

Patterns of ectoenzymatic and heterotrophic bacterial activities along a salinity gradient in a shallow tidal estuary

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ABSTRACT: Bacterial heterotrophic communities play dominant roles in the transference of organic matter between compartments of estuarine ecosystems and, ultimately, between estuaries and the open sea. In this scenario, we investigated the responses of inflowing limnetic bacteria to the salinity gradient and the influence of these patterns on degradation and recycling of organic matter in the Ria de Aveiro. Phytoplankton biomass was maximum (0.65 to 2.10 mg C l⁻¹) in the mid- or inner-estuary and declined sharply at about 30 psu. Bacterial communities reached highest densities in the mid- and inner-sections of the estuary (up to 15.3 × 10⁹ cell l⁻¹), being 2 to 3 times higher than in the outer-estuary during identical tidal conditions. The rates of ectoenzymatic hydrolysis were also maximum in the mid-estuary or the inner-section. Values ranged from 4.3 to 181.3 nmol l⁻¹ h⁻¹ for β-glucosidase and from 490 to 5374 nmol l⁻¹ h⁻¹ for leu-aminopeptidase. At the transition from the limnetic environment to the main body of the lagoon an exceptional increase in the utilisation of carbohydrate was observed (β-glucosidase activity). This was accompanied by major increases in glucose incorporation, with maximum values of 17.7 nmol l⁻¹ h⁻¹. Turnover rate ranged from 0.3 to 4.1 % h⁻¹ in the outer estuarine section increasing to maximum values in the inner section (10.4 to 39.4 % h⁻¹). Statistical analysis revealed that the variation in ectoenzymatic activities could be significantly related to bacterial abundance, which in turn, was highly associated with variations in salinity, temperature and chlorophyll *a*. Within the salinity gradient, the profiles of bacterial abundance, glucose uptake and ectoenzymatic activity generally agreed with a curvilinear pattern, with a peak at ~25 to 30 psu. Ectoenzymatic activity showed fairly conservative behaviour during tidal transport along the salinity gradient compared to the more reactive parameters of glucose metabolism.

KEY WORDS: Bacterioplankton · Ectoenzymatic activity · Heterotrophic activity · Salinity gradient · Estuaries

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INTRODUCTION

An estuary is a transition system governed by complex interacting elements that change over space and season. When subjected to tidal currents, the entire estuary experiences fluxes that interfere with the transport and expression of biological activities and with the distribution of biomass in the water column. One can, however, assess its metabolic structure by

locating the main sources of nutrients and of organic carbon and by determining the potential for autotrophic and heterotrophic growth as well as the potential for mineralisation.

Allochthonous carbon sources, in addition to the elevated primary production generally observed in estuaries, tend to enhance the heterotrophic component in the metabolism of the plankton as opposed to the autotrophic component. In various estuaries of the Iberian Peninsula, for example, it was observed that the external source of total organic carbon (TOC) was much larger than primary production within the estu-

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aries (Vallespinós & Mallo 1990). Microheterotrophs, especially bacteria, are the only biological populations capable of significantly altering both dissolved organic carbon (DOC) and particulate organic carbon (POC) (Chróst 1990), and because of this have a great impact

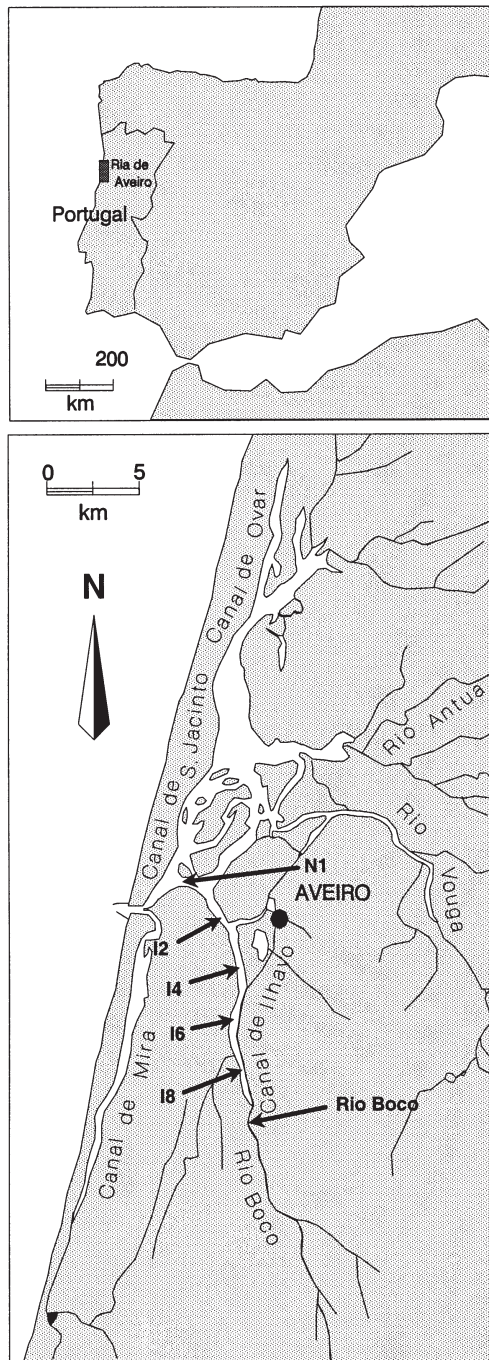


Fig. 1. Ria de Aveiro (Portugal); sampling stations indicated with arrows: Stns N1 and I2 in Canal de Navegação, Stns I4, I6 and I8 in Canal de Ílhavo; Stn Rio Boco at mouth of fresh water stream

on the cycling of organic matter and on energy flux. These processes require extensive extracellular substrate hydrolysis by different ectoenzymes, activities that play a key role in microbial ecology (Hoppe 1991, Vetter et al. 1998). The importance of ectoenzymatic activity is evidenced by the coupling to decomposition of POC and DOC during seasonal and spatial fluctuations of such activity (Chróst 1990).

