β-gal (nM h$^{-1}$)</td>
<td>1.5 3.6 0.9</td>
<td>1.6 1.4 2.0</td>
<td>7.2 2.3 1.2</td>
<td>19.6 2.9 5.6</td>
</tr>
<tr>
<td>NAG (nM h$^{-1}$)</td>
<td>2.2 5.0 1.0</td>
<td>4.3 5.8 5.5</td>
<td>13.7 16.6 12.2</td>
<td>61.0 5.6 7.7</td>
</tr>
<tr>
<td>APA/AMA</td>
<td>0.021 0.048 0.016</td>
<td>0.018 0.036 0.065</td>
<td>0.013 0.114 0.340</td>
<td>0.109 0.106 0.222</td>
</tr>
</tbody>
</table>

See text for abbreviations.
attained the highest values at st. 2 in spring. Considering the whole data set, DPP was correlated with both TPP \( r = 0.76, P < 0.05, n = 12 \) and PPP \( r = 0.74, P < 0.05, n = 12; \) Fig. 2). No significant relationships were found between PER and PPP (Fig. 2) or TPP.

BCD (Tables 2 and 3) increased at every station from winter to summer, then decreasing in autumn. The highest values, from spring to autumn, were recorded at st. 1. On a daily basis, DPP (Table 3) represented only a small fraction \( (\text{<}5\%) \) of BCD and it was therefore never sufficient to completely sustain the BCD. Also, the maximum potential daily DPP (DPP\(_{\text{pico}}\)) was never sufficient to meet BCD on a daily basis. Taking into account the TPP, as a non-contemporaneous DOC pool potentially available for bacteria, BCD was met by TPP only in summer and autumn at st. 1. No significant correlations were found between BCD and DPP or TPP (Fig. 3) and between BCP and DPP (Fig. 3).

### Enzymatic activities and potential organic carbon mobilization

Among the assayed extracellular enzymatic activities (Table 2), aminopeptidase activity (AMA) showed the highest values. The maxima were always recorded at st. 1 and in summer at every station. The alkaline phosphatase activity (APA) was quite low and it showed the same similar pattern as for AMA only at st. 2 and 3, while it attained the maximum in autumn at st. 1, appearing decoupled from the seasonal pattern of phytoplankton and bacteria biomass here (Table 2). The APA/AMA ratio, proposed as a proxy of phosphorus deficiency in microbial assemblages (Sala et al., 2001), was always \(<1\), indicating good phosphorus availability in the lagoon waters. The specific activity of APA and AMA (APA and AMA/HBB and APA/chl, Table 2) showed a very irregular pattern among both seasons and stations. The lipase- and the enzymatic-degrading polysaccharides activities varied irregularly, without showing a clearly identifiable pattern among stations and through seasons (Table 2).

The potential bacterial carbon mobilization (C\(_{\text{mob}}\), Table 3), calculated from bacterial enzymatic activity, increased from winter to summer at every station. The highest values were measured at st. 1. Daily BCD always appeared to be

<table>
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<th>Table 3. Daily metabolic rates at the three stations, in the four seasons</th>
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<td>TPP (µg C L(^{-1}) day(^{-1}))</td>
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<tr>
<td>DPP (µg C L(^{-1}) day(^{-1}))</td>
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<td>Leu-BCP (µg C L(^{-1}) day(^{-1}))</td>
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<td>BCD (µg C L(^{-1}) day(^{-1}))</td>
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<td>C(_{\text{mob}}) (µg C L(^{-1}) day(^{-1}))</td>
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<td>CR/TPP</td>
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<td>DOC/C(_{\text{mob}}) (days)</td>
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![Fig. 2.](https://academic.oup.com/femsec/article-abstract/72/2/153/485829)
satisfied by \( C_{\text{mob}} \) daily rates, at every station and season (Table 3).

