Universidade de Aveiro Departamento de Biologia

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Maria Ângela Sousa Dias Alves Cunha

Bacterioplâncton da Ria de Aveiro: actividade ectoenzimática e metabolismo de monómeros

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Dissertação apresentada à Universidade de Aveiro para cumprimento dos requisitos necessários à obtenção do grau de Doutor em Biologia, realizada sob a orientação científica da Prof. Dr.ª Fernanda Alcântara, Professora Associada Aposentada do Departamento de Biologia da Universidade de Aveiro

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resumo

As comunidades estuarinas de bacterioplâncton desenvolvem-se sob condições ambientais instáveis e respondem às variações espaciais e temporais de pequena escala das características da coluna de água com alterações dos níveis de actividade heterotrófica.

A reactividade bacteriana foi avaliada no campo, em diferentes interfaces estuarinas e foi também testada em laboratório. A degradação ectoenzimática de péptidos (actividade da leucina-aminopeptidase E.C. 3.4.11) e hidratos de carbono (actividade da ?-glucosidase E.C. 3.2.21), bem como parâmetros de caracterização da absorção de glucose (Vm e Tr), foram usados como descritores das respostas metabólicas das bactérias do plâncton.

Os perfis estuarinos de abundância bacteriana, absorção e incorporação de glucose e actividade ectoenzimática corresponderam globalmente a um padrão curvilíneo com máximos na gama de 20-30 UPS. Associada à transição entre os ambientes dulciaquícola e estuarino, registou-se uma alteração no padrão de utilização de substratos orgânicos traduzida pelo aumento da preponderância de proteína sobre hidratos de carbono, como substratos para o crescimento bacteriano, em relação directa com a proximidade do mar.

Os decréscimos de actividade do bacterioplâncton nas fronteiras com o rio e com o oceano foram mais pronunciados do que o esperado por diluição conservativa, confirmando assim a natureza reactiva do metabolismo bacteriano sujeito às condições naturais de instabilidade associadas a estas interfaces estuarinas.

As respostas de comunidades bacterianas naturais, obtidas em secções contrastantes do sistema estuarino, a variações de curto prazo das características da água foram testadas em laboratório com recurso a câmaras de difusão. O elevado grau de susceptibilidade das taxas de actividade bacteriana à modulação ambiental traduziu-se em variações rápidas e intensas dos níveis de actividade. Os resultados laboratoriais sugerem que o controle ambiental é exercido fundamentalmente através dos compostos dissolvidos. Não ficou claramente demonstrada a contribuição de partículas ressuspensas dos fundos sedimentares ou transportadas de zonas intertidais para a actividade heterotrófica das bactérias da coluna de água.

Embora os biótopos pouco profundos predominem no sistema estuarino da Ria de Aveiro, a maior parte da mineralização aeróbia da matéria orgânica ocorre na coluna de água. A troca de água entre o estuário e o oceano durante o ciclo de maré resulta num fluxo líquido de comunidades bacterianas e actividades associadas da Ria para o mar, com formação de uma pluma costeira de elevada biomassa bacteriana e potencial heterotrófico.

abstract

Estuarine bacterioplankton communities develop under unstable environmental conditions and respond to short-term or short-distance variations of water properties with changes in activity rates. Bacterial reactivity was evaluated in the field, at distinct estuarine boundaries, and further tested in the laboratory. The ectoenzymatic degradation of peptides (activity of the leucine-aminopeptidase E.C. 3.4.11) and carbohydrates (activity of the ?-glucosidase E.C. 3.2.21) and the parameters describing the uptake of glucose (Vm and Tr), were used as proxies for the assessment of bacterial metabolic responses.
Within the estuarine gradient, the profiles of bacterial abundance, glucose uptake and ectoenzymatic activity generally agreed with a curvilinear pattern with maxima within the 20-30 PSU range. At the transition from the limnetic environment to the main body of the estuary, a shift in the utilization of organic substrates occurred with an increasing importance of proteins over carbohydrates with decreasing distance to the sea.
Bacterioplankton activity decreased at both riverine and oceanic boundaries more than expected from conservative dilution, therefore confirming the reactive nature of bacterial metabolism at these highly unstable estuarine interfaces.
Diffusion chambers were used to test in the laboratory the responses of natural bacterial assemblages from distinct estuarine sections to short-term variations in water properties. Rapid and intense shifts between levels of activity confirmed the high degree of susceptibility of bacterial activity rates to environmental modulation and suggest that the control is mostly exerted through the dissolved pool of substrates.
The influence of sediment resuspension and of the input of particles from the intertidal zones on the rates of bacterioplankton activity in the water column could not be clearly demonstrated.
Although shallow biotopes account for the majority of the area corresponding to the complex estuarine system of Ria de Aveiro, most of the aerobic mineralization of organic matter occurs in the planktonic compartment. The tidal exchange of water between the estuary and sea results in a net seaward flux of bacterial communities and associated activities expressed by a coastal plume of enhanced bacterioplankton standing stocks and heterotrophic potential.

Bacterioplâncton da Ria de Aveiro: actividade ectoenzimática e metabolismo de monómeros

(Bacterioplankton ectoenzymatic activity and monomer uptake in the Ria de Aveiro)

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Justification and objectives

The dynamic behaviour of the estuarine environments is a long recognised attribute of these particular environments. The metabolic flexibility of the estuarine biota results in high rates of primary and secondary production (Cadeé, 1986; Sherr and Sherr, 1996). Estuaries have always been preferential settlement sites: (1) they are semi-enclosed natural harbours; (2) they effectively trap nutrients and are rich in sources of food; (3) they are natural transport centres connecting oceans and inland waters (Cronin, 1967). However, human activities may significantly change the organic matter loading, and physical factors, such as wind and water circulation, are capable of changing estuarine conditions over small temporal scales (Schulz Jr and Ducklow, 2000).

Coastal eutrophication is a problem that assumes a particular relevance in estuarine ecology. If the definition of estuary is extended to include all the transition zones between rivers and oceans, estuaries are the major contributors to the loading of open oceans with organic and inorganic materials (Martin and Gordeev, 1982). There is however, an increasing awareness of the modulating role of system-specific attributes that lead to marked differences in the sensibility of the distinct estuarine systems (Cloern, 2001). The understanding of estuaries as physical, chemical and biological filters (Morris *et al.*, 1995; Billen and Garnier, 1997; Cloern, 2001) enhances the importance of research programs that provide fundamental information on mechanistic models connecting biological processes and natural or human-related environmental variability.

The understanding of the importance of planktonic bacterial and viral communities of the Ria de Aveiro, either as indicators of sanitary quality or as mediators of biological processes of self-purification, justified the development of some preliminary investigation on the dynamics of bacterioplankton and viral indicators of environmental contamination: Research Project JNICT 87/122 – Geographic variation and seasonal fluctuation of bacterial populations of the Ria de Aveiro (1987-1990); Research Project INIC-2H - Geographic variation and seasonal fluctuation of bacterial populations of the Ria de Aveiro II. Effects of toxic pollutants (1991-1992); Research Project ENVIREG – Study of the virological situation of the Ria de Aveiro and of the impacts of urban sewage effluents.

The project Polaveiro - Microbiological and chemical quality of the water of Ria de Aveiro, corresponded to a combined effort of regular monitoring of biological and chemical indicators of water quality between 1989 and 1994. Even though the microbiological component of this study was focused on the evaluation of the seasonal and

geographic variability of bacterioplankton standing stocks and on the abundance of specific groups of bacterial indicators, it provided valuable information on the microbiology of the Ria and is still a reference for comparative analysis and assessment of anthropogenic impacts.

The contribution of bacteria to total planktonic respiration and secondary biomass production can vary significantly between different systems but their importance generally increases with increasing eutrophication and organic loading (Hoppe *et al.*, 1998). Bacterioplankton communities in the estuarine environment respond to changes in ambient temperature (Christian and Karl, 1995), salinity (Hoppe *et al.*, 1996), concentration of particles (Fuks *et al.*, 1991), light intensity (Pakulski *et al.*, 1998), and general nutritional environment (Rath *et al.*, 1993; Christian and Karl, 1995; Hoppe *et al.*, 1998). Bacterial responses to changing water properties are generally stronger in terms of total and specific activity rates than in terms of abundance (Chin-Leo and Benner, 1992; del Giorgio *et al.*, 1996; Hoppe *et al.*, 1996). Therefore, the high spatial and temporal variability within the estuarine environment should be expressed by marked shifts in the rates of biological processes mediated by the heterotrophic bacteria of the plankton.

The general objective of this work was the understanding of the mechanisms of reactivity of estuarine bacterioplankton to short time and/or short distance variability of environmental factors at the boundaries of the estuarine system of Ria de Aveiro. Processes of polymer hydrolysis and monomer uptake were taken as a proxy to the potential role of bacterioplankton in the turnover of autochthonous and allochthonous organic matter within the estuary.

The accomplishment of the general objective was attempted by specifically addressing the following questions:

a) What are the relative contributions of superficial sediments and overlaying water to the mineralization of organic matter in a shallow estuarine system?

b) How do the patterns of bacterioplankton degradation and recycling of organic matter respond to an estuarine gradient of changing environmental conditions?

c) Can bacterioplankton from extreme estuarine sections adapt to changing environmental conditions to the point of evolving into functionally distinct communities or are the rates of heterotrophic processes mostly modulated by water properties?

d) What is the contribution of particulate materials resuspended from bottom

sediments or washed out from the salt marshes to the reactivity of bacterial heterotrophic activities in the water column?

e) How do bacterioplankton communities respond to marine influences at the interface with the sea and in what way do these responses affect the patterns of exchanges between the estuary and the ocean?

CHAPTER I
INTRODUCTION

Bacterioplankton in the estuarine environment

Bacteria have been recognized as a part of the plankton for approximately a century. The initial emphasis of research was set on the determination of the numbers of organisms and on the identification and physiological characterization of species that grew in the laboratory. Latter techniques allowed a more realistic estimation of bacterial abundance and brought new interest into the role of planktonic bacteria in the degradation and recycling of organic matter and as food sources for higher levels of pelagic food chains. During the last 20 years, important information on the contribution of bacterioplankton to biogeochemical cycles in diverse environments has allowed a better understanding of the complex net of variables that intervene in the regulation of bacterial dynamics. Molecular techniques are now being used to characterize the large unculturable fraction of species in the bacterioplankton communities. The integration of knowledge on bacterial physiology gathered from laboratory approaches with information on community composition and diversity obtained through culture-independent techniques will probably, in a near future, provide new insights into regulatory factors of bacterioplankton abundance and activity in nature.

1. Bacterioplankton abundance and diversity

Bacteria are the least variable component of plankton in terms of both total density and biomass (del Giorgio *et al.*, 1996) contributing with approximately 20 % to total plankton biomass (Fuhrman *et al.*, 1980; Williams, 1984). However, the density of communities of marine and estuarine bacterioplankton can vary considerably between distinct environments.

The lower limit of the range of reported bacterial densities can be set by values below 10^9 cells l^{-1}, found in Arctic water (Ritzrau *et al.*, 1997), in Eastern Antarctica (Leaky *et al.*, 1996), in a cold temperate coastal Japanese lagoon (Sime-Ngando *et al.*, 1999) or in marine water layers below the photic zone (Kuipers *et al.*, 2000). At the upper limit of the

range, bacterioplankton densities above 10^{10} cells l^{1} have been observed in temperate estuarine systems (Shiah and Ducklow, 1994), tidal salt-marsh creeks (Shiah and Ducklow, 1995) and coastal lagoons (Barbosa, 1991; Hoppe *et al.*, 1996).

In open oceanic waters, the variation of bacterial abundance defines clear vertical patterns characterised by the decrease of the community density below the photic zone (Künnis, 1991; Ritzrau *et al.*, 1997; Kuipers *et al.*, 2000), or below the thermocline (Gast and Gocke, 1988) and, on the contrary, some increase within the chemoclines at the interface between oxic and anoxic waters (Gast and Gocke, 1988; Karrasch and Hoppe, 1991) and at the benthic boundary layer (Ritzrau *et al.*, 1997; Drake *et al.*, 1998).

In estuaries, longitudinal patterns of variation often develop corresponding to a seaward decrease of bacterial abundance. Estuarine maxima are more often found at the upper (Palumbo and Fergusson, 1978) or mid (Wright and Coffin, 1983) estuarine sections. Similarly, bacterial abundance within estuarine plumes tends to decrease with increasing distance from shore (Fuhrman *et al.*, 1980). Estuarine systems also exhibit strong short-term temporal fluctuations of bacterioplankton abundance associated to tidal effects and generally characterised by increasing density of the communities in low tide, relatively to high tide (Shiah and Ducklow, 1995; Hoppe *et al.*, 1996).

The major factors involved in the regulation of bacterioplankton standing stocks may vary significantly, both seasonal and geographically. Strong positive relations with phytoplankton biomass are often found in costal environments (Väätänen, 1980; Ritzrau *et al.*, 1997; Poremba *et al.*, 1999; Fandino *et al.*, 2001) but in estuaries bacterial growth may be uncoupled from phytoplankton biomass since nutrient supply is related to other sources rather than local primary production (Shiah and Ducklow, 1995; Hoppe *et al.*, 1996; Zdanowski and Figueiras, 1999).

Hydrographical variability driven by physical factors may significantly affect bacterial abundance. Zdanowski and Figueiras (1997) found that 60 % of the variability in the density of bacterial communities in surface waters of Ria de Vígo could be explained by physical factors such as solar radiation, water temperature, runoff or coastal upwelling. Temperature is probably the most referred controlling factor of bacterial standing stocks. The positive influence of increasing temperature on bacterial growth is expressed by increasing bacterial abundance during warm seasons (Kirstein, 1991) or by denser communities found above marine thermoclines (Künnis, 1991). Temperature limitation may even be stronger than nutrient limitation in the estuarine environment (Shiah and Ducklow, 1994; Shiah and Ducklow, 1995). The regulatory effect of temperature over community size is stronger on the free-living fraction whereas the proportion of particle-

attached cells is more related to the concentration of particulate matter (Unanue *et al*., 1992; Crump *et al*., 1998).

The effect of salinity on bacterial density in estuarine waters is sometimes referred as minor, compared to biological, physical and other chemical factors. However, negative relations between salinity and either total bacterioplankton abundance or, in particular, the particle-attached fraction, have been found in estuarine gradients (Väätänen, 1980; Valdés and Albright, 1981; Painchaud *et al*., 1987; Chin-Leo and Benner, 1992).

Biological factors such as grazing or viral lysis may also assume relevant roles in the regulation of bacterioplankton standing stocks. Increased grazing-related bacterial mortality has been found in cold marine waters during austral summer (Leakey *et al*., 1996) and at the lower limit of salinity gradients (Guixa-Boixareu *et al*., 1996) and the effects are more pronounced in the active fraction of the community, which is often coincident with the larger cell-size class (Gasol *et al*., 1995). Viral lysis has been cited as a mortality factor particularly important in the estuarine environment. It was found that aproximatly 40 % of estuarine bacteria are lysogenic and that lysis induction occurs more frequently in estuarine and coastal waters that in the offshore (Jiang and Paul, 1996).

Concomitantly with geographic and temporal variations in the total numbers of cells, it has been demonstrated that significant shifts in the composition of bacterioplankton communities may occur. Culture-independent approaches to the composition of communities, such as the analysis of 16S rDNA sequences or fluorescence *in situ* hybridisation with specific oligonucleotide probes (FISH), show that the structure of bacterial assemblages may reflect changes in water column stability, depth, season or trophic status (Donner *et al*., 1996; Murray *et al*., 1998).

The degree of bacterial diversity in estuarine environments is expected to be high due to the dynamic nature of these particular environments and to the inputs of colonised particles from various sources (Crump *et al*., 1999). Members of the essentially unculturable *Cytophaga-Flavobacterium* cluster compose one of the most abundant groups in marine ecosystems (Cottrell and Kirchman, 2000; Eilers *et al*., 2000; Pinhassi and Hagström, 2000). Contrastingly, members of the beta subclass of the *Proteobacteria* are mostly found in fresh water systems and members of the alpha and gamma subclasses of the *Proteobacteria* have been detected both in marine and in freshwater assemblages (Glöckner *et al*., 1999).

The variability in the composition of bacterioplankton communities over small horizontal distances was found to be minimal (Murray *et al*., 1998) but significant differences between communities were found over larger geographic scales (Lee and

Fuhrman, 1991; Murray *et al*., 1996; Hagström *et al*., 2000). Shifts in the composition of bacterioplankton communities have been related to seasonal fluctuations of abundance in coastal waters (Murray *et al*., 1998; Pinhassi and Hagström, 2000) and longitudinal profiles of bacterial abundance and activity along estuarine gradients (Murray *et al*., 1996). In vertical profiles along the water column, layers of increased turnover of organic matter are characterised by increased bacterial diversity (Höfle and Brettar, 1995) and marked shifts in community composition are also related to chemoclines (Donner *et al*., 1996; Ramsing *et al*., 1996).

Culture-independent techniques have also been used to compare the free-living and the particle-attached estuarine bacterial communities. The contribution of particle-attached cells to total abundance can be rather variable but usually below 10 % (Almeida and Alcântara, 1992; Unanue *et al*., 1992; Fandino *et al*., 2001) and the proportion increases at the estuarine turbidity maximum (ETM) section (Crump *et al*., 1998). High-resolution electrophoresis of low molecular weight RNAs (5S rRNA and tRNA) showed that the particle-attached and the free-living communities of bacterioplankton of Chesapeake Bay were different in composition (Bidle and Fletcher, 1995). A similar conclusion was redrawn from terminal restriction fragment length polymorphism analysis (T-RFLP) of bacterioplankton from Eastern Mediterranean (Moeseneder *et al*., 2001). Results have also demonstrated that the particle-attached estuarine bacterial communities are geographically more stable in composition than free-living assemblages, which include a large fraction of bacterial groups also found in coastal or riverine waters (Crump *et al*., 1999; Hollibaugh *et al*., 2000). In this scenario, particle-attached bacterioplankton would be more representative of the "true" estuarine communities than the more variable free-living fraction.

2. Bacterioplankton heterotrophic activity

2.1. Substrate sources for estuarine heterotrophic bacterioplankton

Estuaries are important sites for the degradation of terrestrial, riverine, oceanic and estuarine organic matter. Heterotrophic bacteria intervene in the processes of organic matter transformation within the planktonic compartment by performing two major functions: they respire organic compounds to inorganic forms and they incorporate organic matter into new bacterial biomass.

The nutritional environment for estuarine bacteria is chemically complex, spatially heterogeneous and subject to temporal and geographic variations. Although seasonal variations of bacterioplankton abundance and activity are sometimes correlated with phytoplankton standing stocks with a lag time of a few weeks (Jonas and Tuttle, 1990) autochthonous phytoplankton primary production is often insufficient to supply the potential demand of estuarine bacterioplankton for organic forms of carbon and nitrogen (Jugnia *et al.*, 1999; Bode *et al.*, 2001; Almeida* *et al.*, submitted).