Variations in ectohydrolase profiles (types and levels of activity) along an estuarine trophic gradient reflect the trophic status of the environment, and this changes with the season (Karner et al. 1992, Hoppe et al. 1998). It has been argued that such variations could be caused by shifts in the dominant species or in the level of enzyme expression by the same species in response to changes in the spectrum of organic substrates (Martinez et al. 1996)

Generally, the Ria de Aveiro is dominated by microbial heterotrophic processes not only dielly, but also probably annually (Hoppe et al. 1996). In the warm season, during daylight, the balance between autotrophy and heterotrophy, as judged from the ratio of primary production to bacterial production, shifts towards clear net autotrophy, particularly at high tide (Hoppe et al. 1996). In the cold season, however, even during daylight, at low tide there is only general equilibrium of the 2 activities in the inner and outer estuary sections (Cunha et al. 1999).

In light of the observed importance of heterotrophy in the lagoon of Aveiro, we attempt to demonstrate the patterns of degradation of major macromolecules and the turnover patterns of monomers along the salinity gradient. The tidal effects on bacterial activity are explored in order to assess the conservative or reactive transport of cells with tidal currents and to identify the main sources of bacterioplankton heterotrophic activity in the estuary.

MATERIALS AND METHODS

Study site. The Ria de Aveiro (Fig. 1) is a bar-built estuary (Pritchard 1967) on the northwest coast of Portugal separated from the sea by a sand barrier. The freshwater input to the lagoon during the flood-tide period varies throughout the year around an average value of 1.8 Mm^3 , i.e. $\sim 2\%$ of the total low-tide volume of the lagoon (Silva 1994).

The Ria is, in fact, a mesotidal coastal lagoon with a complex topography representing a multi-estuarine ecosystem associated with different inflowing rivers. Canal de Navegação is the main navigation channel and Rio Boco is a small stream that continuously supplies freshwater to the south end of Canal de Ílhavo (Fig. 1).

Investigations were conducted along a longitudinal profile, extending across the inner-estuary (River Boco) down to the outer segment of the lagoon, at 6 sampling sites spaced at regular intervals of at 3 km (Fig. 1). The stations were numbered from north to south: Stns N1 and I2 in Canal de Navegação; and Stns I4, I6 and I8 in Canal de Ílhavo, and Rio Boco (RB) at the mouth of this small freshwater stream.

For later comparisons, 3 segments were considered along this profile, a segment being defined as the distance over which a particle is transported during flood tide (Silva 1994). The outer-estuary (Stns N1, I2), in the transition to the coastal zone, had salinities ranging from 33 to 35.5 psu at high tide. The mid-estuary (Stns I4, I6, and I8) had salinities in the 20 to 33 psu range on the same tide. The inner-estuary (Stn RB) was defined as the mixing zone between fresh and marine waters, with salinities at high tide below 20 psu.

The main sources of contamination along this profile are waste waters from the city of Aveiro and pollution associated with harbour activities in the outer- and mid-segments, some industries, aquaculture plants and diffuse domestic sewage drains in the mid segment, and run-off from agriculture fields in the inner segment.

Sampling. The 6 sampling sites were visited in early summer 1997, at neap tide (NT) (12th July) and spring tide (ST) (20th July). Samples were always collected during the daytime in the centre of the channels, 0.2 m below the surface. Collection was performed at slack high (HT) and at low (LT) tides using 5 l plastic bottles. Collected samples were kept cold and in the shade during transport to the laboratory, where they were processed within the next 2 to 3 h.

Physical and chemical parameters. Salinity and temperature were measured with a WTW LF 196 conductivity meter. Dissolved oxygen, expressed as the percentage of saturation, was determined with a WTW OXI 96 oxygen meter equipped with a WTW BR 190 stirrer. The depth of the water column was determined with a Sonar probe, and turbidity was evaluated with a Secchi disk. The determination of the concentration of suspended solids (seston) was performed after filtration of 500 ml aliquots through Whatman GF/C (47 mm diam.) pre-weighted, pre-combusted filters. The filters were dried at 60°C for 24 h and seston content was calculated as the increase in weight. Particulate organic matter (POM) was determined by difference in the weight of the dry seston filters after 4 h incineration of the dry seston at 525°C (Parsons et al. 1989). POC was calculated as 50% of the POM (Rodier 1996).

Microbiological parameters. Total bacterial numbers (TBN) and bacterial organic carbon (BOC) were determined by cell counting under epifluorescence

microscopy after fixation of the water samples with 2% formaldehyde (final conc.). The sample was filtered through 0.2 μm , black, polycarbonate membranes (Poretics) and stained with 0.03% acridine orange (Hobbie et al. 1977). On each filter, 50 cells were measured with a reticule (Graticules Ltd, Model G2) placed in the microscope eyepiece for the determination of the average cell volume. Bacterial biomass was calculated from the averaged biovolume after conversion ($350 \text{ fg C } \mu\text{m}^{-3}$ according to Bjørnsen 1986).

The heterotrophic metabolism of glucose was described by the parameters V_m (maximum uptake velocity) and Tr (turnover rate), following the procedure described by Gocke (1977). For V_m determinations, a final saturation concentration of 430 nM of ^{14}C -glucose was added to 10 ml aliquots. For Tr , the final concentration of ^{14}C -glucose was 43 nM. Substrate concentrations were chosen after kinetic analysis. Incubations were carried out for 2 to 3 h at *in situ* temperature. Cells were collected on 0.2 μm Poretics polycarbonate membranes, and radioactivity was read in a liquid scintillation counter (Beckman LS 6000 IC) using Universol as the scintillation cocktail. Radioactive-labelled glucose ($\text{SA } 11.5 \text{ GBq mmol}^{-1}$, 310 mCi mmol^{-1}) was obtained from Amersham.