Plotting all the data together, \( C_{\text{mob}} \) was correlated with Leu-BCP \((r = 0.64, P < 0.05, n = 12)\), TPP \((r = 0.76, n = 12, P < 0.005)\) and DPP \((r = 0.88, P < 0.001, n = 12)\). The correlation of \( C_{\text{mob}} \) with Leu-BCP and with BCD suggests that the carbon potentially mobilized by enzymatic activity could be used for the bacterial carbon requirements. On the other hand, the relationships between \( C_{\text{mob}} \) and DPP confirm the trophic link between heterotrophic bacteria and algae mediated through DPP, usually composed of easy-to-degrade molecules (Amon et al., 2001). \( C_{\text{mob}} \) was also positively correlated with TPP even if TPP needs a variable lag phase before it is available for bacterial uptake.

**Dissolved and particulate organic carbon**

The main part of the total organic carbon in the lagoon waters was in the dissolved form (64–87%) at each station (Table 1). The highest POC concentrations were measured at st. 1, where the relative incidence on TOC was lower (64–76%). POC concentrations were generally the highest in spring–summer, while DOC did not show a clearly identifiable pattern among stations and through seasons.

The magnitude of chromophoric dissolved organic carbon (aCDOM\(_{355}\)) varied on average from 0.3 to 3.9 nm\(^{-1}\), with minimum values in winter (mean: 0.6 ± 0.3 nm\(^{-1}\)). The highest values were generally recorded at st. 1 and the minima at st. 3. aCDOM\(_{355}\) was inversely correlated with salinity \((r = 0.73, n = 11, P < 0.05)\) and directly with chl \((r = 0.65, n = 11, P < 0.05)\) and DPP \((r = 0.74, n = 11, P < 0.01)\), indicating that possibly both riverine inputs of organic matter and phytoplankton exudation could contribute to the formation of CDOM. The amount of nonabsorbing DOC concentration was extrapolated from the positive correlation \((r = 0.74, n = 11, P < 0.01, \text{DOC}-\text{25.1/ccd}_{355}+170)\) between aCDOM and DOC. The CDOM, on average, contributed to 19% of the mean DOC concentration.

The significant \((r = 0.86, n = 11, P < 0.001)\) linear relationship between aCDOM\(_{355}\) and \( C_{\text{mob}} \) and of aCDOM\(_{355}\) and DPP \((r = 0.74, n = 11, P < 0.01)\) suggests an important role of the bacteria in the transformation and elaboration of CDOM.

Considering the potential turnover rate of DOC mobilizable by enzyme activities (DOC/\( C_{\text{mob}} \) Table 2), they ranged from 0.4 to 3.7 days, in autumn and winter, respectively, with minima at st. 2 and maxima at st. 3.
Discussion

This investigation was carried out at three stations in the Lagoon of Venice, characterized by a large heterogeneity in salinity and nutrient concentration. The different trophic state was revealed by substantial differences with regard to algal biomass and production. The photosynthetic activity of the phytoplankton, in particular, was characterized by a very wide seasonal range (up to four orders of magnitude at st. 1 and to one at st. 2 and 3), comparable in any case with the few PP data available in the literature for the lagoon, gathered during the 1980s and the 1990s (Degobbis et al., 1986; Bianchi et al., 2000). The seasonal phytoplankton production and biomass appear to be mainly related to that of temperature and solar irradiance. Specific production ($P_N$) was comparable with that of other transitional ecosystems, characterized by an elevated nutrient availability (Macedo et al., 2001; Montes-Hugo & Alvarez-Borrego, 2003; Azevedo et al., 2006). The bacterial community, on the contrary, showed a quite constant metabolism (BCP from 1.5- to 4-fold throughout the seasons) when compared with the widely ranging PP and, apparently, bacterial biomass and production do not respond with the same intensity to the trophic variations.