During warm periods, amino acid incorporation (Rosenstock and Simon, 1993) and bacterial biomass production (Chin-Leo and Benner, 1992) may exceed planktonic primary production and under low productivity and high estuarine flushing conditions material derived derived from higher plants gains relevance (Otero *et al.*, 2000).

Findlay *et al.* (1992) estimated that the amount of allochthonous carbon necessary to support bacterial productivity at the Hudson River estuary (USA) would correspond to 3-6 times the net carbon fixation by phytoplankton. A similar carbon budget calculated for the Westerschelde estuary (The Netherlands) also shows a strong imbalance between auto- and heterotrophic processes with a yearly community respiration corresponding to 4-35 times the net production (Soetaert and Herman, 1995). However, experimental results indicate that phytoplankton-derived organic compounds are preferred as substrates for bacterial growth, producing the highest bacterial growth efficiencies (Cherrier *et al.*, 1996).

There is a general consensus around the preponderance of dissolved and polymeric substrates in aquatic environments (Chróst *et al.*, 1989; Chróst, 1991). The concentration of simple molecules that can directly enter bacterial cells is often in the pico-nanomolar range (Chróst, 1991; Keil and Kirchman, 1999). In estuaries, the concentration of total amino acids, peptides, proteins and simple carbohydrates ranges from < 10 µgC l^{-1} to several mgC l^{-1} (review by Pomeroy and Wiebe, 2001).

Polysaccharides account for approximately 50% of marine dissolved organic matter (DOM) in surface waters but the proportion is reduced to half this value below the photic zone (Benner *et al.*, 1992). Glucose may account for 50-80 % of the monosaccharide pool and supply for approximately 25 % of C-demand for bacterial biomass production in the ocean (Rich *et al.*, 1996). It is estimated that neutral sugars and amino acids account for 14% and 7% of marine DOM, respectively. These two classes of molecules are highly bioreactive and their selective removal from the water column by heterotrophic bacterioplankton may account for half the early consumption of organic carbon (Amon *et al.*, 2001).

2.2. Ectoenzymatic activity and monomer uptake

The general model of transference of organic matter to bacterial cells corresponds to a flux from polymeric to simple substrates that are later up taken by bacteria. The remote sources of DOM for heterotrophic bacterioplankton are particulate (algae, zooplankton, organic detritus, other bacterial cells) so, gradients of nutrient availability in aquatic environments are a common feature (Azam and Cho, 1987). These sources may provide substrates in a continuous manner or in pulses and the heterotrophic bacterioplankton must take advantage of gradients of nutrients and of intermittent periods of enhanced substrate inputs.

Since only low-molecular-weight (LMW) compounds and simple molecules can be directly transferred from the environment into the cells, the first step of the DOM-bacteria pathway is dependent upon bacterial hydrolytic enzymes. Ectoenzymes develop their activity as close as possible to permeases in order to take maximum advantage of monomer-rich micro zones surrounding intense polymer degradation sites. Several experimental works have demonstrated that bacterial hydrolytic enzymes are physically bound to the cell surface or occur in the periplasmic space of gram-negative bacteria. Martinez and Azam (1993) found that approximately 30 % of aminopeptidase activity in a marine bacterium was associated to the cell-surface and 70 % was periplasmic. This is consistent with a model in which surface enzymes directly react with polymers and generate oligomers that diffuse into the periplasm. Further hydrolysis occurs in a high ectoenzymatic activity environment where monomers may directly interact with substrate-binding proteins or with permeases (Martinez and Azam, 1993). Extracellular degradation of organic compounds has important implications in the processing of particulate substrates because the conversion of particles into solutes prevents substrates sources from sinking and being lost from the water column and makes them inaccessible to particle feeders (Azam and Cho, 1987).

Coupling polymer hydrolysis and monomer uptake allows bacterioplankton to take maximum profit from punctual sources o DOM. This coupling is particularly tight in oligotrophic environments or during low productivity periods and the ratio between uptake and hydrolysis rates provides a measure of the pool size ratio between monomers and polymers (Hoppe *et al.*, 1988). Considering that the relative importance of polymers increases with increasing eutrophication, uncoupling between hydrolysis and uptake can

occur during spring blooms (Middelboe *et al.*, 1995) or in highly eutrophied coastal ecosystems (Hoppe *et al.*, 1988).

The co-existence of high and low affinity hydrolytic and uptake enzymatic systems is one important strategy in the interaction of bacterioplankton and organic matter because it provides bacterioplankton communities with the metabolic flexibility to utilize low bulk-phase concentrations of substrates and high nutrient concentrations in the vicinity of discrete sources of DOM (Hollibaugh and Azam, 1983; Tholosan *et al.*, 1999; Unanue *et al.*, 1999). The maintenance of distinct enzymatic systems allows rapid responses to shifts in substrate availability that are expressed by changes in the levels of per-cell activity rates (Wehr *et al.*, 1999). Transition between systems occurs at different concentration ranges for different enzymes and is more pronounced for hydrolytic than for uptake systems (Unanue *et al.*, 1999). Studies of biphasic kinetics in natural bacterioplankton assemblages show that the coupling between hydrolysis and uptake observed under low ambient concentrations of substrates corresponds to situations of high affinity hydrolytic ectoenzymes and low affinity uptake systems operating simultaneously (Unanue *et al.*, 1999).

2.3. Environmental regulation of bacterial activity

Environmental factors interact in the regulation of bacterioplankton activity in such a way that it is often difficult to discriminate between individual effects and to assess their relative contribution.

Substrate availability and temperature interact in all bacterial communities, at different temperature and substrate concentration ranges (Psenner and Sommaruga, 1992; Jugnia *et al.*, 1999; Pomeroy and Wiebe, 2001).

Bacteria respond to changes in organic matter availability by shifting between levels of activity. As a general trend, increasing rates of bacterial biomass production, ectoenzymatic activity and uptake rates have been extensively reported during phytoplankton blooms (Chróst *et al.*, 1989; Chróst, 1991; Middelboe *et al.*, 1995), in the proximity of particulate sources of DOM (Martinez *et al.*, 1996; Ritzrau *et al.*, 1997; Tholosan *et al.*, 1999; Grossart and Ploug, 2001) or along gradients of increasing eutrophication (Rath *et al.*, 1993; Christian and Karl, 1995; Hoppe *et al.*, 1998).

Experimental addition of organic matter to bacterioplankton communities shows that responses are not only related to the concentration of available organic carbon but also to

the quality of substrates. Terrestrial detritus were found to be relatively refractory whereas algal-derived material produces significant stimulation of growth (Wehr *et al.*, 1999). Dissolved substrates produced stronger stimulation than particulate material of identical sources (Cherrier *et al.*, 1996; Ferrier-Pagès *et al.*, 2000).

Monomer incorporation and ectoenzymatic activity measure different cellular processes. Uptake of simple substrates involves membrane transport and intracellular metabolic functions and is interpreted as an indicator of growth. Ectoenzymatic activity measures the expression of cell-bound enzymes and the potential for the hydrolysis of polymeric substrates that ultimately reflects the spectra of recent substrates to which bacteria have been exposed. Therefore, additions of simple substrates may trigger distinct responses of these two metabolic indicators. Additions of monosaccharides and amino acids stimulate monomer uptake (Berman *et al.*, 1994) but decrease ectoenzymatic activity rates either by end-product inhibition or by catabolic repression (Chróst, 1991).

In the estuarine environment bacterial growth is generally not limited by carbon availability but rather by nitrogen (Chin-Leo and Benner, 1992). In oligotrophic waters bacteria compete with phytoplankton for dissolved inorganic nitrogen (DIN) whereas in eutrophic waters they mineralise dissolved organic nitrogen (DON) to DIN (Jørgensen *et al.*, 1999). Dissolved free amino acids and ammonia are the dominant N sources for bacterial growth (Jørgensen *et al.*, 1999; Middelburg and Nieuwenhuize, 2000) but the hydrolysis of dissolved combined amino acids (DCAA) coupled to amino acid uptake can support approximately 50% of bacterial nitrogen demand and 25% of the carbon demand in estuarine and coastal waters (Keil and Kirchman, 1999).

The regulatory effect of temperature on bacterial metabolism may prevail over nutrient supply during the cold season (Shiah and Ducklow, 1995) or in stratified environments (Patel *et al.*, 2000) and generally bacterial activity rates are positively correlated with temperature (Christian and Karl, 1995; Simon and Wünsch, 1998; Patel *et al.*, 2000). One important indirect effect of temperature is that it affects the affinity of enzymatic systems. Under low temperature the affinity of enzymatic systems decreases significantly and labile substrates may accumulate in the environment because they become less bioavailable (Zweifel, 1999; Pomeroy and Wiebe, 2001).

Salinity generally correlates poorly with bacterioplankton metabolic activity measures in estuarine bacterioplankton (Murrell *et al.*, 1999) and since in estuaries salinity gradients also correspond to trophic gradients, individual effects become difficult to interpret. A relatively high salinity optimum (30 PSU) was reported for ?-glucosidase (E.C. 3.2.21) activity in intertidal sediments (King, 1986) but the potential activity of the

same ectoenzyme was negatively related to salinity in coastal waters (Murrell *et al.*, 1999). The relation between leucine-aminopeptidase (E.C. 3.4.11) activity and salinity in coastal systems was found to be positive in some environments (Murrell *et al.*, 1999) and negative in others (Hoppe *et al.*, 1996; Pattel *et al.*, 2000). Strong decreases of monomer uptake with increasing salinity have also been observed (Valdés and Albright, 1981; Hoppe *et al.*, 1996).

Light is generally not among the major factors controlling estuarine bacterioplankton activity rates. However photo inhibition of thymidine and leucine uptake was observed in a shallow coastal system (Pakulski *et al.*, 1998). Contrastingly, the photodegradation of recalcitrant DOM into more labile compounds has been related to the stimulation of the growth of marine bacterioplankton populations (Benner and Biddanda, 1998). Light intensity also influences rates of primary production and therefore causes indirect effects on the availability of substrates for bacterial growth and influences the outcome of the competition between phyto- and bacterioplankton for inorganic nutrients (Sanford *et al.*, 2001). These indirect effects of light and the concomitant variations in the concentration of organic and inorganic nutrients may originate diel rhythms of variation of bacterial activity rates in environments where primary production and bacterial organic matter utilization are tightly coupled (Gasol *et al.*, 1998; Kormas *et al.*, 1998; Kuipers *et al.*, 2000).

2.4. Bacterioplankton dynamics at estuarine boundaries

The water column of estuarine systems is physically and functionally connected to the terrestrial, riverine and oceanic environments and establishes also tight relations with intertidal margins. Bacterioplankton experiences spatial and temporal variations according to the intensity of environmental pressure at these interfaces.

The benthic boundary

Resuspension of bottom sediments caused by tidal currents, wind-induced waves or anthropogenic disturbance is an important process of exchange of materials between the benthic and the planktonic compartments in shallow coastal systems (Demers *et al.*, 1987; Trousselier *et al.*, 1993; Sloth *et al.*, 1996).

The susceptibility to resuspension is subjected to seasonal and geographic fluctuations in relation to sediment texture and microphytobenthic colonization (de Jonge and van den Bergs, 1987). Experimental results show that the critical current speed

increases considerably in fine sediments highly colonised by diatoms when compared to sandy sediments with low microphytobenthic biomass (Lucas *et al.*, 2000).

Pulses of nutrients associated to resuspension events result in increased rates of heterotrophic activity of bacteria in the adjacent water column. Experimental disturbance of benthic sediments in either flume or mesocosm experiments produced significant increases of bacterial abundance, biomass productivity and potential ectoenzymatic activity (Wainright, 1987; Chróst and Riemann, 1994; Sloth *et al.*, 1996).

The superimposition of tidally-induced sediment resuspension, salinity-related flocculation of riverine materials and density gradients, results in the maintenance of suspended particles inside the estuaries and in the occurrence of estuarine turbidity maxima (Vale and Sundby, 1982; Brenon and Le Hir, 1999). These estuarine sections are characterised by high rates of bacterial activity (Fuks *et al.*, 1991) and by an increase in the contribution of particle-attached bacteria to total activity rates (Griffith *et al.*, 1990; Crump *et al.*, 1998; Hollibaugh and Wong, 1999; Murrell *et al.*, 1999). Attachment to particles also prevents estuarine planktonic bacteria from being washed out of a favourable nutritional environment (Murrell *et al.*, 1999).

Processes of transference between sediments and the water column under low current velocities are mostly driven by salinity gradients and involve exchanges of solutes rather than particulate materials. Differences of salinity between interstitial and overlaying water cause the density displacement and advection of solutes from estuarine sediments into the water column (von Bodungen *et al.*, 1995; Webster *et al.*, 1996). Bacterioplankton growing in water experimentally exposed to sediments showed higher biomass production rates and the stimulation corresponded to a net flux of DOM from the sediments to the water column (Hopkinson *et al.*, 1998; Middelboe *et al.*, 1998). Field results also show that bacterial abundance and activity at the water layer directly above the sediment are higher that at the intermediate layer in open ocean waters (Ritzrau *et al.*, 1997) or than in surface waters of shallow estuarine systems (Goosen *et al.*, 1995) suggesting that benthic-derived substrates may fuel heterotrophic activity in the water column.

The fresh-water boundary

Variations of the fresh-water fluxes are one of the major factors of seasonal control of estuarine biological activity (Cloern and Nichols, 1985). High inputs of particulate materials occur during periods of elevated fresh-water fluxes (Otero *et al.*, 2000) and the nutritional quality of seston (Murrell *et al.*, 1999), as well as the contribution of

phytoplankton and bacterial biomass to total particulate organic carbon (POC), (Hollibaugh and Wong, 1999) decrease during dry periods. Plant-derived material transported by rivers may assume a dominant role as source of organic carbon for estuarine heterotrophs during periods of low autochthonous production (Otero *et al.*, 2000). Fresh-water flow, taken as a proxy for organic matter flux, was positively correlated with several bacterioplankton metabolic indicators such as ectoenzymatic activity, monomer uptake, growth rates and growth efficiency (Benner *et al.*, 1995; Murrell *et al.*, 1999).

Upper sections of estuarine systems also receive inputs of fresh-water bacterial communities. Although their growth may experience some stimulation at the upper estuarine sections, the increase of salinity causes sharp decrease of monomer uptake (Valdés and Albright, 1981) and growth rates (Painchaud *et al.*, 1995).

The inter-tidal boundary

Salt marshes and mud-flats are generally considered as estuarine compartments of enhanced primary production (Buzzelli *et al.*, 1999; Serôdio and Catarino, 2000) and respiration rates (Taylor and Allanson, 1995; Cai *et al.*, 1999). Exchanges of materials between the estuarine margins and the water column result from cyclic flooding and exposure of sediments in relation to tidal currents.

Early attempts to assess the relative contribution of salt-marshes as sources of organic matter to adjacent coastal waters led to the formulation of the "outwelling hypothesis" according to which considerable amounts of materials would be exported from the salt-marshes to the main estuarine water body (Odum, 1968; Dame *et al.*, 1986). More recently, the exchange of particulate forms of organic carbon and dissolved forms of inorganic nitrogen between the littoral zone and main estuarine channel has been demonstrated in shallow estuarine ecosystems (Buzzelli *et al.*, 1999). However, salt-marshes are found to act predominatly as sinks for particulate matter whereas they export DOM forms (Taylor and Allanson, 1995). When respiration rates are high, the exports to the water column are dominated by inorganic respiratory products (Cai *et al.*, 1999) and inorganic forms of nitrogen and phosphorus that are used by phyto- and bacterioplankton (Heinle and Flemer, 1976).

The ocean boundary

The oceanic influences on the total size of bacterioplankton communities and on the

activity rates are often related to patterns of variation with distinct periodicity. The decrease in abundance and activity of estuarine bacterioplankton during the flood-tide is a feature of tidal estuaries (Kirchman *et al.*, 1984; Hoppe *et al.*, 1996; Cunha *et al.*, 2000). However, field results often show that the tidal-associated variations of abundance and activity within the bacterial communities of the water column cannot be fully explained by conservative dilution effects suggesting that bacterial cells react also in terms of the levels of individual activity (Hoppe *et al.*, 1996; Cunha *et al.*, 2000).

Fortnight cycles of spring-neap tides affect the vertical mixture of the water column and the longitudinal extension of the estuarine turbidity maximum (Murrell *et al.*, 1999), therefore influencing the supply or organic and inorganic nutrients and the relations between phytoplankton and bacterioplankton biomass production.

Seasonal variations on the direction and intensity of coastal winds and on oceanic upwelling conditions also shape biological gradients at the estuary-ocean boundary (Cloern and Nichols, 1985). Coastal upwelling was positively associated with the colony-forming bacterioplankton fraction (Zdanowski and Figueiras, 1999) and since it changes the degree of mixing of the water column and the supply of nutrients, upwelling may shift the limitations of bacterioplankton growth from organic to inorganic sources (Kirchman *et al.*, 2000).

Estuarine plumes correspond to estuarine-ocean interfaces of elevated standing stocks of phyto- and bacterioplankton (Malone and Ducklow, 1990) as well as intense heterotrophic activity (Robertson *et al.*, 1998; Jørgenson *et al.*, 1999; Pakulski *et al.*, 2000). Bacterial activity along estuarine gradients and transition zones considerably alters the size and quality of the DOM pool that ultimately reaches oceanic waters (Raymond and Bauer, 2000).

The specific biological attributes of the marine-influenced lower sections of estuarine systems and the dynamics of heterotrophic bacterioplankton within the plume zone may act as filters and considerably attenuate the effects of coastal eutrophication (Morris *et al.*, 1995; Billen and Garnier, 1997; Cloern, 2001).

CHAPTER II
COMPARTMENTS OF OXYGEN CONSUMPTION IN A TIDAL MESOTROPHIC ESTUARY (RIA DE AVEIRO, PORTUGAL)

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Abstract - Oxygen consumption rates were determined, in parallel with primary production and bacterial biomass production, as an approach to the analysis of carbon cycling in the estuarine community of the Ria de Aveiro. The water column of the marine zone was the major contributor (64-99 %) for the total aerobic carbon remineralisation in which O_2 uptake rates averaged from 80 to 127 $mgO_2m^{-2}h^{-1}$, respectively at low tide and high tide. The planktonic consumption of O_2 varied from 0.010 to 0.041 $mg\,O_2l^{-1}h^{-1}$ with the highest values in the brackish zone. Small water column depths in this zone reduced, however, the integrated average consumption of the plankton, per surface unit, to 57 % (LT) and 66 % (HT) of that observed in the marine zone. Benthic O_2 consumption rates, 5.1 to 22.0 $mgO_2m^{-2}h^{-1}$, were 2 to 4 times higher in the brackish zone when compared to the rates in the marine zone. It represented 1 to 31% of the total surface integrated values in different areas and at different tides. From the ratios of the primary production and bacterial biomass production, on a per surface unit basis, it is concluded that, in late autumn, the Ria de Aveiro was mostly an heterotrophic system with a feeble recover of primary production at HT in the marine zone and at LT in the brackish water zone.

Key words: estuary, oxygen consumption, bacterial respiration, sediment respiration, bacterial productivity, primary production.