Ectoenzymatic activity was determined fluorimetrically (Jasco FP-777 fluorometer) as the maximum hydrolysis rate (H_m) of model substrates for β -glucosidase (4-methylumbelliferyl- β -D-glucoside) and leu-aminopeptidase (L-leucine-7-amido-4-methyl-coumarin) added at the saturating concentration of 1 mM (Hoppe 1983). Wavelengths for excitation and emission were 380 to 440 nm for MCA (7-amino-4-methylcoumarine) and 360 to 450 nm for MUF (4-methylumbelliferone). Measurements were made in 3 replicates for each sample after 1 to 2 h incubations at *in situ* temperature. Calibration was performed by adding a series of 6 to 8 concentrations of the fluorescent products (0 to 500 nM for MUF and 0 to 6 μM for MCA) to a pool of water from the 6 sampling stations.

Chlorophyll *a* was determined fluorimetrically (Yentsch & Menzel 1963) after extraction by 90% acetone. In the absence of a calculated factor for chlorophyll to carbon conversion, the generally accepted value of 50 (Eppley et al. 1977) was used.

Statistical methods. In an attempt to explain the variation in the microbiological parameters, stepwise multiple regression was followed using chlorophyll *a*, seston, POM, percentage of POM in the total seston, temperature, salinity, total depth, Secchi depth and oxygen concentration as independent variables. TBN was also included as independent variable for the regression analysis of microbial activity parameters. Statistical procedures were performed with SPSSWIN 7.1.

RESULTS

Physical and chemical parameters

Physical and chemical data are shown in Table 1. Salinity ranged from 2.3 psu at Stn RB to 35.6 psu at Stn N1. The variation of salinity was strongest at the mouth of the freshwater stream, at Stn RB, with an amplitude of 13.2 psu in NT and 5.0 psu in ST.

The temperature of the water column (Table 1) varied between 16.6 and 26.6°C, increasing towards the inner estuary. Water depth varied in the range 0.3 to 8.6 m (Table 1) with tidal amplitudes of 0.5 to 2.6 m.

The dissolved oxygen concentration corresponded, to at least 76% of the saturation value (Table 1). In the mid- and inner-sections of the longitudinal profile, par-

ticularly at LT on neap tide, oversaturation was frequent.

Seston concentration ranged from 36 to 66 mg l⁻¹ (Table 1), with no distinct longitudinal pattern of variation. At HT, however, a considerable decrease in the amount of seston could be observed in the inner-estuary compared to the downstream stations. Nevertheless, Secchi depth values remained quite low. In the outer- and mid-estuary, the seston content at LT was up to 16% greater than at HT. In the inner-estuary the corresponding increase was greater (37 or 44%), denoting a richer particle content of the inflowing river.

The organic component of the seston (POC) varied between 5.5 and 7.5 mg l⁻¹, and remained quite stable irrespective of salinity or tide (Table 2). POC represented 11 to 18% of the seston in the inner-estuary and 9 to 14% in the mid- and outer-estuary water.

Table 1. Total depth, Secchi depth, salinity, temperature, dissolved oxygen (% of saturation conc.) and seston concentration registered at the surface of water column (0.2 m) at high and low tide under neap- and spring-tide conditions. Standard deviation, when calculated, is given in parentheses

Station	Total depth (m)	Secchi depth (m)	Salinity (psu)	Temperature (°C)	Dissolved oxygen (% sat.)	Seston (mg l ⁻¹)
12 July 1997 (neap tide)						
High tide						
N1	7.5	4.2	35.6	16.6	78	49 (3)
I2	7.7	3.5	34.7	18.5	78	48 (1)
I4	2.0	2.0	33.0	20.6	78	50 (2)
I6	2.4	2.4	32.3	21.3	78	51 (3)
I8	3.0	1.0	29.0	23.3	78	53 (1)
Rio Boco	1.0	0.5	7.3	24.1	116	36 (1)
Low tide						
N1	6.7	2.5	32.7	20.7	88	49 (6)
I2	6.1	2.2	32.6	22.2	84	48 (3)
I4	1.5	1.3	30.0	24.5	109	58 (1)
I6	1.6	0.9	28.2	25.1	115	59 (1)
I8	2.5	0.5	24.6	26.1	162	55 (4)
Rio Boco	0.3	0.3	2.3	25.3	100	52 (6)
20 July 1997 (spring tide)						
High tide						
N1	8.0	3.5	35.5	17.8	99	57 (4)
I2	8.6	4.5	35.5	18.4	103	53 (2)
I4	4.1	3.0	34.4	21.2	96	58 (2)
I6	3.2	3.0	34.2	19.5	92	60 (4)
I8	4.1	1.5	33.2	25.5	91	53 (1)
Rio Boco	1.8	0.8	17.7	26.6	90	38 (1)
Low tide						
N1	5.8	2.0	33.5	20.8	96	61 (6)
I2	6.0	1.2	33.3	22.1	92	60 (1)
I4	2.3	0.5	30.2	24.3	79	66 (4)
I6	1.5	0.8	28.9	24.9	76	62 (1)
I8	2.0	0.7	25.2	25.9	nd	57 (7)
Rio Boco	0.5	0.5	4.5	26.6	nd	52 (7)

Table 2. POC calculated from POM, assuming carbon content of 50%; phytoplankton biomass calculated from chlorophyll *a* concentration assuming conversion factor of 50 (Eppley et al. 1977); bacterial biomass and phytoplankton biomass calculated as percentage of POM. Average values for 3 main sections of estuary are presented in parentheses