The ratio between PP and BCP indicates whether secondary pelagic production is based on bacterial mobilization of chemically bound energy or on phytoplankton photosynthesis, with consequences on the structure of the trophic web (Berglund et al., 2007). In our study, the base of the planktonic trophic appears to be largely independent from the different trophic state, while a rather common seasonal pattern could be recognized. A definite shift of the plankton food web toward autotrophy (TPP/BCP $\gg$ 1) occurs in correspondence to a production efficiency ($P_N$) higher than 9 $\mu$g C $\mu$g chl $^{-1}$ h $^{-1}$.

BCP and TPP were significantly, although weakly, correlated. The correlation between BCP and PP is often accepted as a sufficient evidence of phytoplankton–bacterioplankton coupling. However, the contemporaneous dependence of bacteria on algae can only be mediated through DPP, while TPP needs a time lag before the assimilation, giving rise to a decoupling between bacterial and phytoplankton production. The DPP at the three stations examined represents, generally, a very small fraction of TPP (prevalently < 7%, with just two peaks exceeding this value), mostly within the lowest range reported in the literature (Baines & Pace, 1991). The PER does not show a decreasing trend at increasing PPP, as it is, in contrast to what is reported in other ecosystems (Moran et al., 2002; Teira et al., 2003): indeed, it spanned from 0% to 30%, independent of the wide range of PPP (from 0.1 to 90 $\mu$g C L $^{-1}$ h $^{-1}$) and of trophic conditions. BCD, on both an hourly and a daily basis, was, at every station and season, always extremely greater than the actual supply of DPP from algae, leading to the conclusion that bacteria and phytoplankton were uncoupled. In this investigation, the possibility to underestimate DPP with short-term, end-point incubation, due to bacterial recycling of recently produced DOC (Moran & Estrada, 2002), has been taken into account by calculating the potential DPP, which also includes the organic $^{14}$C in the 0.2–2 $\mu$m fraction. This fraction will include both the $^{14}$C recycled by bacteria and the autotrophic picoplankton production (APP) that, in this environment, might be consistent, the APP biomass representing, on average, > 5% of the total phytoplankton biomass in the lagoon with peaks up to 20% (Coppola et al., 2006, 2007). Anyway, BCD was always too high to be supported by DPP, even when considering this DPP potential rate (DPP+PP pico).

Both the occurrence of conditions in which BCP is larger than TPP and the continuous prevalence of BCD over DPP imply that further sources of organic C, more than phytoplankton production alone, are necessary to sustain the bacterial metabolism. The carbon that is made available by the ectoenzymatic mobilization always satisfies the BCD and the correlation of $C_{mob}$ with Leu-BCP suggests that bacterial metabolism was mainly used for enzyme synthesis. On the other hand, the relationship between $C_{mob}$ and DPP and TPP confirms the trophic link of heterotrophic bacteria on algae even if the phytoplankton alone are unable to support BCD.

The major autochthonous, nonphytoplanktonic sources of DOM in wetlands are leachates from plants, exudates from benthic microalgae, or macrophytes, and pore water from sediments and soils (Ziegler & Benner, 1999; Bertilsson & Jones, 2003; Maie et al., 2006). Macrophytes generate DOM either through extracellular release of photosynthetic or upon senescence (Bertilsson & Jones, 2003; Maie et al., 2006; Wang et al., 2007 and references therein). In the three shallow areas considered in this study, organic matter from the sediments may also make an important contribution to the organic matter in the waters (Sfriso et al., 2005), as a nonreadily assimilable OM, mostly composed by high-molecular-weight (HMW) molecules, must be hydrolyzed by bacteria ectoenzymes before the assimilation, giving rise to a decoupling between bacterial and phytoplankton production. The DPP at the three stations examined represents, generally, a very small fraction of TPP (prevalently < 7%, with just two peaks exceeding this value), mostly within the lowest range reported in the literature (Baines & Pace, 1991). The PER does not show a decreasing trend at increasing PPP, as it is, in contrast to what is reported in other ecosystems (Moran et al., 2002; Teira et al., 2003): indeed, it spanned from 0% to 30%, independent of the wide range of PPP (from 0.1 to 90 $\mu$g C L $^{-1}$ h $^{-1}$) and of trophic conditions. BCD, on both an hourly and a daily basis, was, at every station and season, always extremely greater than the actual supply of DPP from algae, leading to the conclusion that bacteria and phytoplankton were uncoupled. In this investigation, the possibility to underestimate DPP with short-term, end-point incubation, due to bacterial recycling of recently produced DOC (Moran & Estrada, 2002), has been taken into account by calculating the potential DPP, which also includes the organic $^{14}$C in the 0.2–2 $\mu$m fraction. This fraction will include both the $^{14}$C recycled by bacteria and the autotrophic picoplankton production (APP) that, in this environment, might be consistent, the APP biomass representing, on average, > 5% of the total phytoplankton biomass in the lagoon with peaks up to 20% (Coppola et al., 2006, 2007). Anyway, BCD was always too high to be supported by DPP, even when considering this DPP potential rate (DPP+PP pico).