1. Introduction

In estuarine systems, autochthonous and allochthonous organic matter are transported and deposited in different geographical patterns, stimulating respiration in the water column and in the benthos. The partition of the aerobic metabolism among the different spatial compartments and physiological groups in the community is an important tool for the evaluation of the estuarine carbon cycle.

The sediment-water interface is a site of intense heterotrophic activity where nutrients are generated, percolated into the sediment and diffused out to the water column. It gives support to bacterial populations, which are 2-3 orders of magnitude more dense than in the water column (Alcântara *et al*., 1996).

The determination of the metabolic pattern of an ecosystem on the basis of oxygen uptake is relatively simple (Hargrave, 1969; Fallon and Boylen, 1990) and can be directly applied to the water column and to the sediments, at *quasi in situ* conditions (Gocke *et al*., 1981; Roos and Eriksen, 1995). Although the Winkler titration method is criticised, particularly when used in cases of low rates of respiration (Pamatmat, 1997), the method is still considered valuable in a community approach.

Community respiration represents the transfer of carbon between the organic and inorganic reservoirs of a system. The subtraction of bacterial respiration from autotrophic production, gives a direct estimate of the organic carbon available for export in the system or incorporation by non-bacterial consumers (Yahnke and Craven, 1995). Community respiration defines limits to the overall partition of bacterial metabolism of dissolved organic carbon in secondary production of biomass and aerobic respiration (Yahnke and Craven, 1995).

The system variation in the rate of O_2 consumption involves seasonal fluctuation, with maxima in summer, of the respiration rate of planktonic bacteria as was observed in the continental shelf (Grifith and Pomeroy, 1995). Estuarine sediments also show mineralization seasonality (Hargrave, 1969; Mallo *et al.*, 1993) due to changing temperature and organic loading variation, but this fluctuation is not common to other marine systems, specially to those affected by low concentrations of dissolved oxygen (Conley *et al*., 1997). Positive effects of benthic bioturbation on the rate of O_2 consumption are frequent and probably due to activation of the bacterial community (Hansen and Kristensen, 1997).

Aerobic respiration in coastal sediments is limited by oxygen penetration due to the resistance of the diffusive boundary layer to the flux of O_2 (Yørgensen and Revsberg, 1985). The penetration is variable with the composition of the sediment but, generally, it is restricted to a few millimetres.

The profiles of respiratory and production activities in estuaries are shaped by many environmental factors including those related to the short-term tidal changes in water properties.

This work intends to establish preliminary values for the rates of biotic mineralisation and carbon fixation in the marine and brackish water zones of the Ria de Aveiro. It is also directed to the determination of the relative contributions of the different compartments, namely plankton and benthos, to the total oxidation of organic carbon within this ecosystem.

2. Material and methods

Samples were collected in 1997/98 along two transects defined one in the marine zone at the main navigation channel (transect N1) and other in the inner brackish water section of the estuary (transect I6) (Fig.1). Transect I6 was visited on the 28th October (low tide) and on the 7th November (high tide). Samples from transect N1 were collected on the 16th December (low tide) and on the 9th January (high tide). All samples were collected under spring tide conditions.

Along each transect 5 equidistant subtidal stations were defined and further identified by their relative position: stations South, South-Centre, Centre, North-Centre and North at transect N1 and stations West, West-Centre, Centre, East-Centre and East at transect I6.

For the determination of the rate of oxygen consumption two cores of sediment plus overlaying water were collected by scuba divers from each station directly into cylindrical respiration chambers (14 cm in diameter and 8 cm high) similar to those used by Gocke *et al*. (1981). The closed chambers were immediately immersed in water from the station and transported to the laboratory. Water from the bottom layer of the water column (0.5 m from sediment surface) was collected on boat with a Niskin bottle for later adjustments and preparation of the respiration chambers. In the laboratory the water from the station was allowed to flow through the chambers before taking the first sample for determination of the initial oxygen concentration. The completely filled chambers were sealed and incubated at *in situ* temperature for 5-7 hours. BOD bottles were also filled with bottom water for determination of planktonic respiration. Total aerobic respiration (sediment plus water oxygen consumption per m^2) was calculated from the values of respiration (per volume) in the

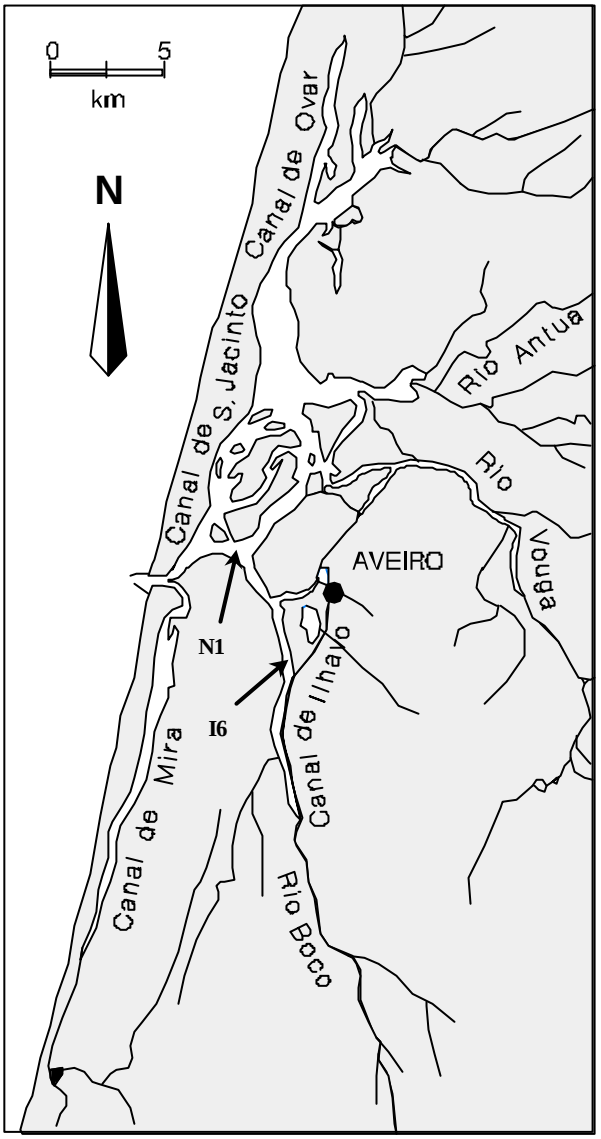

Figure 1: Ria de Aveiro, a coastal estuarine ecosystem. Sampling transects (N1, marine zone and I6, brackish zone) are indicated with arrows.

chambers and in the water bottles, taking into account the total depth of the station, and the surface area of the sediment in the chamber.

The sediments used for respiration assays were later used for granulometric analysis after treatment with 100 V hydrogen peroxide and 20 % sodium pyrophosphate, according to the categories used by Rodrigues and Quintino (1985). Organic matter content was determined through loss of dry weight after 48 hours treatment with 100 V hydrogen peroxide.

For the determination of physical, chemical and microbiological parameters of the water column, only the 3 stations corresponding to the margins and to the centre of the channel were studied. At transect N1 (total depth of 2.0-20.6 m at HT and 2.5-12.3 m at LT) the water samples were collected at surface (0.2 m from surface) and bottom (0.5 m from de sediment-water interface) layers of the water column but at the shallower transect I6 (total depth of 1.7-3.0 m at HT and 0.3-1.8 m at LT), bottom samples were worth collecting only at the centre of the channel.

Bacterial biomass productivity was determined according to Simon and Azam (1989) after addition of a saturating concentration of 30 nM ^{3}H-leucine. Samples were incubated at *in situ* temperature for 1 hour. The metabolism of glucose was determined by the radioisotopic technique (Gocke, 1977) with a tracing concentration of ^{14}C-glucose (43 nM) and after 2 hours of incubation at *in situ* temperature. Percentage of glucose respiration was calculated as the ratio between the respired fraction and the total uptake of glucose (sum of respired and incorporated fractions). Primary production was determined by incorporation of ^{14}C-bicarbonate (Steemann-Nielsen, 1952) in simulated *in situ* conditions. All radioactive substrates were purchased from Amersham.

Salinity, temperature and dissolved oxygen were determined in the field with WTW equipment.

3. Results

A brief characterisation of the marine and brackish-water zones of the Ria de Aveiro can be derived from the values of salinity, temperature, dissolved oxygen, granulometry and organic content of the sediments at, respectively, transects N1 and I6 (Tables 1 and 2). In the marine zone, the average values of salinity at the surface water for the 3 sampling stations of the transect were 33.3 UPS at HT and 23.0 UPS at LT, while at the brackish-water zone the corresponding values were 27.4 and 19.1 UPS. Temperature values determined at transect I6 were higher than at transect N1. Temperature at surface (average values from 3 stations in each transect) was, at

transect N1, 14.9 ºC (HT) and 12.0 ºC (LT) and at transect I6 16.0 ºC (HT) and 18.6 ºC (LT). Oxygen saturation ranged from 72 to 102 % at transect N1 and from 44 to 85 % at transect I6 (Figures 2D and 3D).

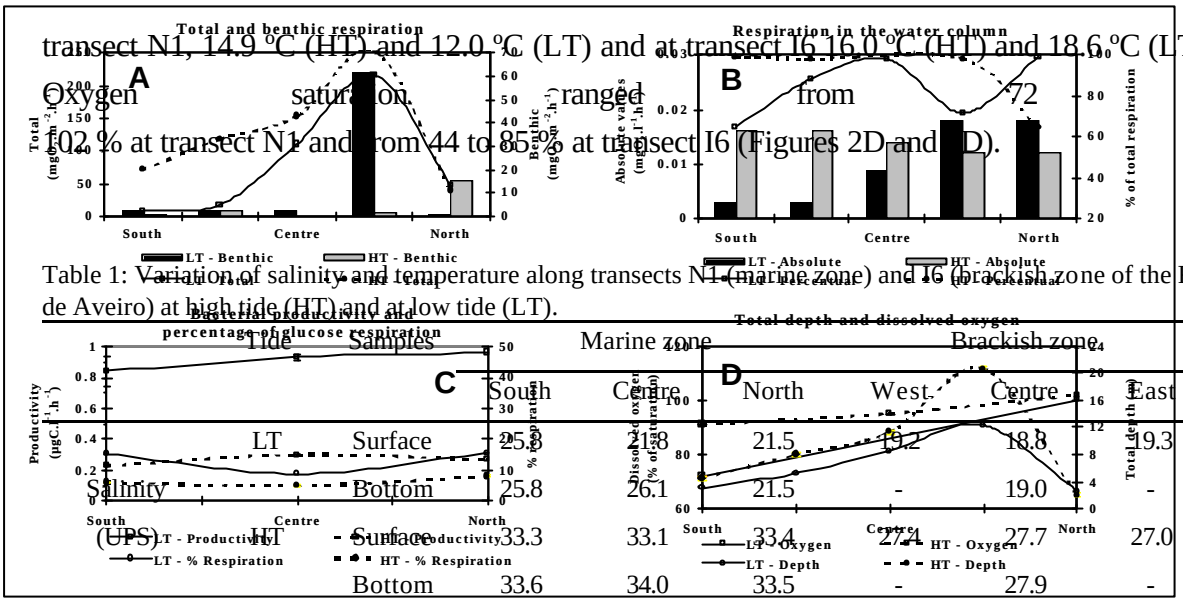

Table 1: Variation of salinity and temperature along transects N1 (marine zone) and I6 (brackish zone of the Ria de Aveiro) at high tide (HT) and at low tide (LT).

	Tide	Samples	Marine zone			Brackish zone		
			South	Centre	North	West	Centre	East
Salinity (UPS)	LT	Surface	25.8	25.8	21.5	19.2	18.8	19.3
		Bottom	25.8	26.1	21.5	-	19.0	-
	HT	Surface	33.3	33.1	33.4	27.4	27.7	27.0
		Bottom	33.6	34.0	33.5	-	27.9	-
Temperature (ºC)	LT	Surface	12.7	11.2	12.1	18.6	18.5	18.6
		Bottom	12.1	12.7	12.1	-	18.5	-
	HT	Surface	14.8	14.9	14.9	16.0	16.0	16.1
		Bottom	14.8	14.9	14.9	-	16.0	-

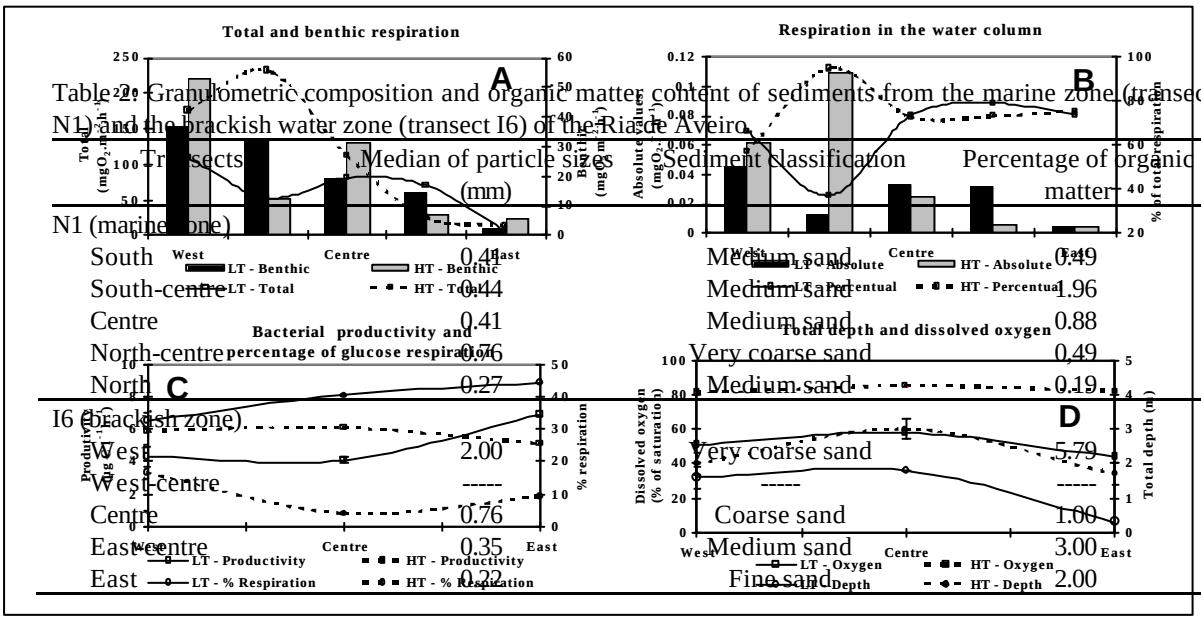

Table 2: Granulometric composition and organic matter content of sediments from the marine zone (transects N1) and the brackish water zone (transect I6) of the Ria de Aveiro.

Transects	Median of particle sizes (mm)	Sediment classification	Percentage of organic matter
N1 (marine zone)			
South	0.48	Medium sand	0.49
South-centre	0.44	Medium sand	1.96
Centre	0.41	Medium sand	0.88
North-centre	0.76	Very coarse sand	0,49
North	0.27	Medium sand	0.19
I6 (brackish zone)			
West	2.00	Very coarse sand	5.79
West-centre	-----	-----	-----
Centre	0.76	Coarse sand	1.00
East-centre	0.35	Medium sand	3.00
East	0.22	Fine sand	2.00

Figure 2: Variation of respiration, bacterial biomass productivity, percentage of glucose respiration, dissolved oxygen and total depth along transect N1 (marine zone of the Ria de Aveiro), determined at high tide (HT) and low tide (LT). (A) total respiration (sediment and water column) and benthic respiration (sediment); (B) respiration in the water column expressed as absolute values (per volume) and as a percentage corresponding to the contribution of the water column for total respiration (sediment and water column); (C) bacterial biomass productivity and percentage of glucose respiration in the water column (0.5 m above surface); (D) dissolved oxygen in the water (0.5 m above surface) and total depth.

Figure 3: Variation of respiration, bacterial biomass productivity, percentage of glucose respiration, dissolved oxygen and total depth along transect I6 (brackish zone of the Ria de Aveiro), determined at high tide (HT) and low tide (LT). (A) total respiration (sediment and water column) and benthic respiration (sediment); (B) respiration in the water column expressed as absolute values (per volume) and as a percentage corresponding to the contribution of the water column for total respiration (sediment and water column); (C) bacterial biomass productivity and percentage of glucose respiration in the water column (0.5 m above surface); (D) dissolved oxygen in the water (0.5 m above surface) and total depth.

Transect N1 is included in the deeper zone of the Ria with maximal HT water depth values of 20.6 m at the north-centre station. The average water depth (considering 5 stations) at this transect was, however, 9.3 m at HT and 6.3 m at LT. Average depth (from 5 sampling stations) at transect I6 was 2.2 m at HT and 1.2 m at LT.

The granulometry of the sub-tidal sediments of the two transects did not differ drastically (Table 2). The coarser type of sediment (very coarse sand) was found at the north-centre station of transect N1 and at the west station of transect I6 and the finer (fine sand) correponded to the east margin of transect I6. The organic matter content of the sediments was higher at transect I6 where it reached an average value (5 stations) of 2.9 % of the dry weight, compared to 0.8 % in transect N1.

At transect I6, the aerobic respiration of the benthos was more intensive and quite stable in contrasting tidal conditions. The average values of O_2 consumption were 22.0 and 21.3 $mgO_2m^{-2}h^{-1}$ at HT and LT, respectively. In the marine zone the average values of respiration in the sediment were 5.1 and 13.8 $mgO_2m^{-2}h^{-1}$, respectively at HT and LT.

Table 3: Variation of primary production (PP), total respiration (R) and bacterial biomass production (BBP) along transects N1 (marine zone) and I6 (brackish zone) of the Ria de Aveiro at high tide (HT) and at low tide (LT) expressed as $mgCm^{-2}h^{-1}$. An RQ of 0.85 was assumed for converting respiration (R) values from O_2 consumption to carbon released.

Transect	Tide	Parameter	South	Centre-south	Centre	Centre-north	North	Transect average
	HT	PP	1.9	-	1.5	-	1.0	1.5
		R	23.0	38.4	48.9	62.3	12.8	28.2
		BBP	0.4	-	4.1	-	0.5	0.8
N1	LT	PP	3.8	-	5.3	-	1.3	3.5
		R	2.4	5.7	35.8	70.5	15.1	17.8
		BBP	2.3	-	6.7	-	2.6	3.9
			West	Centre-west	Centre	Centre-east	East	
	HT	PP	4.5	-	5.0	-	2.4	4.0
		R	57.2	75.5	36.6	8.2	4.6	32.8
		BBP	11.8	-	17.7	-	18.9	16.1

I6	LT	PP	2.9	-	6.0	-	-	4.5
		R	35.5	17.0	26.2	22.9	2.1	21.3
		BBP	6.9	-	6.1	-	2.1	5.0

Planktonic respiration values are shown in Figures 2B and 3B. The averages of O_2 consumption at transect I6 were 0.030 and 0.041 $mgO_2l^{-1}h^{-1}$, respectively at LT and HT. In the marine zone the values decreased to 0.010 and 0.014 $mgO_2l^{-1}h^{-1}$, at corresponding tides.