Station	POC (mg C l ⁻¹)	Phytoplankton biomass (mg C l ⁻¹)	Bacterial biomass (µg C l ⁻¹)	Phytoplankton biomass/POC (%)
12 July 1997 (neap tide)				
High tide				
N1	6.0 (6.0)	0.35 (0.38)	45.0 (60.0)	5.8 (6.3)
I2	6.0	0.41	75.1	6.8
I4	6.0 (6.3)	0.44 (0.91)	121.1 (182.2)	7.3 (14.1)
I6	6.5	0.61	180.8	9.3
I8	6.5	1.68	244.8	25.8
Rio Boco	6.5 (6.5)	1.13 (1.13)	260.0 (260.0)	17.4 (17.4)
Low tide				
N1	7.0 (6.5)	0.57 (0.61)	108.4 (141.0)	8.1 (9.5)
I2	6.0	0.65	173.5	10.8
I4	7.0 (6.8)	1.52 (1.82)	220.3 (277.1)	21.6 (27.4)
I6	6.5	1.92	259.0	29.5
I8	6.5	2.03	352.2	31.2
Rio Boco	6.5 (6.5)	2.10 (2.10)	152.8 (152.8)	32.2 (32.2)
20 July 1997 (spring tide)				
High tide				
N1	7.0 (7.0)	0.19 (0.19)	55.8 (57.7)	2.6 (2.7)
I2	7.0	0.19	59.6	2.7
I4	7.0 (7.3)	0.39 (0.44)	117.1 (124.2)	5.6 (6.4)
I6	7.5	0.29	102.9	3.8
I8	6.5	0.65	152.5	9.9
Rio Boco	6.5 (6.5)	1.64 (1.64)	418.0 (418.0)	25.2 (25.2)
Low tide				
N1	6.5 (6.3)	0.56 (0.52)	124.0 (234.2)	8.6 (8.3)
I2	6.0	0.48	144.3	8.0
I4	6.0 (6.3)	1.05 (0.98)	168.3 (218.3)	17.4 (15.6)
I6	6.0	0.96	213.3	16.0
I8	7.0	0.93	273.3	13.3
Rio Boco	5.5 (5.5)	0.65 (0.65)	190.0 (190.0)	11.8 (11.8)

Phytoplankton and bacterioplankton abundance

The highest concentrations of phytoplankton biomass (Table 2) were found in the inner- (0.65 to 2.10 mg C l⁻¹) or in the mid- (0.44 to 1.82 mg C l⁻¹) estuary. In the outer estuary, the phytoplankton biomass ranged from 0.19 to 0.57 mg C l⁻¹. The phytoplankton carbon fraction in the POC was also greater (1.9 to 9.3 times, average 4.3) in the inner- and/or the mid-estuaries, depending on tidal state.

Total bacterial number (TBN) ranged from 2.6 to 15.3×10^9 cells l⁻¹, defining a clear spatial gradient of enrichment towards the inner stations of the mid-estuary, generally (with the exception of HT–ST) followed by a decline in the transition to Stn RB (Fig. 2). Bacterial organic carbon (Table 2) followed approximately the same pattern as TBN, and varied from 45 to 418 µg C l⁻¹ in different sections of the profile and at different tides.

Compared to neap tide, spring tide increased the HT biomass value in the inner estuary by 60%.

Bacterial heterotrophic activity

As demonstrated for bacterial numbers, the heterotrophic metabolism of glucose also showed major amplifications in the upper mid- or inner-sections of the lagoon (Fig. 2). It reached a maximum value of 17.7 nmol l⁻¹ h⁻¹ at Stn I8. Glucose Tr (Fig. 2) was lowest at the outer-estuary (0.3 to 4.1% h⁻¹), increasing inwards. The maximum value (39.4% h⁻¹) was registered at RB at low tide, under spring-tide conditions. The respired fraction was ≤34% (average 13.8%) of the glucose uptake.

The rates of ectoenzymatic hydrolysis were generally higher for leu-aminopeptidase (leu-AMPase) than