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consequence of both natural (e.g. wind, storm, tidal flushing) and anthropogenic (clam harvesting, maritime traffic, capital and maintenance dredging) sediment resuspension. The resuspension was confirmed by the significant relationship found from DOC concentrations in water and those at the water–sediment interface (M. Giani, unpublished data).

The contribution of the CDOM, on average 19% of the mean DOC concentration, probably derives from the bacterial transformation of phytoplankton-derived DOM and from riverine discharges into the lagoon. The highest aCDOM values are usually observed in freshwaters and estuaries and they decrease in coastal and offshore waters (Blough et al., 1993). Bacterial processing of nonchromophoric, algal organic matter, either particulate or dissolved, could be responsible for the CDOM production. The positive relationships found among aCDOM, DPP and Cmod suggest that the bacteria could play an important role in the formation of CDOM in the lagoon waters through the reprocessing of phytoplankton exudates, supporting the Rochelle-Newall & Fisher (2002) findings towards the hypothesis that the noncolored DOM released by phytoplankton could be transformed by bacteria in CDOM.

The relation between bacteria respiration and photosynthesis is expected to be different in relation to the strength of the coupling between the two communities: the availability of the HMW organic matter pool will allow the bacteria to extend their activity and respiration beyond the decline of photosynthesis. In this study, the relations between CR, TPP and BCP are quite contradictory in this respect. CR fell within the values generally reported for estuarine and coastal waters (e.g. Jensen et al., 1990; Smith & Kemp, 1995; Iriarte et al., 1996). A close coupling between CR and TPP, but not between CR and BCP, was observed, suggesting that phytoplankton may be responsible for a significant fraction of CR. On the other hand, the rates of oxygen consumption were much less variable than those of PP: this may indicate that CR and PP do not respond with the same intensity to the environmental variations and that the CR is fuelled by sources other than phytoplankton production and are also supplied more constantly through time. In particular, in the periods in which CR exceeded PP, stored or imported organic matter should prevailently support the planktonic system. However, in the present study, the periods in which the system was heterotrophic (CR > PP) were not related either to a concomitant increase of BCP or of DOC concentrations or to BCP > PP.

In conclusion, this study evidenced that the large within-system trophic heterogeneity corresponded to elevated phytoplankton biomass and production variability, while bacteria standing stock and production appeared to be much more constant. The relationships between bacteria and phytoplankton community could not be associated with the trophic state in a straightforward way. Rather, a common seasonal variability could be evidenced, occurring quite independent of the trophic state, confirming the hypothesis of a general loose bacterioplankton–phytoplankton coupling in coastal and transitional areas. The two communities appeared, indeed, uncoupled, with BCD largely exceeding DPP: this characteristic, which is common to other coastal systems, in this investigation appeared to be constant and independent of the station and the season considered. The variations of the microbial metabolism and the shift from autotrophy to heterotrophy seem to be mainly related to variations in phytoplankton activity, rather than on bacteria one. However, the interplay of trophic conditions, organic carbon availability and seasonality makes it arduous to find a within-system clear pattern of variability related to the spatial and trophic differences.

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