Taking into consideration the total depth of the water column at each station, the calculations show that the respiration in the planktonic compartment amounted, in average and as a fraction of total consumption, to 69 % in the brackish zone and to 87 % in the marine zone (Figures 2B and 3B).

Primary production showed to be more intense at the brackish water zone. Average values at transect I6 were 4.0 and 4.5 $mgCm^{-2}h^{-1}$ respectively at HT and at LT while at transect N1 the average values decreased to 1.5 and 3.5 $mgCm^{-2}h^{-1}$ at corresponding tidal conditions (Table 3).

The values of bacterial biomass productivity and of the fraction of respired glucose indicate the occurrence of higher levels of bacterial activity at the brackish water transect (Figures 2C and 3C). The average values of bacterial biomass productivity at the surface of the water column across this transect (average of 3 stations) were 5.1 and 5.7 $\mu gCl^{-1}h^{-1}$, respectively at LT and HT. The respired fraction of the absorbed glucose corresponded to 10.0 and 39.2 % in the corresponding tides. The highest rates of bacterial activity at the transect N1 were observed at LT. The average bacterial biomass productivity at the surface of the water column was here 0.9 $\mu gCl^{-1}h^{-1}$ at LT and 0.3 $\mu gCl^{-1}h^{-1}$ at HT. Glucose respiration determined on surface samples amounted to 13.2 % of the total uptake at HT and to 5.9 % at LT.

4. Discussion

The lack of detailed bathymetric and hydrological data on the Ria de Aveiro, as well as of a more extensive work on the activity of the community, precludes more precise calculations of the integrated values of biological activity throughout this system. Even so, the results already obtained in this work, regarding the marine and brackish water zones, allow a first approach to the geographical and compartment distribution of carbon cycling in this system.

The rate of oxygen consumption determined, in a per volume unit basis, in the water column of the Ria de Aveiro varied from 10 to 41 $\mu gO_2l^{-1}h^{-1}$ (Table 4), values which are within the range of results published for marine systems. These include a range of 35-47 $\mu gO_2l^{-1}h^{-1}$ in the water of mangrove swamps (Gocke *et al*., 1981) and of 2.7 – 13.3 $\mu gO_2l^{-1}h^{-1}$ in marine water (Kruse, 1993). The values of total consumption of O_2 observed in the water column of the Ria de Aveiro range from 2 to 247 $mgO_2m^{-2}h^{-1}$ (Table 4) and from values of 48 to 5928 $mgO_2m^{-2}d^{-1}$. These high respiration values, indicating eutrophication conditions, are similar to the range of 9 to 289 $mmolO_2m^{-2}d^{-1}$ (288-9248 $mgO_2m^{-2}d^{-1}$) reported by Caffrey *et al*. (1998) during a spring phytoplankton bloom in south San Francisco Bay.

Table 4: Data relative to the compartments of oxygen consumption in the subtidal area of the Ria de Aveiro (autumn/winter 1997/98).

	Marine zone[1]		Brackish zone[1]		Ria de Aveiro	
					Weighted average [2]	
	HT	LT	HT	LT	HT	LT
1.Maximum and minimum depth of the water column (m)	22-6	20-4	6-1	4-0.5	-	-
2.Consumption of O_2 in the water column ($mgO_2l^{-1}h^{-1}$)	0.014	0.010	0.041	0.030	0.035	0.023
3.Total consumption of O_2 in the water column ($mgO_2m^{-2}h^{-1}$)	2-247 (122)	9-153 (67)	9-220 (89)	8-72 (44)	96	52
4. Thickness of aerobic layer of sediment (m) [3]	0.04		0.01		-	-
5.Consumption of O_2 in sediment (average of five points along the transect) ($mgO_2m^{-2}h^{-1}$)	5.1	13.8	22.0	21.3	18.3	18.8
6. Consumption of O_2 in sediment per unit of volume ($mgO_2l^{-1}h^{-1}$)	0.127	0.350	2.200	2.130	1.744	1.604
7. Amplitude of total consumption of O_2 per unit of surface ($mgO_2m^{-2}h^{-1}$)	71-253 (127)	7-217 (80)	14-232 (112)	6-109 (64)	115	70
8. Percentual consumption of O_2 in water column	64-99 (90)	64-99 (84)	57-95 (71)	37-79 (66)	-	-

Average values shown with parentheses.

(1) The marine zone is represented by station N1 and the brackish zone is represented by station I6.
(2) The marine zone represents 22% of wet area of the Ria de Aveiro at HT and 35% at LT (Silva, 1994).
(3) Average values obtained during the course of another research project (Alcântara *et al*., 1996).

Oxygen consumption was 3 times higher in the brackish water zone due to increased respiration of benthos and plankton. However, the differences in the depth of the water column in the two zones reversed the relation of the integrated values (per surface unit). The total rate of O_2 consumption in the water compartment of the marine zone is, in fact, 1.4-1.5 times higher than in the brackish water zone.

Table 5 shows the oxygen uptake rates determined in sediments from freshwater and marine systems, with indication of the method of determination. Our values for the system of the Ria de Aveiro (15-1474 $mgO_2m^{-2}d^{-1}$) are within the range published for estuarine and coastal sediments (15-2500 $mgO_2m^{-2}d^{-1}$) as seen in Table 5.

Table 5: Aerobic respiration rates in sediments published by several authors.

Location	Oxygen consumption ($mg\ O_2m^2d^{-1}$)	Method	Reference
Labe Marion (British Columbia)	64-3840	O_2 microelectrodes	Hargrave, 1969
Mangrove swamp (Costa Rica)	202-893[(a)]	Winkler titration	Gocke *et al*., 1981
Chesapeake Bay	1400-3300	O_2 electrode	Boynton and Kemp, 1985
Oxic brackish water sediments (Baltic Sea)	7-25	Winkler titration	Koop *et al*., 1990
Eutrophic fjord (Denmark)	2500	Winkler titration	Sand-Jensen *et al*., 1990
Fjord sediment (Roskilde Fjord, Denmark)	725-1740	Winkler titration	Flindt and Nielson, 1992
Shallow bay (North Carolina, USA)	1448[(b)]	Winkler titration	Cahoon and Cooke, 1992
Aarhus Bight (Denmark)	1216-1312[(a)]	O_2 microelectrodes	Hansen and Blackburn, 1992
Estuarine sediment (Ebro Delta, Spain)	15-36[(a)]	Gas chromatography and conductivity detection	Mallo *et al.*, 1993
Intertidal sand (Denmark)	320[(a)]	O_2 electrode measure in the water out flow of microcosms	Sloth *et al*., 1995
Baltic Sea (Finland)	355-404[(a)]	O_2 microelectrodes (sediment); oxymeter (water)	Conley *et al*., 1997
Shallow estuary (Denmark)	2240[(a)]	Winkler titration	Hansen and Kristensen, 1997
San Francisco Bay	3-1536[(a)]		Caffrey *et al*., 1998

Sand estuary (Ria de Aveiro, Portugal)	15-1474-marine zone 58-1272-brackish zone	Winkler titration	Cunha *et al*, this work

(a) Values from authors converted to $mgO_2m^{-2}dia^{-1}$.
(b) Values converted from $mgCm^{-2}h^{-1}$ to $mgO_2m^{-2}h^{-1}$ assuming an RQ of 0.85.

The rate of the benthic O_2 consumption per surface area unit was 1.5 to 4.3 times higher in the brackish zone, probably due to the relative increase in the organic matter content. This difference is even greater when it is taken into account the average thickness of the aerobic layer of the sediments (0.04 m in the marine zone and 0.01 m in the brackish water zone) (Alcântara *et al*., 1996). In fact, the surface layer of the sediment respired, in a per volume basis of aerobic sediment, 6 (LT) and 17 (HT) times more intensely in the brackish zone than in the marine zone (Table 4). The wide range of values of community respiration observed along each transect reflects differences in water depth (contribution of planktonic respiration) between sampling stations, differences in sediment characteristics and may also be related to changes in the density of zoobenthos.

The subtidal sediments of the brackish zone of the Ria showed stable rates of oxygen consumption in relation to the tidal currents. The same did not happen in the marine zone, which exhibited 2.7 increases in the average transect values at low tide. This could be due to tidal differences in the bottom hydrodynamism (Hansen and Kristensen, 1997) and to short term instability in the organic content of the interface, associated or not with the deposition of organic loads transported from the brackish zone during ebb tide.

Estuarine sediments are considered as a privileged compartment for the accumulation, deposition and mineralisation of locally produced and transported organic matter. The sediments of the Ria de Aveiro were 9 to 71 times more active than the water, in a per volume basis. The rates of oxygen consumption per surface area showed, however, that aerobic mineralisation runs mostly in the water column, with a relative share of 64-99 % (marine zone) and 37-95 % (brackish zone) of the total. This distribution of O_2 consumption relative to the sediment and water compartments was also observed in other systems (Gocke *et al*., 1981; Madenjian, 1990; Sand-Jensen *et al*., 1990; Roos and Eriksen, 1995) and is indicative of high content of labile organic matter in the water column.

The low values of the rate of O_2 consumption, in a per volume basis, in estuarine water did not allow its fractionation according to plankton size, and, in particular, to bacterioplankton as was intended. Anyhow, one could see that the values of bacterial respiration relative to total uptake of glucose (Figures 2C and 3C), agree with a more intense bacterial mineralisation, in a per volume unit basis, at the brackish zone and, in general, at low tide.

Bacterial biomass productivity, determined as the rate of leucine incorporation, was taken as an index of metabolic activity of the bacterioplankton in spite of the degree of uncertainty involved. The high values observed at the brackish zone are in agreement with the general pattern of activities within the estuary. Our range of values considering all the individual stations at the marine and brackish-water zones (0.05-5.7 $\mu gCl^{-1}h^{-1}$), fits within the range of some published results (0.6-31 $\mu gCl^{-1}h^{-1}$) for productivity in coastal systems (Biddanda *et al*., 1994; Jellett *et al*., 1996) with the exception of the relatively rare lower limit. The ratios of the productivity of bacterial biomass in the two zones (brackish/marine) of this ecosystem are 5.6 at low tide and 21.0 at high tide. These values reflect the fact, already denoted (results for publication), that the productivity of the bacterial community originating from the marine zone is not immediately stimulated after transport to the brackish zone during flood tide. On the contrary, the brackish zone bacterial community moved to the the marine zone at ebb tide can increase local productivity by 237 % at the surface water.

The overall estimates of oxygen consumption rates for the Ria de Aveiro are 115 (HT) and 70 (LT) $mgO_2m^{-2}h^{-1}$ (Table 4). These values take into account the higher weight of the brackish zones in the total subtidal area (65 % at LT; 78 % at HT) of the Ria (Silva, 1994).

In this system, and on a per surface unit basis, the aerobic carbon oxidation in daylight exceeded 5-9 times the carbon fixation by primary producers. This is an indication of large inputs of allochthonous labile organic carbon. Primary production in the Ria (4.3-23.2 $gCm^{-2}y^{-1}$) compares well with production in other coastal systems (25-45 $gCm^{-2}y^{-1}$, reviewed by Sherr and Sherr, 1996) but is at the lower end of the range for more productive estuaries (19.7-2394 $gCm^{-2}y^{-1}$, calculated from Cadée, 1986).

Bacterial metabolism of glucose revealed that only a minor fraction (less than 40%) was respired. However, the evaluation of the total microbial respiration in the water column gives an altogether different picture. Aerobic respiration exceeded (up to 58 times) bacterial biomass productions, denoting either a general unbalance of bacterial metabolism in favour of mineralisation or/and a small contribution of the bacterial population for the total respiration. It is generally accepted, however, that in estuarine and coastal waters the respiration rates are mainly determined by the bacterial community (Grifith and Pomeroy, 1995) and that the importance of this fraction declines with the distance to the coast (Hopkinson, *et al.*, 1989). The separation of bacteria from the total community is not easy or exempt of criticism and was not attempted in this study. The values of the total community respiration in unfractionated samples may reflect the importance of interbacterial DOC cycling as discussed by Yahnke and Craven (1995).

The ratio between primary production and bacterial biomass production at LT and HT reveals the intensity of the shifts towards autotrophy or heterotrophy in dependence of tide.

According to our results, in a per volume basis, referring to late autumn and winter conditions (values not shown), the ratio at LT in the marine (1.0) and brackish water (2.1) zones corresponded to a state of equilibrium between autotrophy and heterotrophy in the first zone and to a slight dominance of autotrophy in the upper estuary. At HT, however, this ratio shifted in opposite directions in the two zones. It increased to 3.3 in the marine zone (mainly due to the decrease in bacterial biomass productivity) and decreased to 0.4 in the brackish water zone (due to the increase in bacterial biomass productivity and the decrease in primary productivity).

In a previous study of the Ria de Aveiro (Hoppe *et al*., 1996), during spring, this ratio was always equal or greater than 4 (4 to 22) in the two zones, denoting the importance of primary productivity in this season. According to these authors, at HT, the ratio exceeded the values at LT by a factor of 3 in the marine zone in a trend similar to that observed during the cold season. Also at HT, but in the brackish water zone, the trend was, however, reversed in relation to our results, showing a 5 times increase in the ratio (due to an enormous increase in primary productivity not observed in the cold season).

During daylight, the shifts towards autotrophy and heterotrophy of the water column, in this estuarine system, are not only tide dependent but seem to be also season dependent. In the marine zone, enhanced heterotrophy happened at LT particularly in the cold season. On the contrary, in the same season, the upper estuary was mostly heterotrophic only at HT.

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CHAPTER III
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 this patterns on the degradation and recycling of organic matter in the Ria de Aveiro.
Phytoplankton biomass was maximal (0.65-2.10 mgC 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 x10^9 cell l^{-1}), being 2-3 times higher than in the outer estuary at identical tidal conditions.
The rates of ectoenzymatic hydrolysis were also maximal at the mid-estuary or at the inner section. Values ranged from 4.3 to 181.3 nmol l^1 h^{-1} for ?-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 (?-glucosidase activity). This was accompanied by major amplifications of glucose incorporation with maximal values of 17.7 nmol l^1 h^{-1}. Tr ranged from 0.3-4.1 % h^{-1} at the outer estuarine section increasing towards maximal values at the inner section (10.4-39.4 % h^{-1}).
Statistical analysis revealed that the variation of ectoenzymatic activities could be significantly related to bacterial abundance, which, in turn, was highly associated to the variation of 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 about 25-30 PSU. Ectoenzymatic activity showed a quite conservative behaviour during tidal transport along the salinity gradient when compared to the more reactive parameters of glucose metabolism.

Key Words: Bacterioplankton; ectoenzymatic activity; heterotrophic activity; salinity gradient; estuaries

1. Introduction

Estuaries are transition systems governed by complex interacting elements that change in space and with season. When subjected to tidal currents, the entire estuary acquires a pulsation that interferes with the transport and expression of biological activities and with the distribution of biomass in the water column. One can, however, assess the

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 of mineralisation.

Allochthonous carbon sources, in accretion to the elevated primary production generally observed in estuaries, tend to enhance the heterotrophic component in the metabolism of the plankton in disfavour of autotrophy. In different 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 (Vallespinós and 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) developing, in this way, a great impact in the cycle of matter and in the 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 the ectoenzymatic activity is brought to evidence by the coupling to the decomposition of POC and DOC during seasonal and spatial fluctuations of activity (Chróst, 1990).

The variation in ectohydrolase profiles (types and levels of activity) along an estuarine trophic gradient reflects the trophic status of the environment and this changes with the season (Hoppe *et al*., 1998, Karner *et al.*, 1992). 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 field of organic matter (Martinez *et al*., 1996)

Generally, the Ria de Aveiro, is dominated by microbial heterotrophic processes not only in a diel scale, but probably all over the year (Hoppe *et al*., 1996). In the warm season, during the daylight period, the balance between autotrophy and heterotrophy, judged from the ratio of primary production and bacterial production, shifted towards clear net autotrophy, particularly at high tide (Hoppe *et al*., 1996). In the cold season, however, even during daylight, low tide only allowed a general equilibrium of the two activities in the inner and outer estuary sections (Cunha *et al*., 1999).

In face of the observed importance of heterotrophy in the lagoon of Aveiro, it is now attempted to put in evidence the patterns of degradation of major macromolecules and the patterns of turnover of monomers along the salinity gradient. The tidal effects on bacterial activity will be explored in order to assess the conservative or reactive response of the supporting metabolism during transport associated to tidal currents and to detect the location of the main activity sources in the estuary.

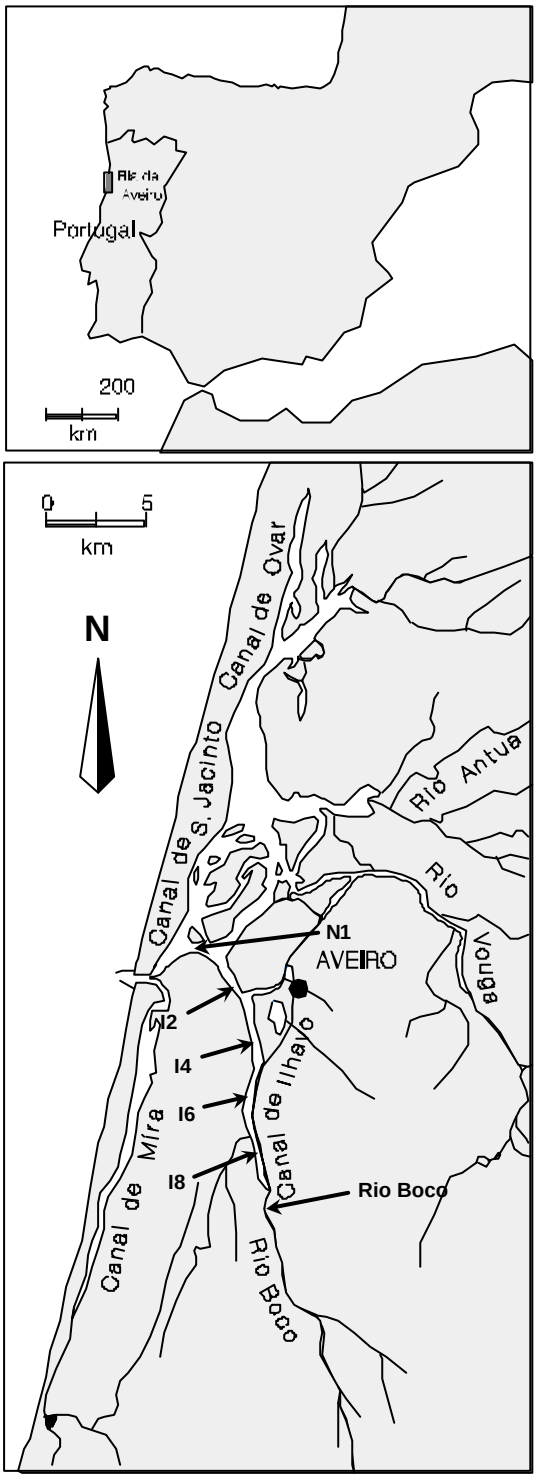

Figure 1: Ria de Aveiro (Portugal) with sampling stations indicated with arrows: Stations N1 and I2 in Canal de Navegação, stations I4, I6 and I8 in Canal de Ílhavo and station Rio Boco at the mouth of the freshwater stream.