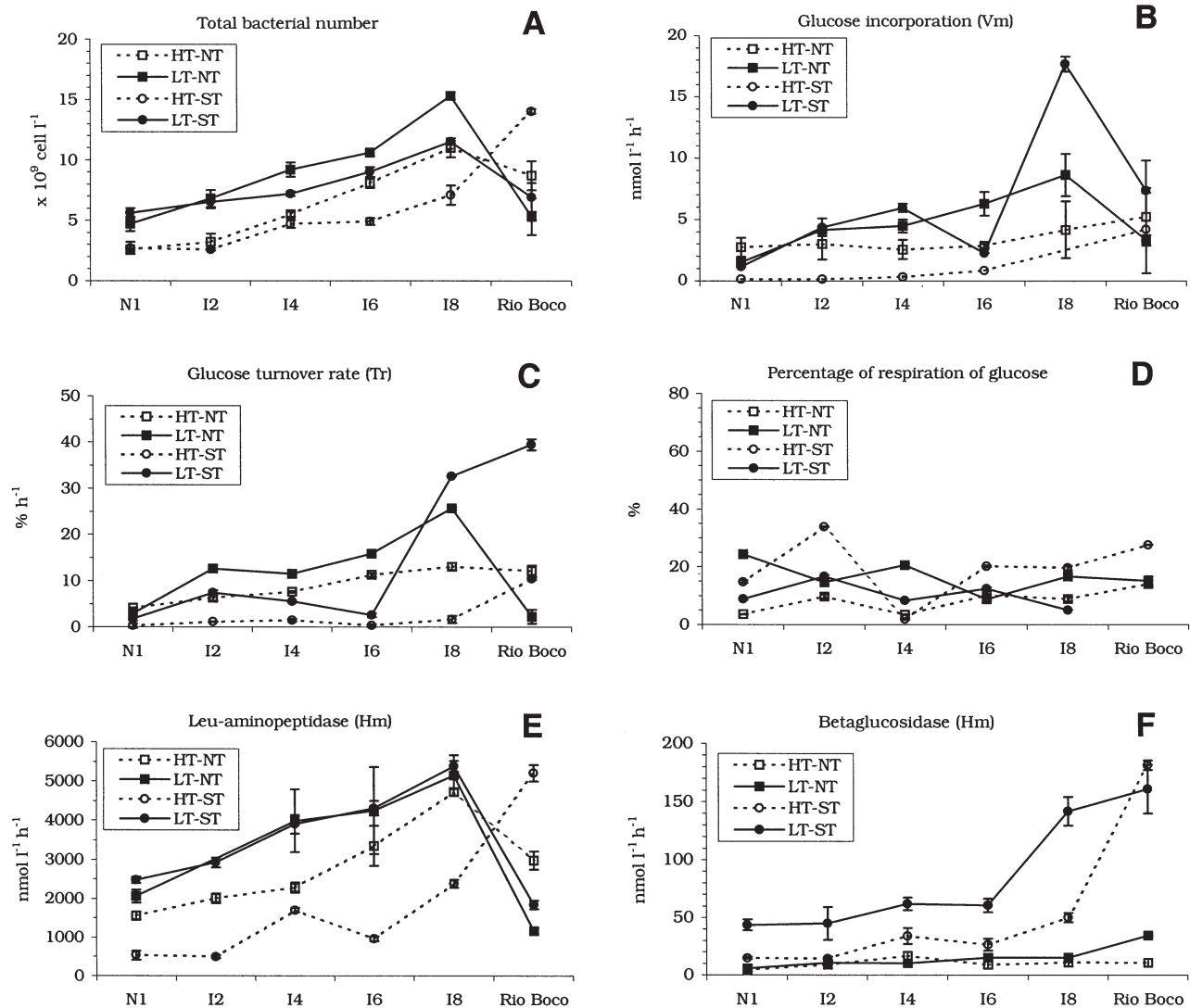


Fig. 2. Variations in parameters of bacterial abundance and activity along longitudinal profile of 6 stations in Ria de Aveiro at high (HT) and low (LT) tide during neap (NT) and spring (ST) tidal conditions

for β -glucosidase (β -GlCase). H_m varied from 490 to 5374 $\text{nmol l}^{-1} \text{ h}^{-1}$ for leu-AMPase and from 4.3 to 181.3 $\text{nmol l}^{-1} \text{ h}^{-1}$ for β -GlCase. Along the profile, leu-AMPase activity was generally highest at Stn I8, while β -GlCase reached its maximum of activity at Stn RB (Fig. 2). At LT and in mid-estuary, the hydrolytic activities were up to 4.5 times (leu-AMPase) and 2.8 times (β -GlCase) higher than at HT. In contrast, at the mouth of RB there was a decrease in leu-AMPase activity at LT to 40% of the HT value. This decrease was not observed in β -GlCase activity.

Cell activities (Table 3) ranged between 0.05 and 1.54 $\text{amol cell}^{-1} \text{ h}^{-1}$ for glucose uptake V_m , 188.5 and 625.6 $\text{amol cell}^{-1} \text{ h}^{-1}$ for leu-AMPase activity, and 5.4 and 25.0 $\text{amol cell}^{-1} \text{ h}^{-1}$ for β -GlCase activity. In spring-tide conditions, values of specific activity were higher

at LT than at HT, particularly in the outer section. At neap tide, in contrast, the outer section displayed higher specific activity values in HT. At Stn RB, values of specific activity were always higher at HT for leu-AMPase, but for β -GlCase the values were always higher at LT. Specific leu-AMPase and β -GlCase activities did not show a constant spatial pattern.

Statistical analysis

The results of the stepwise multiple regressions, presuming a coupling between microbiological parameters and the physico-chemical and biological independent variables are presented in Table 4. The percentage of variation that could be explained with

Table 3. Glucose uptake V_m , leu-AMPase activity H_m , and β -GlCase activity H_m (amol cell⁻¹ h⁻¹) per cell

Station	Glucose V_m	Leu-AMPase H_m	β -GlCase H_m
12 July 1997 (neap tide)			
High tide			
N1	1.04	595.8	12.4
I2	0.94	625.6	11.1
I4	0.47	414.4	6.7
I6	0.36	413.3	6.8
I8	0.38	421.2	6.4
Rio Boco	0.60	341.5	10.2
Low tide			
N1	0.32	436.8	9.7
I2	0.62	279.9	7.9
I4	0.49	433.4	8.2
I6	0.59	400.5	8.9
I8	0.56	336.3	7.1
Rio Boco	0.62	218.1	25.0
20 July 1997 (spring tide)			
High tide			
N1	0.05	196.7	5.5
I2	0.05	188.5	5.5
I4	0.07	361.5	7.0
I6	0.17	196.1	5.4
I8	–	335.4	7.0
Rio Boco	0.30	371.5	12.9
Low tide			
N1	0.21	441.3	7.8
I2	0.67	449.2	6.9
I4	0.83	541.5	8.6
I6	0.25	478.7	6.7
I8	1.54	467.3	12.3
Rio Boco	1.07	265.5	23.3

this set of independent variables was high (>80%) for TBN and ectoenzymatic activities, while only 30 to 40% of the variation of the parameters related to the heterotrophic metabolism of glucose could be explained.

DISCUSSION

Bacterial abundance and heterotrophic activity along estuarine profile

Bacterial biomass and bacterial-dependent activities were in agreement with a curvilinear pattern, with a peak at ~25 to 30 psu. This corresponds in general to the estuarine structure described by Wright & Coffin (1983), Palumbo et al. (1984), Fuks et al. (1991), Bordalo et al. (1998) and but the location of the peak in Canal de Ílhavo was shifted to a comparatively higher salinity level because of the low freshwater input. The Ria contrasts with other estuaries, which showed maximum values for bacterial heterotrophic activities in the inner estuary at low salinity values (3 to 10 psu), followed by a conservative decrease towards higher salinities (Palumbo & Ferguson 1978). In the particular situation of the Ria de Aveiro, it seems clear that the freshwater stream, in the inner part of the estuarine profile studied (Fig. 1) is generally less polluted than the lagoon itself and has higher chlorophyll concentrations but lower seston content, bacterial abundance, and rates of ectoenzymatic protein degradation. The peaks of bacterial abundance, leu-AMPase activity, and glucose heterotrophic metabolism, accompanied by denser (2 to 4 times) phytoplankton communities and increased nitrate concentrations (data not shown),

Table 4. Regression equations for variations in microbiological parameters obtained from stepwise multiple-regression analysis. Dependent variables = total bacterial number (TBN), leu-AMPase (H_m of leu-aminopeptidase), β -GlCase (H_m of β -glucosidase), V_m (V_m of glucose incorporation), Tr (glucose turnover rate) and % Resp (percentage of glucose respiration); independent variables = TBN, Temp (temperature), Sal (salinity) and Chlor (chlorophyll *a*)

Dependent variable	Independent variables	Regression equation	Adj. R ²
LogTBN	Temp ($\beta = 0.715$; $p = 0.000$) Sal ($\beta = 0.321$; $p = 0.011$) LogChlor ($\beta = 0.424$; $p = 0.013$)	LogTBN = $-0.908 + 0.052 \text{Temp} + 0.007127 \text{Sal} + 0.299 \text{LogChlor}$	0.824
Leu-AMPase H_m	TBN ($\beta = 0.906$; $p = 0.000$)	Leu-AMPase $H_m = -333.898 + 414.966 \text{TBN}$	0.813
β -GlCase H_m	Sal ($\beta = -0.634$; $p = 0.000$) TBN ($\beta = 0.478$; $p = 0.000$)	β -GlCase $H_m = 105.170 - 2.934 \text{Sal} + 6.335 \text{TBN}$	0.822
Glucose V_m	Temp ($\beta = 0.584$; $p = 0.003$)	Glucose $V_m = -11.584 + 0.704 \text{Temp}$	0.310
Glucose Tr	Temp ($\beta = 0.577$; $p = 0.003$)	Glucose Tr = $-34.568 + 1.954 \text{Temp}$	0.333
% Resp	Sal ($\beta = -0.558$; $p = 0.013$) LogChlor ($\beta = -0.942$; $p = 0.003$) Temp ($\beta = 0.703$; $p = 0.030$)	% Resp = $13.072 - 0.864 \text{Sal} - 44.253 \text{LogChlor} + 3.572 \text{Temp}$	0.435

usually occurred at Stn I8 and reached Stn RB only in association with the strong flood tide currents experienced under spring-tide conditions.

Values of TBN, β -GlCase activity and glucose Tr obtained during this study generally fit within the range of values published for the Ria de Aveiro and for other temperate coastal ecosystems (Rheinheimer et al. 1989, Chróst & Velimirov 1991, Fuks et al. 1991, Karner et al. 1992, Crump & Baross 1996, Hoppe et al. 1996, 1998). However, the metabolism of glucose along the studied profile was strongly channelled into incorporation as bacterial biomass (72.5 to 98.0%) rather than into respiration. Higher growth efficiencies of estuarine bacteria compared to open-water communities have been reported by Jørgensen et al. (1999), who postulated that the differences are related to the quality (lability) of the available organic matter. Our values are higher than the range of values for estuarine environments (11 to 61%) reviewed by del Giorgio & Cole (1998). However, the single radiotracer approach may produce artificially high values of the incorporated fraction, since the equilibrium of the intracellular pool is not completely achieved during short incubation periods (Yahnke & Craven 1995).

The extracellular degradation of protein was also exceptionally high along the salinity gradient. Leu-AMPase H_m varied in the high range of 490 to 5374 $\text{nmol l}^{-1} \text{h}^{-1}$, greatly exceeding a previous range of values (8.3 to 311 $\text{nmol l}^{-1} \text{h}^{-1}$, after conversion) determined for a less rich region of the Ria de Aveiro (Hoppe et al. 1996). It exceeded also the range of other

published results (4 to 2540 $\text{nmol l}^{-1} \text{h}^{-1}$) for brackish-water systems (Hoppe 1983, Rego et al. 1985, Chróst & Velimirov 1991, Hoppe et al. 1996, 1998), denoting an exceptionally high potential for peptide hydrolysis in the mid-estuary of the studied profile. This is probably related to intense anthropogenic pressure and increased eutrophication of the brackishwater sections of the lagoon.

Changing patterns of organic matter utilisation between freshwater stream and main body of lagoon

One of the most interesting aspects in the variation of bacterial abundance and activity along this estuarine profile is the transition between the inner-station (Rio Boco) and the main body of the lagoon. The gradient of enrichment in bacterial abundance and activity, from the mouth to the inner parts of the lagoon, extends to the river only under spring tide-conditions, when strong flood-tide currents transport a considerable amount of estuarine water upstream. Under different conditions, bacterial abundance decreases at the inner station, as well as leu-AMPase activity, contrasting with an increase of β -GlCase activity. This seems to locate the main source of bacterial potential for the degradation of carbohydrates upstream, and the major sources of bacterial carbon and AMPase activity in the main body of the lagoon. The LT/HT ratios for β -GlCase activity at Stn RB were above or close to 1 (Table 5), as in the mid- and outer-sections. In contrast,

Table 5. Ratios LT/HT for TBN, glucose V_m and Tr, H_m of leu-AMPase, H_m of β -GlCase, and per cell values of glucose V_m , leu-AMPase H_m and β -GlCase H_m calculated for 3 sectors of estuarine profile. Average values are presented in parentheses. NT: neap tide; ST: spring tide