2. Materials and methods

Study site

The Ria de Aveiro (Figure 1) is as a bar-built estuary (Pritchard, 1967) on the Northwest coast of Portugal separated from the sea by a sand barrier. The fresh water input to the lagoon during the period of time equivalent to the flood tide varies throughout the year around an average value of 1.8 Mm^3, which is about 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 (Figure 1).

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

For later comparisons, 3 segments were considered along this profile being a segment defined as the distance over which a particle is transported during flood tide (Silva, 1994). The outer-estuary (stations N1, I2), in the transition

to the coastal zone, showed salinities ranging from 33 to 35.5 PSU at high tide. The mid-estuary (stations I4, I6, I8) showed salinities in the 20-33 PSU range at the same tide. The inner-estuary (station RB) was defined as the mixing zone between the 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 to harbour activities at the outer and mid segments, some industries, aquaculture plants and diffuse domestic sewage drains at the mid segment and run-off from agriculture fields at the inner segment.

Sampling

The six sampling sites were visited in early summer 1997, in neap tide (NT) (12^{th} July) and spring tide (ST) (20^{th} July) conditions. Samples were always collected during daytime, an the centre of the channels, 0.2 m below the surface. Collection was performed at slack high (HT) and 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 2-3 hours.

Physical and chemical parameters

Temperature and salinity 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 WTW BR 190 stirrer. 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 diameter) pre-weighted, pre-combusted filters. The filters were dried at 60 ºC for 24 hours 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 hours incineration at 525 ºC of the dry seston (Parsons *et al.*, 1989). POC was calculated as 50 % of the POM (Rodier, 1996).

Microbiological parameters

Total bacterial number (TBN) and bacterial organic carbon (BOC) were determined by cell counting under epifluorescence microscopy after fixation of the water samples with 2 % formaldehyde (final concentration). The samples were 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 fgC μm^{-3} according to Bjørnsen, 1986).

The heterotrophic metabolism of glucose was described by the parameters Vm (maximum uptake velocity) and Tr (turnover rate) following the procedure described by Gocke (1977). For Vm 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 2-3 hours 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 scintillation cocktail. Radioactive labelled glucose (SA 11.5 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 (Hm) of model substrates for ?-glucosidase (4-methylumbelliferyl-?-D-glucoside) and Leu-aminopeptidase (L-leucine-7-amido-4-methylcoumarin) added at the saturating concentration of 1 mM (Hoppe, 1983). Wavelengths for excitation and emission were respectively 380-440 nm for MCA (7-amino-4-methylcoumarone) and 360-450 nm for MUF (4-methylumbelliferone). Measurements were made in 3 replicates for each sample after 1-2 hours incubations at *in situ* temperature. Calibration was performed by adding a series of 6-8 concentrations of the fluorescent products (0-500 nM for MUF and 0-6 µM for MCA) to a pool of water from the 6 sampling stations.

Chlorophyll *a* was determined fluorimetrically (Yentsch and Menzel, 1963) after extraction with 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

As an attempt to explain the variation of 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 the SPSSWIN 7.1 package.

3. Results

Physical and chemical parameters

Physical and chemical data are shown in Table 1. Values of salinity ranged from 2.3 PSU at RB to 35.6 at station N1. The variation of salinity was strongest at the mouth of RB, with amplitude of 5.0 PSU in ST and 13.2 PSU in NT.

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

Dissolved oxygen concentration corresponded, in the minimum, to 76 % of the saturation value (Table 1). In the mid- and inner-sections of the longitudinal profile, particularly at LT in 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, when compared to the downstream stations. Nevertheless, values of Secchi depth remained quite low. In the outer and mid estuary the seston content at LT was up to 16 % greater than at HT. At 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} remaining quite stable irrespectively of salinity or tide (Table 2). POC represented a fraction of 11-18 % of the seston at inner estuary and of 9-14 % at mid and outer estuary.

Phytoplankton and bacterioplankton abundance

The highest concentrations of phytoplankton biomass (Table 2) were found at inner estuary (0.65-2.10 mgC l^{-1}) or at mid estuary (0.44-1.82 mgC l^{-1}). At outer estuary phytoplankton biomass ranged from 0.19 to 0.57 mgC l^{-1}. The phytoplankton carbon fraction in the POC was also greater (1.9 to 9.3 times, average 4.3) in the inner- or the mid-estuary, depending on tide.

Table 1: Values of total depth, Secchi depth, salinity, temperature, dissolved oxygen and seston concentration registered at the surface of the water column (0.2 m), at high and low tide under neap and spring tide conditions. Standard deviation, when calculated, is presented between parentheses.

		Total depth (m)	Secchi depth (m)	Salinity (PSU)	Temperature (ºC)	Dissolved Oxygen (% sat.)	Seston (mg l^{-1})
12th 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)
20th 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	n.d.	57 (7)
	Rio Boco	0.5	0.5	4.5	26.6	n.d.	52 (7)

Total bacterial number (TBN) ranged from 2.6 to 15.3 x 10^9 cell 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 RB (Figure 2). Bacterial organic carbon (Table 2) followed approximately the same pattern as TBN and varied from 45 to 418 µgC l^{-1} in different sections of the profile and in different tides. When compared to neap tide, spring tide increased by 60 % the HT biomass value at inner estuary.

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

		POC ($mgC\ l^{-1}$)		Phytoplankton biomass ($mgC\ l^{-1}$)		Bacterial biomass ($\mu gC\ l^{-1}$)		Phytoplankton biomass/POC (%)	
12th July 1997 (neap tide)									
High tide									
	N1	6.0		0.35		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)
20th 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)

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 (Figure 2). It reached a maximum value of 17.7 nmol l^{1} h^{-1} at station I8. Glucose Tr (Figure 2) was lowest at the outer-estuary (0.3-4.1 % h^{-1}) increasing inward. The maximal value (39.4 % h^{1}) was registered at RB al low tide, under spring tide conditions. The respired fraction was 34 % or less (average 13.8 %) of the glucose uptake.

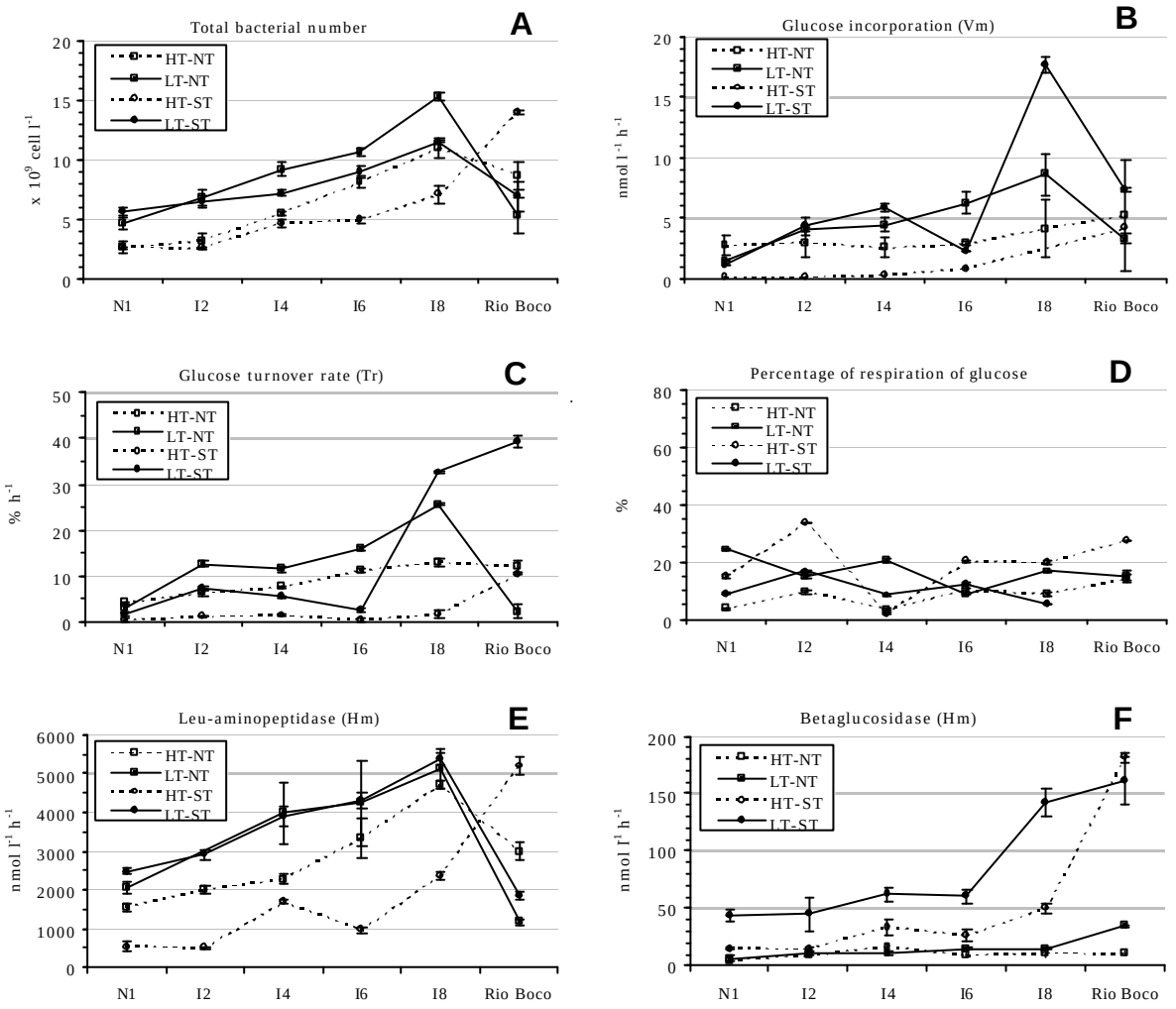

Figure 2: Variation of parameters of bacterial abundance and activity along the longitudinal profile of 6 stations in the Ria de Aveiro at high tide (HT) and low tide (LT) during spring (ST) and neap (NT) tide conditions.

The rates of ectoenzymatic hydrolysis were generally higher for Leu-aminopeptidase (Leu-AMPase) than for ?-glucosidase (?-GLCase). Hm varied from 490 to 5374 nmol l^{1} h^{-1} for Leu-AMPase and from 4.3 to 181.3 nmol l^{1} h^{-1} for ?-GLCase. Along the profile, Leu-AMPase activity was generally highest at station I8 whereas ?-GLCase reached its maximum of activity at RB (Figure 2). At LT and in mid-estuary, the hydrolytic activities were up to 4.5 times (Leu-AMPase) and 2.8 times (?-GLCase) higher than at HT. On the contrary, 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 ?-GLCase activity.

Table 3: Values of per cell glucose uptake Vm, Leu-AMPase Hm and ?-GLCase Hm (attomol cell^{-1} h^{-1}).

		Glucose Vm	Leu-AMPase Hm	?-GLCase Hm
12th 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
20th 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

Per cell activities (Table 3) ranged between 0.05 and 1.54 attomol cell^{-1} h^{-1} for glucose uptake Vm, 188.5 and 625.6 attomol cell^{-1} h^{-1} for Leu-AMPase activity and 5.4 and 25.0 attomol cell^{-1} h^{-1} for ?-GLCase activity. At spring tide conditions, values of specific activity were higher at LT, when compared to HT, particularly at the outer section. In neap tide, on the contrary, the outer section registered higher specific activity values in HT. At RB, values of specific activity were always higher at HT for Leu-AMPase but for ?-GLCase the values were always higher at LT. Specific Leu-AMPase and ?-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 this set of independent variables was high (> 80 %) for TBN and ectoenzymatic activities while only 30-40 % of the variation of the parameters related with the heterotrophic metabolism of glucose could be explained.

Table 4: Regression equations for the explanation of the variation of microbiological parameters obtained from the stepwise multiple regression analysis. Dependent variables are identified as TBN, Leu-AMPase (Hm of Leu-aminopeptidase), ?-GLCase (Hm of ?-glucosidase), Vm (Vm of glucose incorporation), Tr (glucose turnover rate) and % Resp (percentage of glucose respiration). Independent variables are identified as TBN, Temp (temperature), Sal (salinity) and Chlor (chlorophyll *a*).

Dependent variable	Independent variables	Regression equation	Adj. R^2
LogTBN	Temp (? = 0.715; p = 0.000) Sal (? = 0.321; p = 0.011) LogChlor (? = 0.424; p = 0.013)	LogTBN =-0.908 + 0.052 Temp + 0.007127 Sal + 0.299 LogChlor	0.824
Leu-AMPase Hm	TBN (? = 0.906; p = 0.000)	Leu-AMPase Hm = -333.898 + 414.966 TBN	0.813
?-GLCase Hm	Sal (? = -0.634; p = 0.000) TBN (? = 0.478; p = 0.000)	?-GLCasel Hm = 105.170 – 2.934 Sal + 6.335 TBN	0.822
Glucose Vm	Temp (? = 0.584; p = 0.003)	Glucose Vm = -11.584 + 0.704 Temp	0.310
Glucose Tr	Temp (? = 0.577; p = 0.003)	Glucose Tr = -34.568 + 1.954 Temp	0.333
% Resp	Sal (? = -0.558; p = 0.013) LogChlor (? = -0.942; p = 0.003) Temp (? = 0.703; p = 0.030)	% Resp = 13.072 – 0.864 Sal – 44.253 LogChlor + 3.572 Temp	0.435

4. Discussion

Bacterial abundance and heterotrophic activity along the estuarine profile

Bacterial biomass and bacterial dependent activities were in agreement with a curvilinear pattern with a peak at about 25-30 PSU. This corresponds, in general, to the estuarine structure described by Wright and Coffin (1983), Palumbo *et al*. (1984), Fuks *et al*. (1991) and Bordalo *et al*. (1998) but the location of the peak in Canal de Ílhavo was shifted to a comparatively higher salinity level because of the low fresh water input. The Ria contrasts with other estuaries that showed maximal values for bacterial heterotrophic activities in the inner estuary, at low salinity values (3-10 PSU), followed by a conservative decrease towards higher salinities (Palumbo and Ferguson, 1978). In the particular situation of the Ria de Aveiro, it seems clear that the freshwater stream, at the inner part of the studied estuarine profile (Figure 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 (2-4 times) phytoplankton communities and increased nitrate concentrations (data not shown), usually occurred at station I8 and were pushed up to RB only with the strong flood tide currents, under spring tide conditions.

Values of TBN, ?-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 and Veliminov, 1991, Fuks *et al*., 1991, Karner *et al*., 1992, Crump and Baross, 1996, Hoppe e*t al*., 1996, Hoppe *et al*., 1998). However, the metabolism of glucose along the studied profile was strongly diverted to incorporation in bacterial biomass (72.5-98.0 %) rather than to respiration. Higher growth efficiencies of estuarine bacteria, when compared to open-water communities, have been reported by Jørgensen *et al.* (1999). The authors postulate 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 and Cole (1998). However, it must be taken into consideration that the single radiotracer approach may produce artificially high values of the incorporated fraction because the equilibrium of the intracellular pool is not completely achieved during the short incubation periods (Yahnke and Craven, 1995).

The extracellular degradation of protein was also exceptionally high along the salinity gradient. Leu-AMPase Hm varied in the high range of 490 to 5374 nmol l^{1} h^{-1}, greatly exceeding a previous range of values (8.3 to 311 nmol l^{1} h^{-1}, after conversion) determined in 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 nmol l^{1} h^{-1}) for brackish water systems (Chróst and Velimirov, 1991, Hoppe, 1983, Hoppe *et al*., 1996, Hoppe *et al*., 1998, Rego *et al*., 1985) denoting an exceptional 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 brackish water sections of the lagoon.

Changing pattern of organic matter utilisation between the fresh water stream and the main body of the 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 push up a considerable amount of estuarine water. Under different conditions, bacterial abundance decreases at the inner station, as well as Leu-AMPase activity, contrasting with an increase of ?-GLCase activity. This seems to locate upstream the main source of bacterial potential for the degradation of carbohydrates and in the main body of the lagoon the major sources of bacterial carbon and AMPase activity. The LT/HT ratios for ?-GLCase activity at RB were above or, at least, close to 1 (Table 5), as occurred at the mid and outer sections. On the contrary, 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).

Table 5: Ratios LT/HT of the values of TBN, glucose Vm and Tr, Hm of Leu-AMPase, Hm of ?-GLCase, and per cell values of glucose Vm, Leu-AMPase Hm and ?-GLCase Hm calculated for the three sectors of the estuarine profile. Average values are presented in parentheses.

Parameter	Outer estuary (Stations N1, I2)		Mid estuary (Stations I4, I6, I8)		Inner estuary (Station 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 Vm	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 Hm	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
?-GLCase Hm	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 Gluc Vm	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 Hm	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 ?-GLCase Hm	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

Bacterioplankton of RB seems to be more dependent on carbohydrates and results indicate that ?-GLCase activity may limit the availability of simple substrates and constrain glucose uptake. LT/HT ratios greater than 1 should be expected for other parameters related to glucose metabolism (Glucose Vm and Tr) but at RB, the LT stimulation of glucose metabolism was only obvious in spring tide (Table 5). The intense mixture with water from the mid section of the estuary during ST worked as a reinforcement of the supply of simple molecules and allowed the intensification of glucose metabolism at LT, when the negative effect of salinity was reduced.

In terms of specific activities, ectoenzymatic activities exhibited distinct tidal responses. Specific values of ?-GLCase Hm (and glucose uptake Vm) showed, at RB, higher values at LT while, on the contrary, specific AMPase Hm at RB was higher at HT. However, it must be considered that 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 alternatively 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).

Fresh water residence time at the inner section of Canal de Ílhavo ranges from 6 days 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 brackish water sections during 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 for carbohydrate degradation and an estuarine reservoir of bacterial biomass and potential for protein utilisation.

Reactive *versus* conservative bacterial heterotrophic capacities

During the relatively slow transport from the inner sections, processes of mixing of communities, dilution with poorer seawater and adaptation to different organic substrates will considerably change the characteristics of bacterioplankton that reaches the mouth of the lagoon. Some parameters showed a quite conservative behaviour during tidal transport while others, more reactive, were 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 stations I8 and N1 (I8 *versus* N1) for different tidal conditions (Figure 3).