Parameter	Outer-estuary (Stns N1, I2)		Mid-estuary (Stns I4, I6, I8)		Inner-estuary (Stn Rio Boco)	
	NT	ST	NT	ST	NT	ST
TBN	1.8–2.1 (1.9)	2.1–2.5 (2.3)	1.3–1.7 (1.5)	1.5–1.8 (1.7)	0.6	0.5
Glucose V_m	0.6–1.4 (1.0)	8.4–31.2 (19.8)	0.6–2.2 (2.0)	1.8–18.6 (10.7)	0.6	1.8
Glucose Tr	0.7–2.0 (1.3)	6.8–6.9 (6.9)	1.4–2.0 (1.6)	3.9–20.1 (10.7)	0.2	3.8
Leu-AMPase H_m	1.0–1.3 (1.2)	4.7–6.0 (5.3)	1.1–1.7 (1.4)	2.3–4.5 (3.0)	0.4	0.4
β -GlCase H_m	1.4–1.5 (1.5)	3.0–3.1 (3.1)	1.5–2.1 (1.8)	1.9–2.8 (2.3)	1.5	0.9
Specific glucose V_m	0.3–0.7 (0.5)	4.0–12.5 (8.3)	1.0–1.7 (1.4)	1.5–12.1 (6.3)	1.0	3.6
Specific leu-AMPase H_m	0.4–0.7 (0.6)	2.2–2.4 (2.3)	0.8–1.0 (1.9)	1.4–2.4 (1.8)	0.6	0.7
Specific β -GlCase H_m	0.7–0.8 (0.8)	1.3–1.4 (1.4)	1.1–1.3 (1.2)	1.2–1.8 (1.4)	2.4	1.8

bacterial abundance and protease activity at RB were lower at LT. Relative increase of glucosidase in comparison to peptidase in low salinity sections was also observed by Murrell et al. (1999) in another estuarine system (northern San Francisco Bay).

Bacterioplankton at Stn RB seems to be more dependent on carbohydrates, and the results indicate that β -GlCase activity may limit the availability of simple substrates and limit glucose uptake. LT/HT ratios >1 would be expected for other parameters related to glucose metabolism (Glucose V_m and Tr) but at RB, the LT stimulation of glucose metabolism was only obvious at spring tide (Table 5). Intense mixing with water from the mid-section of the estuary during ST replenishes the supply of simple molecules and allows increased glucose metabolism at LT when the negative effect of salinity is thus reduced.

In terms of specific activities, ectoenzymatic activities exhibited distinct tidal responses. At Stn RB, specific values of β -GlCase H_m (and glucose uptake V_m) were higher at LT, while specific AMPase H_m was higher at HT. However, cell-specific activity is a statistical concept that may not represent the whole reality of a changing bacterial community in an unstable environment, since it can reflect an intrinsic difference in the composition (Martinez et al. 1996) or/and in the activity of marine and estuarine bacterial communities (Karner et al. 1992).

Freshwater residence time in the inner section of Canal de Ílhavo ranges from 6 d in spring to several weeks during periods of low freshwater input (Silva 1994). In these conditions, bacterial communities that reach the uppermost sections of the estuary may remain in the brackishwater sections for considerable periods. This allows us to hypothesise that the shift in the patterns of heterotrophic utilisation of protein and carbohydrates reflects the existence of distinct microbial communities adapted to the different environmental conditions and nutrient sources. The observed longitudinal pattern would then result from the combined contributions of a riverine reservoir of potential carbohydrate degradation and an estuarine reservoir of bacterial biomass and potential protein utilisation.

Reactive versus conservative bacterial heterotrophic capacities

During the relatively slow transport from the inner sections, the processes of mixing of communities of different densities, dilution with less-saline and nutrient-poor seawater and adaptation to different organic substrates will considerably change the characteristics of bacterioplankton that reach the mouth of the lagoon. Some parameters were fairly stable during tidal trans-

port, while others, distinctly shifted up and down, resulting in changes in the slope of the profile established along the salinity gradient. These changes can be easily compared by the ratios between the values observed at Stns I8 and N1 (I8 versus N1) for different tidal conditions in (Fig. 3).

As a general trend, bacterial abundance and activity increased inwards during flood-tide transport (HT shift up) and decreased during ebbing (LT shift down) as a combined effect of dilution by poorer marine water and the mixing of communities with different specific-activity rates. Overall, ST results in an increase in the slope of the longitudinal profile, but the most striking feature is the distinct behaviour of ectoenzymes in regard to the heterotrophic metabolism of glucose. Both ectoenzymes seemed to be transported/diluted up and down the estuary, maintaining a fairly good balance between shifting up at HT (I8/N1 ratios of 2.2 to 4.5) and shifting down at LT (I8/N1 ratios of 2.2 to 3.3). In contrast, the negative effects on glucose metabolism (V_m and Tr) of increasing salinity and impoverishment associated with ebb tide transport were stronger than the stimulation of marine communities being transported to a richer environment. The I8/N1 ratios for V_m and Tr of glucose were higher at LT (5.7 to 18.1) than at HT (1.6 to 6.2). This reinforces the scenario of a limnetic source of carbohydrate-utilising bacterial communities.

The steepest trophic gradient, evaluated by the I8/N1 ratios of phytoplankton biomass (4.8) and bacterial abundance (4.3), occurred at HT-NT. However, in the same conditions, the corresponding ratios for glucose V_m and Tr were only 1.6 and 3.2, respectively. This means that at HT-NT, bacterial abundance shifted up more than the metabolism of glucose, and explains the decrease in specific activity from the outer to the inner sections (Table 3). If, in this case, resuspension was related to the increase in bacterial abundance, then newly suspended benthic bacterial communities were characterised by lower specific-activity rates.

The results of multiple regression analysis (Table 4) help to clarify the distinct behaviour of different heterotrophic capacities during tidal transport. A close relationship between ectoenzymatic activity and bacterial abundance can be confirmed. More than 80% of the variation of bacterial abundance was associated with the combined effects of salinity, temperature and chlorophyll *a* concentration. The relationship with salinity is, however, strongly influenced by the contrast between Rio Boco and the brackishwater stations. A similar statistical treatment excluding Stn RB reveals a clearly negative relationship between bacterial abundance and salinity (results not shown).