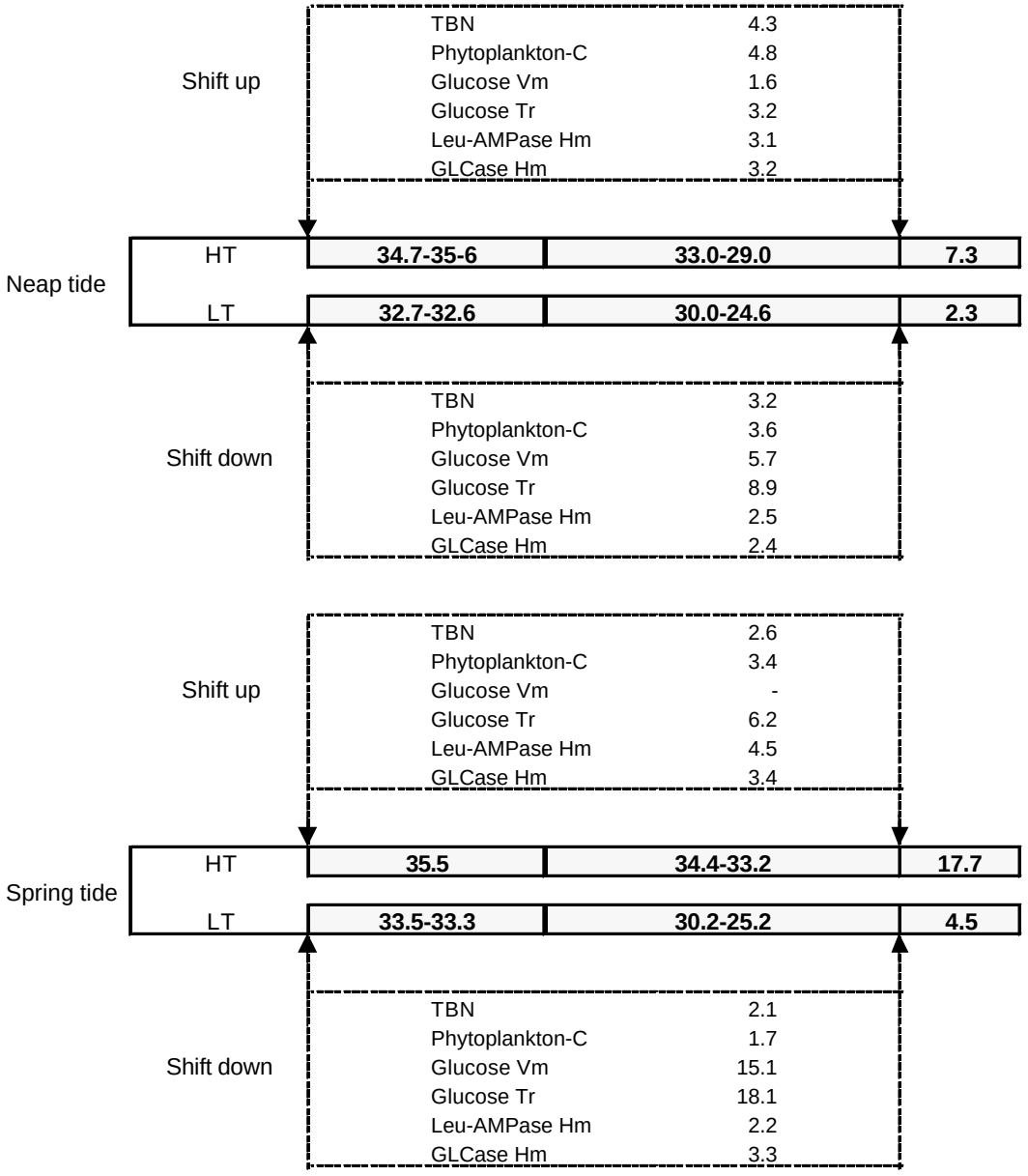

Figure 3: Schematic representation of tidal responses (shift up and shift down) of biological parameters. Values inside the grey boxes indicate the ranges of salinity values in each one of the three segments of Canal de Ílhavo. Inside the dotted-line boxes are presented the ratios calculated between the values registered at stations I8 and N1 (I8/N1) for TBN, phytoplankton-C, and bacterial activity parameters, under different tidal conditions.

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 mixture of communities with different specific activity rates. For the overall of the parameters, ST results in an increase in the slope of the longitudinal profile but the most striking feature is the distinct behaviour of ectoenzymes when compared to the heterotrophic metabolism of glucose. Both ectoenzymes seemed to be transported/diluted up and down the estuary maintaining a quite good balance between shifting up at HT (I8/N1 ratios of 2.2-4.5) and shifting down at LT (I8/N1 ratios of 2.2-3.3). On the contrary, the negative effects of increasing salinity and impoverishment, associated to ebb tide transport, on glucose metabolism (Vm and Tr) were stronger than the stimulation of marine communities being transported to a richer environment. The I8/N1 ratios for Vm and Tr of glucose were higher at LT (5.7-18.1) than the corresponding values calculated for HT (1.6-6.2). This reinforces the scenery 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 Vm and Tr were only 1.6 and 3.2, respectively. This means that at HT-NT, bacterial abundance was more shifted up 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 clarifying the distinct behaviour of different heterotrophic capacities during tidal transport. A close relation between ectoenzymatic activity and bacterial abundance can be confirmed. More than 80 % of the variation of bacterial abundance was associated to the combined effects of salinity, temperature and chlorophyll *a* concentration. The relation with salinity is, however, strongly influenced by the contrast between Rio Boco and the brackish water stations. A similar statistical treatment excluding the RB produces a clear negative relation between bacterial abundance and salinity (results not shown).

An important fraction (>80 %) the variation in Leu-AMPase activity could be explained by the variation of TBN suggesting that this ectohydrolytic capacity is common and a widespread characteristic of estuarine bacterioplankton. Since it is closely associated to bacterial cells, processes of mixing and dilution mainly control the variation of Leu-AMPase activity during tidal transport. In the case of ?-GLCase, the two 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 30-40% of the variation in glucose Vm and Tr suggesting the greater importance of other variables, like the size and quality of the dissolved organic matter pool, and justifies the reactivity 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 one order of magnitude higher than the particulate fraction (Zdanowski and Figueiras 1997). Results from Murrel *at al*. (1999) indicate that during the warm season, when flux of fresh water and the supply of particles to the estuaries are reduced, seston suffers from low nutritional quality. We believe that in the main body of the Ria de Aveiro, like in other estuarine environments, (Azam and 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 the utilisation of complex substrates, eventually associated to high-quality seston at RB, to the increasing importance of simple dissolved substrates as nutrient sources, at the brackish water sections of the estuary, may contribute to the high reactivity of parameters related to the incorporation of monomers.

5. Conclusions

The profiles of variation of abundance and activity along an estuarine profile in the Ria de Aveiro, revealed a shifting from a more N-associated metabolism at the main body of the lagoon to an increase of the importance of the C-utilising community at the mouth of the fresh water stream Rio Boco. Ectoenzymatic activity followed more closely the variation of bacterial abundance than monomer uptake and seemed to be mainly determined by the variation of salinity, temperature and phytoplankton biomass. However, the activity of ?-GLCase was strongly associated to 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, following a quite conservative pattern and responding probably mainly to dilutions effects associated to the entrance of poorer marine water during flood tide. The heterotrophic metabolism of glucose showed a more reactive behaviour. This parameter responded negatively to the

ebb-tide transport. On the contrary, during transport to inner stations of the lagoon there was a comparably less pronounced and, in 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 allochtonous origins. However, ectoenzymatic activity could be limiting the turnover of monomers, at least at 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 an increased importance of autotrophic processes as a source of substrates during the warm season and in the day light period.

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CHAPTER IV
SHORT-TIME RESPONSES OF THE NATURAL PLANKTONIC BACTERIAL COMMUNITY TO THE CHANGING WATER PROPERTIES IN AN ESTUARINE ENVIRONMENT: ECTOENZYMATIC ACTIVITY, GLUCOSE INCORPORATION AND BIOMASS PRODUCTION

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Abstract - The possibility that two principal bacterial communities expressing different levels of heterotrophic activity might co-exist in an estuarine ecosystem (Ria de Aveiro, Portugal), and could quickly respond to tidal fluctuations of environmental factors, was experimentally tested in diffusion chambers by swapping the dissolved components of the natural water between the two communities and comparing their reactivity against the unaltered controls.

The results for ectoenzymatic activity (Leu-aminopeptidase and ?-glucosidase), glucose incorporation and biomass production after transference of the marine bacterial community to brackish water showed maxima in the range of 241-384 % of the control values. The opposite transference of the brackish-water bacterial community to marine water produced maximal decreases to 0.14-0.58 % of the control values.

In a reverse experiment, designed as the return to the initial conditions after 2 hours of the first exposure, the marine community rapidly re-acquired the characteristic low levels of activity. Contrastingly, the negative effects of 2 hours of exposure to marine water on the activity of the brackish water bacteria persisted, at least for 4 hours, after return to their natural water.

The apparent short-term irreversibility of the decline in activity of the brackish water bacteria when exposed to marine water, in parallel with the quick and reversible positive response of the marine water bacteria to the brackish water, suggests the development of two distinct bacterioplankton communities adapted to the environmental conditions prevailing at distinct sections of the estuary. The reactivity to environmental changes demonstrated by the two communities allows the prediction of estuarine profiles of bacterial activity steeper than those expected from the conservative transport of bacterial cells associated with tidal currents.

1. Introduction

Estuarine bacterioplankton communities develop and evolve under unstable environmental conditions. Along estuarine gradients, marine and fluvial influences may combine to produce different patterns of bacterial abundance and heterotrophic activity. Denser and more active bacterioplankton communities may occur at intermediate salinities ?5, 12, 26, 38? frequently associated with the maximum turbidity zone. However, under the particular environmental and morphological characteristics of an estuarine system, peaks of abundance and activity may be found at low salinity values (3-10 PSU), followed by conservative decrease towards higher salinity sections ?25?; contrastingly, maximum

activity may occur at the outer estuary [6]. Generally, at low salinity levels bacterioplankton communities exhibit higher growth efficiencies [14, 20] and express relatively higher rates of β-glucosidase activity [24]. On the contrary, closer to the interface with the sea, bacterioplankton seems to be more adapted to the degradation of protein [24].

Considerable work has been developed in attempts to determine the environmental factors controlling the activity profiles of estuarine bacterioplankton. The availability of inorganic nutrients [37] and the quantity and quality (lability) of the pool of organic matter may exert control over the production of bacterial biomass [14, 21], growth yield [3, 20], ectoenzymatic activity [24] and monomer uptake [8]. Processes involving longitudinal transport of particles along the estuary and exchange of materials between the sediments and the water column may ultimately modify the quality of the substrates available for bacterial utilisation. In ecosystems where rivers are responsible for the major inputs of particles, there is a decrease in the quality of particulate organic carbon (POC) from the inner sections to the mouth of the estuary and a corresponding decrease in bacterial heterotrophic activity [24]. Closer to the riverine source of rich particulate substrates, bacterial activity was frequently found to be uncoupled from primary production since the *in situ* supply of organic substrates was not acting as a limiting factor [32]. It has also been demonstrated that fluxes of dissolved organic carbon (DOC) from the sediments may stimulate bacterial biomass production [23] and increase growth rates [16]. The impact of the release of dissolved organic matter (DOM) from the sediments on bacterial activity in the water column can be more important in shallow areas, particularly those with fine sediments and high rates of benthic primary production [23].

The dynamics of bacterial carbon metabolism respond to the shifting properties of the water column between different sections of an estuary [31]. One question about the establishment of patterned estuarine gradients of bacterial activity is whether these gradients reflect reversible responses of versatile communities and/or processes of mixing of communities adapted to different prevailing environmental conditions and nutrient sources along this gradient. Another question is: do these responses occur over a time scale that can allow observable effects during ebb and flood tides?

To answer these questions and to predict the effects of changing estuarine hydrodynamics, an experimental set-up was devised to challenge bacterioplankton populations, from the outer and mid estuary, to respond to contrasting estuarine water. This work reports on the short-term changes in heterotrophic bacterial activity.

2. Methods

Study site

The Ria de Aveiro (Fig. 1) is a branched estuarine ecosystem in the Northwest coast of Portugal with maximum length and width of 45 and 10 Km, respectively. The tidal water exchange with the ocean is about 89 Mm^3 while the average fresh water input during an equivalent period of time is, on average, 1.8 Mm^3 ?33?. The ecosystem is frequently described as a coastal lagoon within which is distinguished a deeper marine zone (10 to >20 m deep at high tide) encompassing 22-35 % of the total water volume of the Ria ?33? and a progressively shallower brackish water zone, branched in several channels along which salinity gradients develop. For this study, 2 sampling stations were chosen in order to represent contrasting water characteristics within the estuary: station N1 in Canal de Navegação, close to the mouth of the lagoon, representing the deep marine zone, and station I6 in Canal de Ílhavo, representing the shallow inner brackish water zone.

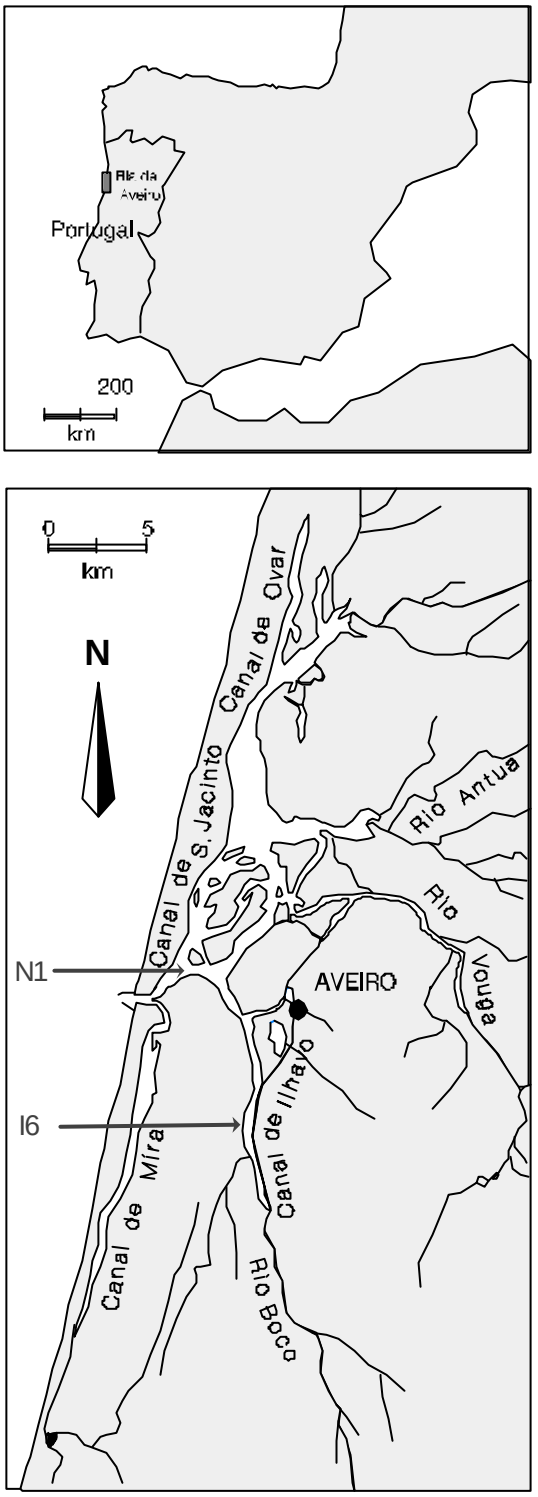

Fig. 1: Ria de Aveiro lagoon (Portugal) with sampling stations indicated by arrows: station N1 in Canal de Navegação, 1.5 Km from the mouth, and station I6 in Canal de Ílhavo, 9 Km from the mouth.

Sampling

The sampling sites and the moment of collection in relation to the tidal cycle were chosen in order to represent sharp contrasting environmental conditions within the estuary. Station N1 was sampled 2 hours before high tide (HT). As the volume of water that enters this section during flood tide (89 Mm^3) is much

larger than the 31 mM^3 that remain at low tide [33], we assumed that the samples obtained when ocean water was in full flow into the estuary would fairly represent marine environmental conditions and the corresponding bacterioplankton communities. At station I6 sampling was performed late in the ebb flux, 2 hours before low tide (LT). It was assumed that in these samples, the characteristics of the water and of the bacterioplankton communities of the mid section of the estuary would be fairly represented.

Stations N1 and I6 were visited in February, March and June 1998. A total volume of 60 l of water was collected from 0.2 m below the surface, by consecutive immersions of a 10 l plastic bottle. Samples were transported to the laboratory in 100 l dark plastic containers.

Water quality variables

The physical, chemical and microbiological characteristics of the marine and brackish water samples were determined within 6 hours after collection.

Salinity was measured with a conductivity meter (WTW – Wissenschaftlich Technische Werkstätten, Model LF 196). The determination of the concentration of suspended solids (seston) was performed after filtration of 500 ml aliquots through Whatman GF/C (47 mm diameter) pre-weighted, pre-combusted filters. The filters were dried at 60 ºC for 24 hours and the seston content was calculated as the increase in weight. The loss of weight by ignition, as a measure of the particulate organic matter content (POM), was achieved by 4 hours of incineration of dry seston at 525 ºC [27]. POC was calculated as 50 % of the POM [30]. Chlorophyll *a* concentrations were determined fluorimetrically [39] after extraction with 90 % acetone.

In the February samples the concentrations of dissolved organic carbon (DOC) and inorganic nutrients were also determined. Samples for DOC analyses were filtered through pre-combusted Whatman GF/C filters and kept frozen (-20ºC). An independent laboratory (Ambio, Ltd) carried out DOC determinations by combustion and infrared detection [2] in a Dohrman DC-180 Analyser. For nutrient analyses, 25 ml aliquots were filtered through cellulose acetate membranes with a pore size of 0.45 µm (MSI - Micron Separation Inc.) and stored at –20 ºC until analysis. The concentration of NO_3^- + NO_2^- was determined by the sulfanilic acid method after the reduction of NO_3^- to NO_2^- in a cadmium column. The analysis of PO_4^{3-} followed the molybdate method. For these determinations, the standard procedures for nutrient analyses [35] were adapted to the Segmented Flow Injection Automatic Analyser, Alliance Instruments, France – Evolution II [1].

Bacterioplankton parameters

Total bacterial number (TBN) was determined by direct counting under epifluorescence microscopy after fixation of the water samples with 2 % formaldehyde (final concentration), followed by collection of cells on 0.2 µm black polycarbonate membranes (Poretics) and staining with 0.03% acridine orange ?15?.

Ectoenzymatic activity was determined fluorimetrically (Jasco FP-777 Fluorometer) as the maximum hydrolysis rate (Hm) of two model substrates, 4-methylumbelliferyl-?-D-glucoside for for ?-glucosidase (?-GLCase) and L-leucine-7-amido-4-methyl-coumarin for Leu-aminopeptidase, (Leu-AMPase) ?17?, at a saturating concentration of 1 mM. For each sample, 3 replicates were analysed. Wavelengths for excitation and emission were respectively 380-440 nm for MCA (7-amino-4-methylcoumarone) and 360-450 nm for MUF (4-methylumbelliferone). The incubation period varied between 1 and 2 hours and took place at a standard temperature of 20º C. Fluorescent substrates and standards were purchased from Sigma Co.

Glucose incorporation (Glucose Vm) ?13? was determined in 3 replicates and one blank fixed with 2 % formaline at a final saturation concentration of 430 nM of ^{14}C-glucose (Amersham, SA 11.5 GBq mmol^{-1}, 310 mCi mmol^{-1}). Incubations were carried out for 2 hours at 20ºC. Cells were collected on 0.2 µm Poretics polycarbonate membranes and radioactivity was measured in a liquid scintillation counter Beckman LS 6000 IC using UniverSol (ICN Biomedicals, USA) as the scintillation cocktail.

Bacterial biomass productivity (BBP) was determined according to Simon and Azam ?34? in 10 ml aliquots (3 replicates and one blank fixed with 2 % formaline) by the incorporation of ^{3}H-leucine (Amersham, SA 61 Ci mmol^{-1}) at a final concentration of 30 nM. Incubation took place at 20 ºC for 1 hour. Cells were harvested on 0.2 µm Poretics polycarbonate membranes and washed with 10 ml cold 10 % TCA. Radioactivity was read in a liquid scintillation counter Beckman LS 6000 IC using UniverSol as the scintillation cocktail.

Time course of bacterioplankton response during exposure to contrasting water quality

Two experimental incubation systems were prepared (Fig. 2). Each one consisted of a stirred water bath with a capacity of 20 l completely filled either with water from station N1 (N1 tank representing the marine zone of the estuary) or from station I6 (I6 tank representing the brackish water of the mid estuary). Temperature was kept at 20 ºC. Each

diffusion chamber, with a capacity of 170 ml, consisted of a hollow nylon cylinder with an external height of 4.5 cm closed at the tops with two 12.5 cm diameter polycarbonate membranes (Poretics, 0.2 µm). The membranes were held in place by Viton® fluoroelastomer o-rings attached to nylon frames, tightly screwed to the cylinder. A neck and an opening at the middle of the chamber allowed the initial filling and periodic sampling. The sampling port was kept closed during immersion by a silicone-rubber stopper covered by a screw cap ?28?.

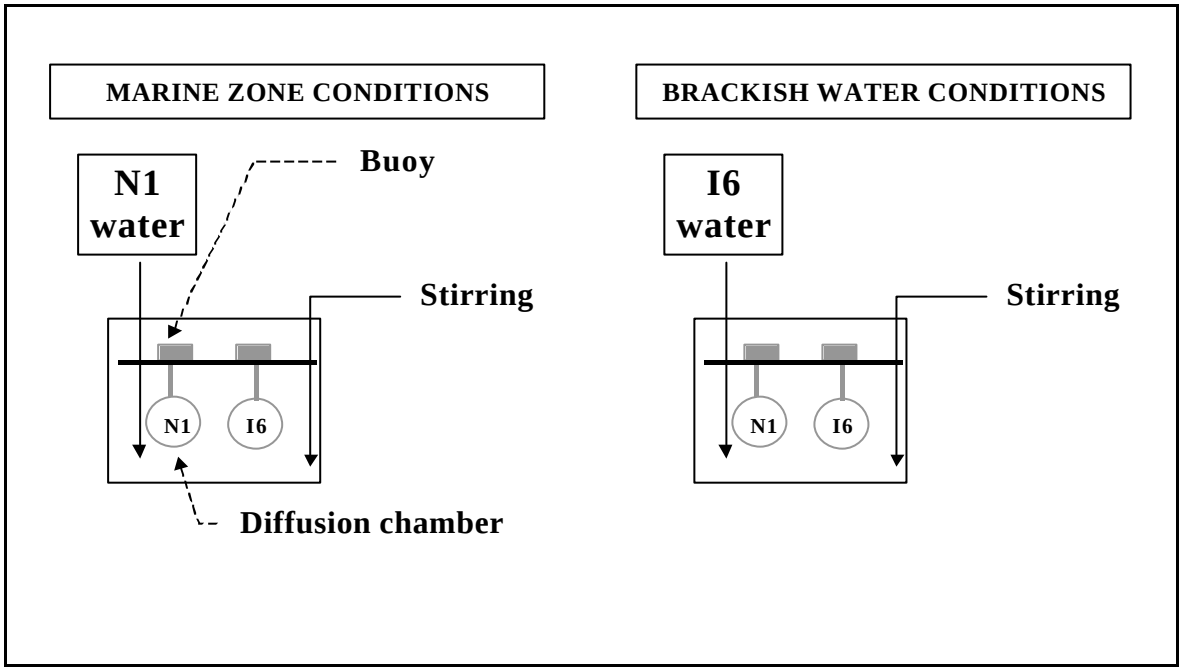

Fig. 2: Schematic representation of the experimental set up: stirred water baths were filled with water from station N1 (marine conditions) or station I6 (brackish water conditions) and diffusion chambers (tests and controls), prepared as described in the text, were immersed hanging from buoys.

One "blank" chamber, filled with the same water as that in each water bath, was used to assess the dilution effect on bacterial abundance and total bacterial activity (entrance of water due to the reduction of pressure inside the chambers associated to the decrease in volume after repeated sampling). Further testing allowed us to assume that the water was entering the chamber only through the surface of the membrane and not through the top opening or through the junctions of the membrane to the body of the chamber (transport of bacterial cells was not observed).

Two sets of 7 diffusion chambers were completely filled with water either from N1 or from I6. The marine N1 tank received four N1 chambers (1 blank + 3 controls) and three I6 chambers (3 tests). In the brackish water incubation system the set-up involved four

chambers with I6 water (1 blank + 3 controls) and three chambers with N1 water (3 tests). All the chambers were kept completely immersed, and in an upright position (with the cap just surfacing) with the help of individual buoys. Time recording was initiated at the moment of immersion. Test samples were exposed to contrasting water characteristics for a maximum of 6 hours, corresponding approximately to the duration of one half of a tidal cycle. After 2 and 4 hours, chambers were emerged and a volume of 30 ml of water was collected from each chamber with the help of a silicone tube connected to a plastic syringe. The three N1 test or control sub-samples and the three I6 test or control sub-samples were pooled to obtain the volume of sample necessary for the determinations of all variables. The blank chamber in each incubation system was also emerged after 2 and 4 hour and the total volume of water inside the chamber was measured. From this volume, an aliquot of 30 ml was withdrawn in order to approach the conditions in the test and control chambers. The remaining volume of water was again inserted in the blank chamber that was then immersed back into the respective tank. At the end of the exposure period, the volumes of water remaining inside the blank, control and test chambers were measured.

The assessment of the variation of bacterial abundance and activity during the period of exposure, was performed through the determination of ectoenzymatic activities (leucine-aminopeptidase and ?-glucosidase), Vm of glucose incorporation, and bacterial biomass production after 2, 4 and 6 hours of incubation. TBN was also determined at the same time intervals with exception of the February experiment, when TBN was determined only at the beginning and at the end of the experiment. The methodologies involved were described above.

Reversion of exposure

In one of the experiments (June 1998), an attempt was made to observe the effects of reverting the marine and brackish water bacteria to the approximate conditions of their natural environment after 2 hours of exposure to contrasting water. For this purpose, one extra set of 3 test chambers was included in each tank during the first 2 hours and then transferred to the tank containing water of the same origin, where incubation continued for a further 4 hours period. The chambers to test reversion effects were also sampled after 2, 4 and 6 hours of exposure.

3. Results

Initial characterisation of marine and brackish water samples

The values corresponding to the initial properties of the marine (station N1) and brackish water (station I6) sections of the lagoon are summarised in Table 1.

Brackish water showed low or medium salinity and higher concentration of POC and chlorophyll *a*. The largest differences in salinity, seston and POC between the two samples were observed in February. On that occasion nutrients and DOC were also analysed. The results confirmed the higher availability of organic and inorganic nutrients in the brackish water samples, particularly nitrite plus nitrate (increase by a factor of 17).

Table 1: Initial physical, chemical and biological properties of the N1 and I6 water samples used in the reciprocal exposure experiments. Values of specific activities were obtained by dividing total activity by bacterial abundance (TBN).

	February 1998		March 1998		June 1998	
	N1	I6	N1	I6	N1	I6
Salinity (PSU)	32.0	10.0	31.8	18.8	30.0	21.0
$N0_2^-$+NO_3^- (µM)	9.5	162.0	ND	ND	ND	ND
PO_4^{3-} (µM)	0.41	0.75	ND	ND	ND	ND
Seston (mg l^{-1})	30.7	60.1	40.3	49.9	46.9	73.5
POC (mg l^{-1})	8.7	13.5	4.2	6.0	4.9	5.7
DOC (mg l^{-1})	7.7	15.3	ND	ND	ND	ND
Chlorophyll *a* (µg l^{-1})	3.6	6.1	1.4	2.6	4.3	6.0
Leu-AMPase Hm (nmol $l^{-1}h^{-1}$)	684	3541	1337	2713	1700	3570
?-GLCase Hm (nmol $l^{-1}h^{-1}$)	21.8	90.7	36.6	79.4	59	155
Glucose Vm (nmol $l^{-1}h^{-1}$)	4.87	9.27	1.01	5.99	2.0	4.0
BBP (µgC $l^{-1}h^{-1}$)	0.49	5.30	0.79	1.84	1.32	2.70
TBN (x 10^9 cell l^{-1})	3.3	4.2	3.1	5.0	4.7	8.2
Specific Leu-AMPase Hm (attomol $cell^{-1}h^{-1}$)	208	843	431	543	362	435
Specific ?-GLCase Hm (attomol $cell^{-1}h^{-1}$)	7	22	12	16	13	19
Specific glucose Vm (attomol $cell^{-1}h^{-1}$)	1.48	2.21	0.33	1.20	0.43	0.49
Specific BBP (fgC $cell^{-1}h^{-1}$)	0.15	1.26	0.25	0.37	0.28	0.39

Bacterioplankton characteristics

Bacterial communities in I6 water, at low tide, were 1.3-1.7 denser and up to 10.8 times more active than the communities from the outer marine zone, at high tide (Table 1). TBN ranged from 4.2 to 8.2 x 10^9 cell l^{-1} in samples from I6 and from 3.1 to 4.7 x 10^9 cell l^{-1} in samples from N1. Leu-AMPase Hm was 2713-3570 nmol $l^{-1}h^{-1}$ in I6, 2.0-5.2 times higher than the corresponding values in N1 water. On a per cell basis, the Leu-AMPase activity in the brackish water zone varied in the range of 435-843 attomol $cell^{-1}$ h^{-1}, 1.2-4.1 times higher than in cells from the marine zone.

?-GLCase Hm at I6 varied from 79.4 to 155.0 nmol $l^{-1}h^{-1}$, an activity that was 2.2-4.2 times higher than at N1 (Table 1). This represented a cell specific activity 1.3-3.1 times greater than at N1.

Initial values of glucose Vm ranged from 1.0-4.9 nmol $l^{-1}h^{-1}$ in N1 samples and from 4.0-9.3 nmol $l^{-1}h^{-1}$ in I6 samples, representing 1.9-5.9 times greater total activity and 1.1-4.0 times greater specific activity (Table 1).

BBP ranged from 1.84 to 5.30 µgC $l^{-1}h^{-1}$ at station I6 and from 0.49 to 1.32 µgC $l^{-1}h^{-1}$ at station N1. Specific BBP in I6 bacterioplankton ranged from 0.87 to 1.26 fgC $cell^{-1}$ h^{-1}, 1.4-8.4 times higher than for N1 bacteria. The largest difference observed in the variables, which described the two initial bacterioplankton communities, was exhibited by BBP for which the I6 values were 2.1 to 10.8 times greater (Table 1).

Time-course of the bacterial response to contrasting water quality

Salinity was used as a tracer to evaluate water exchange during the experiments. The results showed a marked variation in salinity after 2 hours of exposure to contrasting water (Fig. 3). After 4 to 6 hours, salinity equilibrium

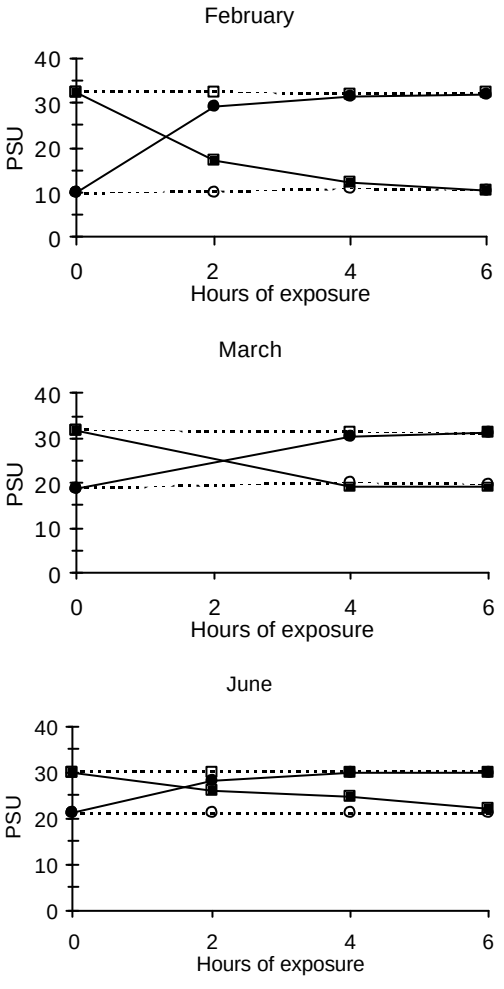

Fig. 3: Salinity values inside the diffusion chambers (tests and controls) during 6 hours of reciprocal exposure to dissimilar water.
(--? -- N1 control; ? ¦ ? N1 test; --✍-- I6 control; ? ☞? I6 test)

was achieved. The variation in the concentration of $NO_2^-+NO_3^-$ and PO_4^{3-} (Fig. 4) indicates that concentrations of dissolved substances followed a pattern similar to that of salinity. Thus, there was effective percolation of water and solutes through the membranes.

Microbiological variables responded quickly to exposure to the contrasting water (Fig. 5). Usually, after only 2 hours, there was a decrease in the activity of the brackish water bacterioplankton incubated in outer-estuary water, and an increase in the activity of the outer-estuary bacterial community incubated in brackish water, relative to the control values.

The amplitude of positive or negative effects was different between experiments and between different variables. In general, however, the absolute magnitudes of the positive and negative effects were similar. Ectoenzymatic activities seemed to be less affected by the shifting of environmental properties. Response coefficients, calculated as the ratios between the values determined in the test and in the corresponding control populations (Fig. 6), indicated lower intensity of the responses of Leu-AMPase Hm (0.49-2.41) and ?-GLCase Hm (0.58-2.53) than those of glucose Vm and BBP (response coefficients of 0.14-3.84 and 0.21-2.90, respectively). In the February experiment, ectoenzymatic activities showed particularly week responses. The values of the response coefficient were 0.8-1.2 for Leu-AMPase Hm and 0.7-1.3 for ?-GLCase Hm. In the same experiment glucose Vm and BBP responded more intensely generating response coefficients of 0.7-2.3 and 0.3-2.9, respectively.

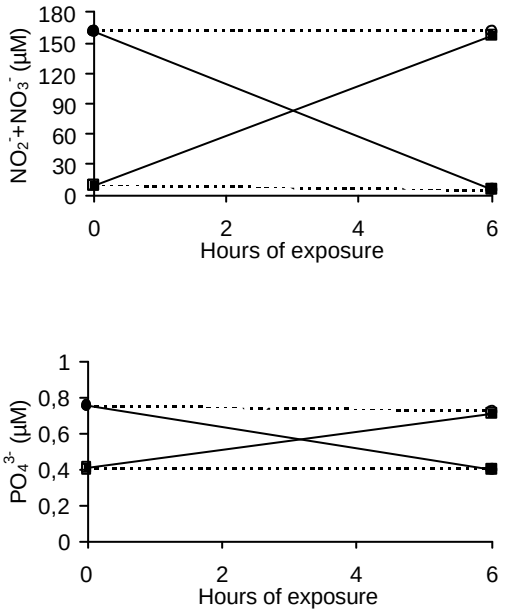

Fig. 4: Concentration of $NO_2^-+NO_3^-$ and PO_4^{3-} inside the exposed chambers (tests and controls) during the February experiment. (--?-- N1 control; ?¦? N1 test; --✍-- I6 control; ?☞? I6 test)

The variation of bacterial abundance (TBN) in response to changes in environmental conditions was low and the differences between tests and controls were not statistically significant. As a consequence, and as a general pattern, the responses in specific activity rates of the marine communities exposed to estuarine water, and of the estuarine communities exposed to marine water (Fig. 7) were, obviously, opposite.

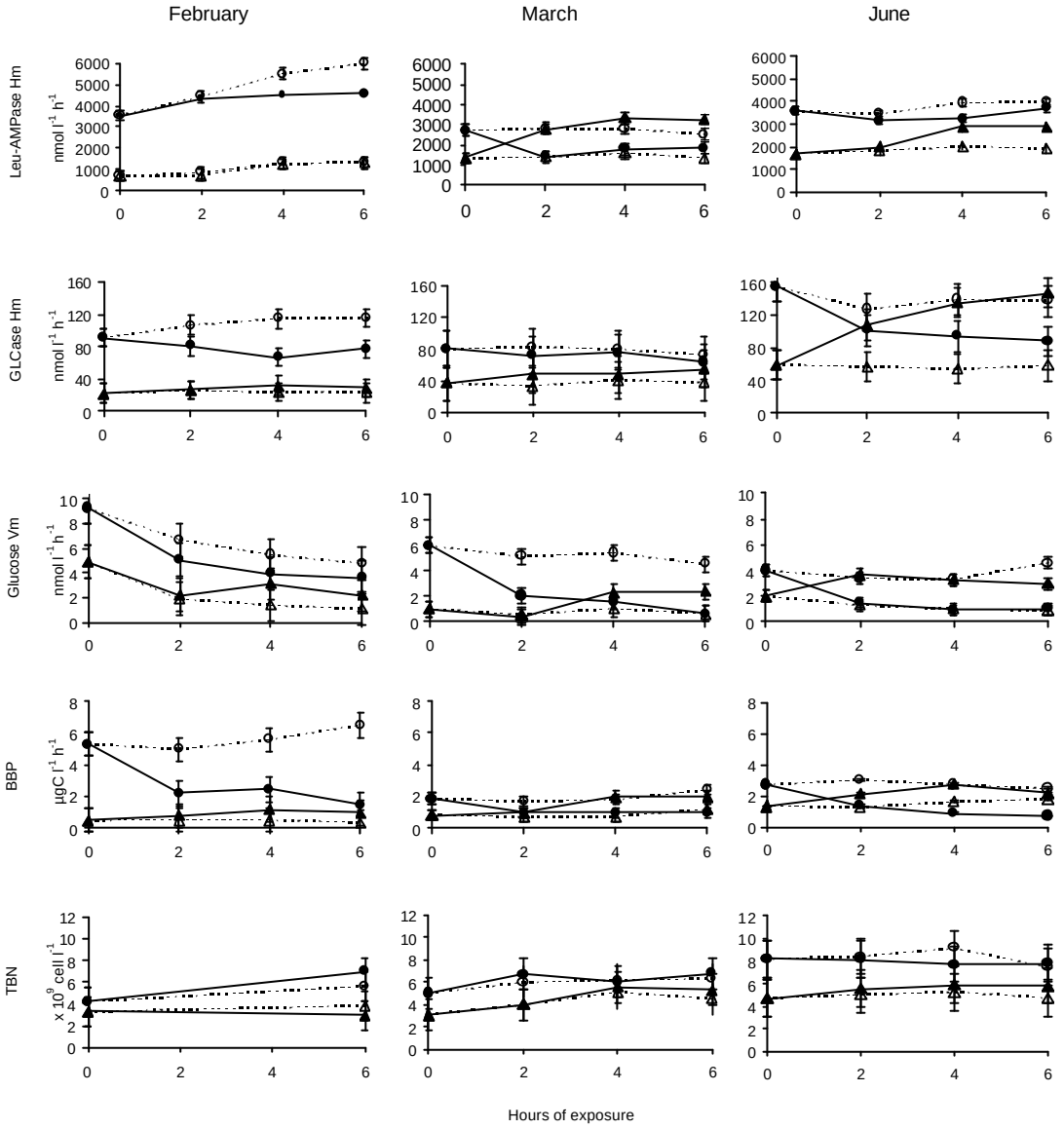

Fig. 5: Variation in activity (Leu-AMPase Hm, ?-GLCase Hm, Glucose Vm, and BBP) and abundance (TBN) of the bacterioplankton inside the diffusion chambers (tests and controls). Error bars represent the least significant difference between samples (LSD) calculated after ANOVA analysis. (--?-- N1 control; ? ? ? N1 test; --✍-- I6 control; ? ☞? I6 test)