An important fraction ($>80\%$) the variation in leu-AMPase activity could be explained by the variation in

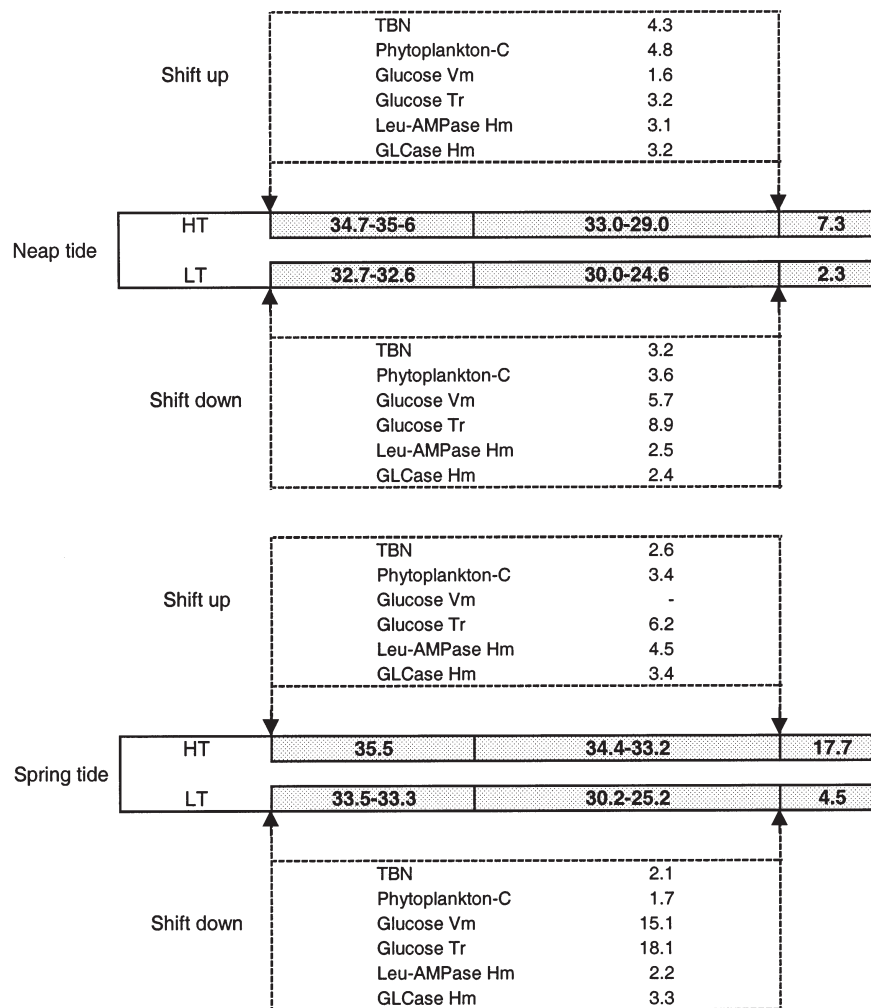


Fig. 3. Schematic representation of tidal responses (shift up and shift down) of biological parameters. Values inside grey boxes indicate ranges of salinity values in each of the 3 segments of Canal de Ílhavo. Dotted frames contain ratios calculated between values registered at Stns I8 and N1 (I8/N1) for total bacterial number (TBN), phytoplankton-C, and bacterial activity parameters under different tidal conditions. V_{max} : max. uptake velocity; H_m : max. hydrolysis rate

TBN, suggesting that this ectohydrolytic capacity is common and a widespread characteristic of estuarine bacterioplankton. Since it is closely associated with bacterial cells, processes of mixing and dilution mainly control the variation of leu-AMPase activity during tidal transport. In the case of β -GLCase, the 2 variables TNB and salinity explained 82% of the variation, denoting an increase in salinity effects in relation to leu-AMPase.

The variation of temperature alone could not explain more than 30 to 40% of the variation in glucose V_m and Tr, suggesting the greater importance of other variables such as the size and quality of the dissolved organic matter pool, and justifies the activity of these parameters during tidal transport. It has been reported that, in aquatic ecosystems, the concentration of dissolved polymers in DOC is usually at least 1 order of magni-

tude higher than the particulate fraction (Zdanowski & Figueiras 1997). The results of Murrell et al. (1999) indicate that during the warm season, when freshwater flux and the supply of particles to the estuaries are reduced, seston is of low nutritional quality. We believe that in the main body of the Ria de Aveiro, as in other estuarine environments (Azam & Hodson 1977, Williams 1981, Ducklow 1983, Murrell et al. 1999) bacteria obtain their organic carbon mainly from the flux of DOC rather than from the decomposition of essentially detrital POM. The shift from utilisation of complex substrates associated with high-quality seston at RB to an increasing quantity of simple dissolved substrates as nutrient sources in the brackishwater sections of the estuary, may contribute to the high activity of parameters related to the incorporation of monomers.

Conclusions

The profiles of variation in abundance and activity along an estuarine profile in the Ria de Aveiro, revealed a shifting from a more N-associated metabolism in the main body of the lagoon to an increasing importance of a C-utilising community at the mouth of the freshwater stream Rio Boco. Ecto enzymatic activity followed more closely variations in bacterial abundance than in monomer uptake, and seemed to be mainly determined by variations in salinity, temperature and phytoplankton biomass. However, the activity of β -GlCase was strongly associated with the interface, with the limnetic environment being negatively affected by increasing salinity.

Along the profile, and under different tidal conditions, ectoenzymatic activity appeared to be transported up (stimulated) or down (depressed) the estuary, in a fairly conservative pattern and probably mainly in response to dilution effects associated with entrance of marine water during flood tide. The heterotrophic metabolism of glucose showed a more reactive behaviour. This parameter responded negatively to ebb-tide transport. In contrast, during transport to inner stations of the lagoon, there was a comparably less pronounced and, to some degree more reversible, stimulation. We did not attempt to characterise the dissolved organic matter that reaches the brackish-water section of the lagoon (mainly from allochthonous origins). However, ectoenzymatic activity could be limiting the turnover of monomers, at least in the inner section of the studied profile. The shifting to a more carbohydrate-utilising bacterial community at the limnetic end of the estuary points to the increased importance of autotrophic processes as a source of substrates during the warm season and during the daylight period.

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