Reversion exposure effects

In June, a parallel test on the reversibility of the effects of exposure was carried out. Samples from the marine station (N1) exposed to water collected at the inner station (I6) responded by increasing the total and specific activity rates, generating, in this way, response coefficients greater than one. However, when after 2 hours the test samples were transferred back to the marine incubation conditions, there was a strong reversion of the

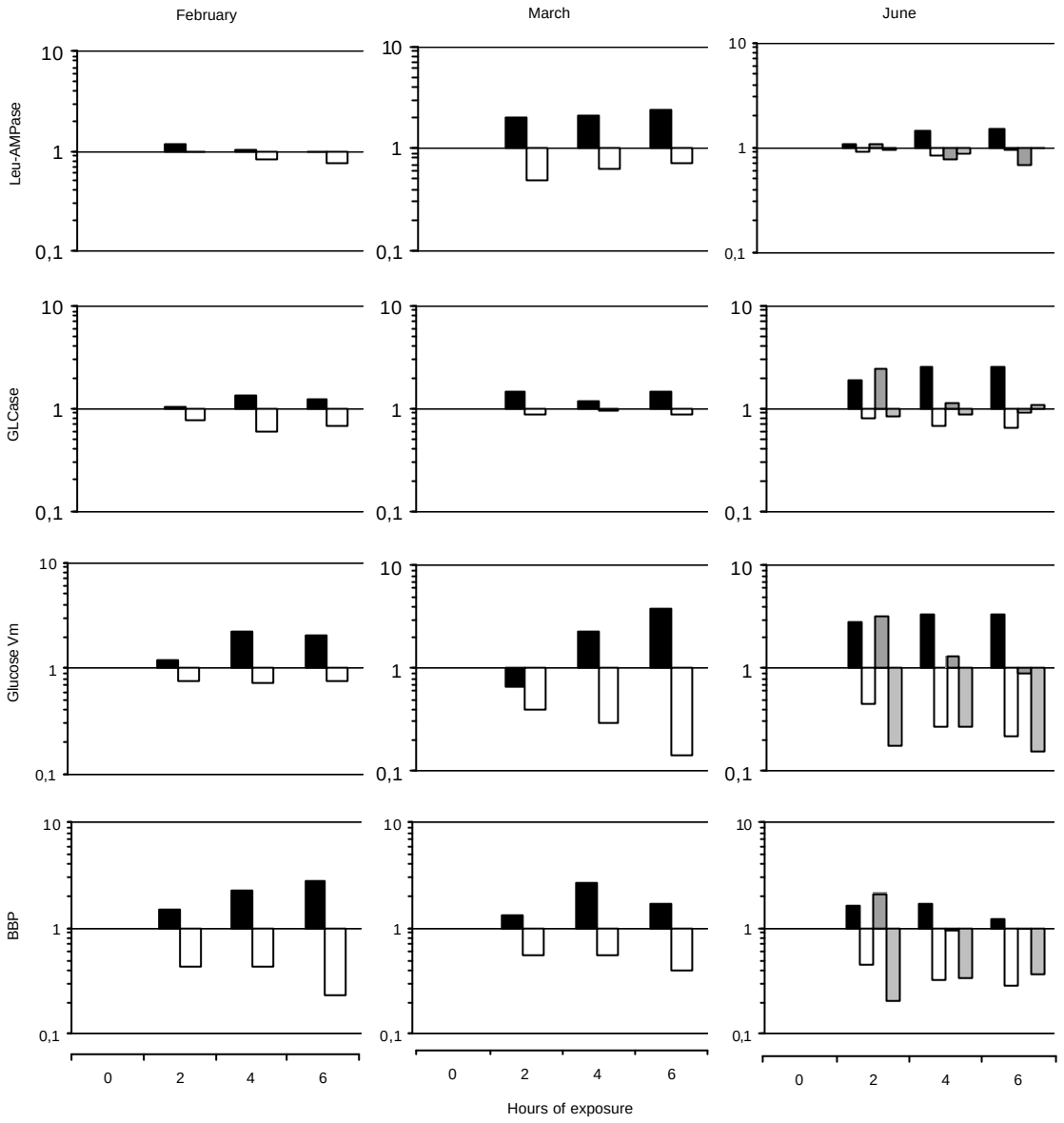

Fig. 6: Response coefficients (test/control ratios) corresponding to the reciprocal exposure experiments expanded to include reversion to the natural conditions after the initial 2 hours in June. Values above 1 indicate increase and values below 1 indicate decrease in the values of the different activities, when compared with the corresponding controls. (☞ N1 test; ☞ I6 test; ■ N1 reversion test; □ I6 reversion test)

responses and, therefore, a decrease of the respective coefficients to values close to one. Both the primary response and the reversion effect occurred within a short period, usually less than 2 hours. Leu-AMPase was particularly sensitive. Reversion produced a reduction in Leu-AMPase activity to values that were lower than in the control N1 chambers.

Samples from the estuarine station I6 exposed to marine water incubation were negatively affected. However, when returned to the estuarine water tank, only ?-GLCase

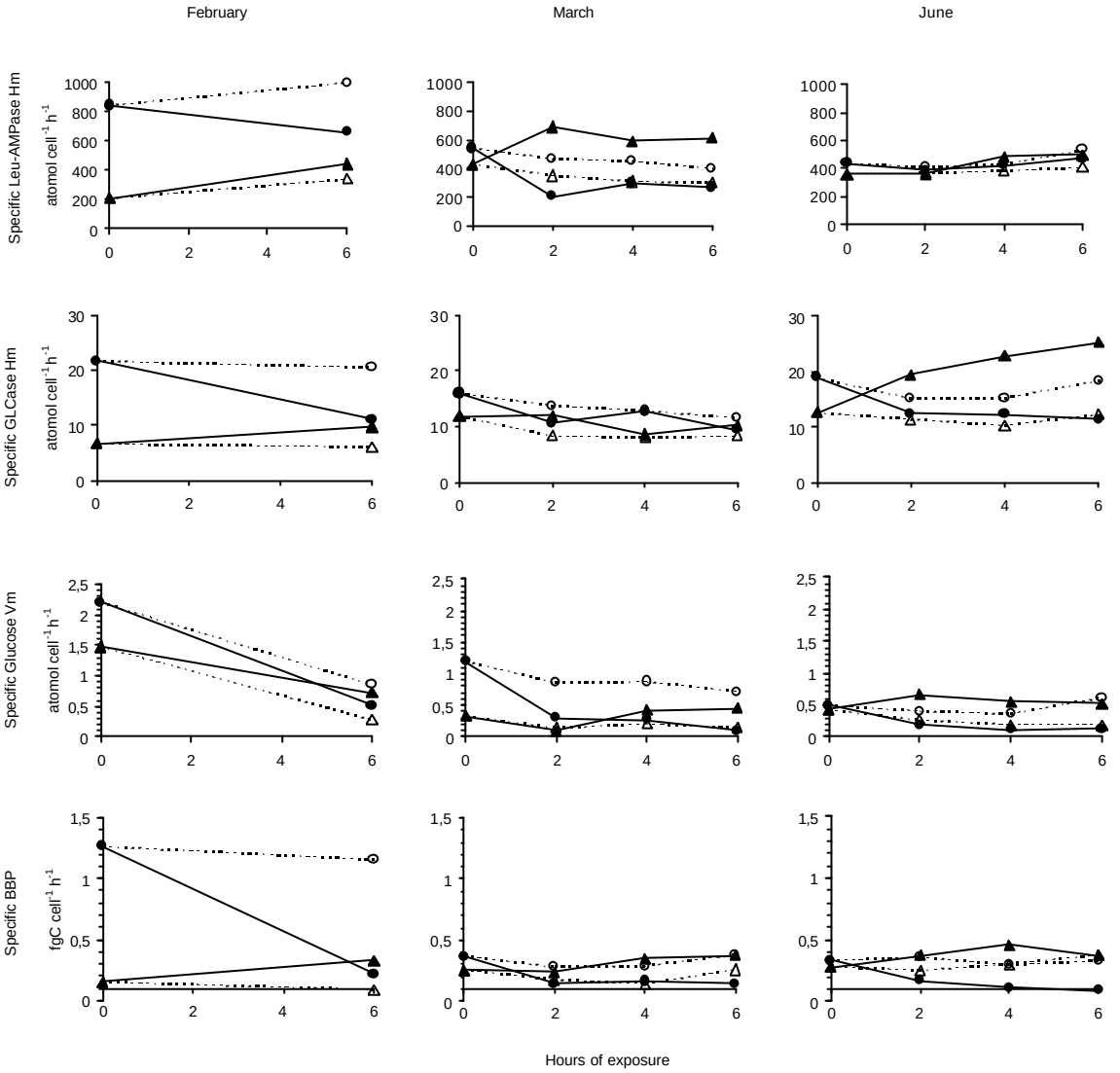

Fig. 7: Variation of bacterial activity per cell (specific activity) inside the diffusion chambers in response to the reciprocal exposure experiments. (--? -- N1 control; ? ? ? N1 test; --✍-- I6 control; ? ☞? I6 test)

activity showed recovery and regained, over the next 4 hours, the level of activity of the I6 control. Other activity variables did not recover from the negative effect of exposure to marine water.

4. Discussion

The available data on the properties of the water column and the sediment along Canal de Ílhavo indicate a pattern of increasing eutrophication towards the inner estuary, a

fact that underlies the responses observed in these experiments. The concentrations of seston and POC were 1.2-2.0 times higher in I6 than in N1, reflecting different inputs of particles from the fresh water stream Rio Boco, at the southeastern end of Canal de Ílhavo, local discharges along the channel and eventual resuspension of bottom sediments. Regardless of some patchiness, sediments from stations N1 and I6 correspond dominantly to medium sand. They differ, however, in the organic content which was estimated as 2.9 % of the dry weight at I6 and 0.8 % at N1 [9]. Higher chlorophyll concentrations give the indication of greater eutrophication in the mid sections of the estuary, also associated with exceptionally high concentrations of $NO_2^- + NO_3^-$ (17 times higher than at station N1). Primary production at the brackish water section was estimated as 3.31 µgC l^{1} h^{-1} at LT while the HT value closer to the mouth was 0.13 µgC l^{-1} h^{-1} (Almeida, unpublished data). I6 water seemed to have a slightly better quality of particulate substrates. Assuming the conversion factor of 50 [11], phytoplankton-C represented 1.7-4.4 % of the total POC at station N1 and 2.2-5.3 % at station I6. The results of the February experiment also showed higher concentrations of dissolved organic substrates in I6 water.

The bacterioplankton communities, which were contrasted in our study, differed less in density than in activity, at the beginning of the experiments. The bacterial communities found at mid-estuary during LT were denser and more active than those from station N1. The ratios derived from the initial values of the two communities (I6/N1) were 1.3-1.7 for TBN, 2.0-5.2 for Leu-AMPase, 2.2-4.2 for ?-GLCase, 2.2-4.2 for glucose Vm and 2.0-10.8 for BBP. Higher rates of total plankton respiration at the brackish water section of the Ria de Aveiro had already been reported [9]. When specific activities were calculated they revealed that the average brackish water bacterial cell was 1.1 to 8.4 times more active than its marine counterpart. Changes in specific activity have been reported as reflecting an intrinsic difference in the composition [22] and/or in the activity of marine and estuarine bacterial communities [21], which could, in this case, be an indication of the presence of communities adapted to different nutritional environments along the estuarine gradient. It has been shown that the experimental addition of protein to estuarine bacterioplankton communities produced, within 1-4 days, considerable changes in the composition of the community, in terms of the relative importance of particular phylogenetic groups, as well as the increase in growth rate [29]. The long residence time of fresh water estimated for the eutrophic mid section of Canal de Ílhavo (10.1-97.4 days) [33] would be compatible with strong changes in the composition of bacterial communities which could also be expressed by different responses to variation in salinity and nutrient

supply.

The values of abundance and activity determined for the two bacterial communities fit within the ranges published for other highly productive coastal systems [4, 12, 17, 18, 19]. High potential for protein degradation (Leu-AMPase) has been found in previous work in the Ria de Aveiro [10].

The primary question regarding the existence of distinct bacterial communities in different sections of the estuary was addressed by characterising the effects of the reciprocal swapping between contrasting conditions in terms of response time, intensity and reversibility.

Both communities responded rapidly, within the initial 2-4 hours of exposure. Samples from the same sites amended with simple (glucose, mixture of 10 amino acids, complex B vitamins) and complex (yeast extract, commercial algae exudates) organic substrates in microcosm experiments showed significant variations of activity within 6 hours of incubation, but the fraction of CTC-active cells remained quite constant (manuscript in preparation). This may indicate that the observed short-term responses are more due to the variation of activity rates of individual active cells than to variation of proportion of active cells in the total community.

Responses expressed by hydrolytic and uptake enzymatic systems were probably not related to the production of the relevant enzymes. Shifting between different enzymatic systems with distinct kinetic characteristics [36], and the direct effects of salinity on the structure and integrity of enzymatic systems, could explain the ready variation of the levels of activity. In fact, preliminary results showed that the increase of salinity of I6 water from 10 to 30 PSU by addition of NaCl produced an immediate reduction of Leu-AMPase and ?-GLCase activities to values corresponding respectively to 53 and 42 % of the initial Hm values (data not shown).

Changes in water properties had a greater effect on the direct uptake of small molecules (Glucose Vm and BBP according to the method) than on ectoenzymatic activities. This may be related to the variability of the organic sources. Monomer uptake responds to variations in the DOM pool [29, 31, 32], which in this study was larger in the brackish water zone. Ectoenzymatic activity, on the contrary, may be regulated by the availability of the more complex substrates which could be partially adsorbed to particles and, in this form, would not exert any effect on the confined test communities since they would not percolate into the chambers through the 0.2 µm pores of the membranes. In addition, the increase in the availability of monomers after transference of marine samples to brackish water could even reduce the activity of ectoenzymatic systems [7] and, in this

way, counterbalance, to some extent, the stimulatory effect of the increase in the availability of dissolved polymers.

In the March and June experiments, when the salinity values at the brackish water section were quite high (> 18 PSU), responses were stronger and bacterial activity in the test samples reached levels identical to those of the natural community of the contrasting water type. This could imply that the communities were not significantly distinct and that the expression of activity would be highly susceptible to environmental control. However, in February, when the salinity difference between the initial N1 and I6 samples was maximal, the reaction of the marine community to brackish water conditions was weak (BBP and glucose Vm) or undetectable (Leu-AMPase and ?-GLCase). This could be expected from a marine community resenting the lower salinity of the brackish water and so being less capable of taking advantage of higher concentration of organic substrates. Some parallel experiments revealed that, during summer, the stimulation of the marine community exposed to water from the inner section of the estuary was enhanced if NaCl was added up to a salinity of 34 PSU (Almeida, unpublished data). These results could indicate the existence of communities with distinct salinity optima.

The different reversibility of the responses was interpreted as a further indication of the distinct nature of the two communities. The stimulation of the marine community rapidly ceased after transference to marine water. On the contrary, brackish communities did not recover from the loss of activity and their inhibition generally persisted even after 4 hours of incubation in their natural water. The unrecoverable loss of activity was not accompanied by significant variation in bacterial abundance. This supports the assertion that viability, rather than the recognisable size of the community, was negatively affected by salinity, eventually in an irreversible way.

The short-term reactivity of bacterioplankton to changes in salinity and in the degree of eutrophication in a shallow estuary such as the Ria de Aveiro, is compatible with the hypothesis of activation and deactivation of bacterial cells during tidal transport and has major implications in the understanding of estuarine profiles of bacterioplankton activity. The stimulation of marine bacterial cells during flood transport, and the opposite effect on brackish-water bacteria during the ebb, will magnify the difference in heterotrophic activity between the outer and mid sections of the estuary and produce steeper gradients than those expected from the conservative mixture of distinct communities.

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CHAPTER V
ECTOENZYMATIC ACTIVITY AND GLUCOSE HETEROTROPHIC METABOLISM IN A SHALLOW ESTUARY (RIA DE AVEIRO, PORTUGAL): INFLUENCE OF BED SEDIMENTS AND SALT MARSHES

Cunha, M. A., Almeida, M. A. and Alcântara, F. (2001)

Acta Oecologica (submitted)

Abstract - In order to assess the relative importance of tidal resuspension of sediments and transport of particles from salt marshes as factors of variability in bacterioplankton abundance and activity, a transect was defined at one of the most productive sections of a shallow estuarine system (Ria de Aveiro, Portugal). Bacterial abundance, ectoenzymatic activity and heterotrophic metabolism of glucose were assayed in samples collected at four sites in the transect during four springtime tidal cycles.

The patterns of variation of particulate materials (seston, POC and chlorophyll *a*) along the transect did not show significant relation with proximity to the margin or to the bottom sediment. Nevertheless, proximity to the salt marsh or to the bottom sediment surface favoured glucose incorporation and aminopeptidase activity. A multiple stepwise regression analysis using temperature, salinity, seston, POC and chlorophyll *a* concentration as independent variables could explain a relatively small proportion (12 – 43 %) of the observed variability in different bacterioplankton parameters, with the exception of the fraction of particle-attached bacteria (66.5 % of the variability was explained).

Resuspension and runoff, in the strict sense of transference of particulate materials, could not be clearly distinguished in the spatial patterns of variation of the particulate variables. This, together with the poor contribution of these parameters to the transversal and tidal variability of bacterial activity, dismisses the importance of inputs of suspended material across the sediment/water interface and from neighbour salt marshes in the control of bacterial activity, that, instead, may be exerted through DOM fluxes.

Key words: Estuarine bacterioplankton; ectoenzymatic activity; heterotrophic activity; sediment resuspension.

1. Introduction

Different processes of transference of materials between benthic and planktonic compartments, as well as between salt marshes and the main estuarine body, may intervene significantly in the supply of substrates to the plankton. This contribution may be decisive in the dynamics of estuarine bacterioplankton. Maxima of bacterial abundance and heterotrophic activity along estuarine gradients are often found in high turbidity zones (Erckenbracher and Stevenson, 1975; Wright and Coffin 1983; Fuks *et al.*, 1991)

indicating a tight coupling between particle inputs and stimulation of bacterial heterotrophic activity. Bacterial communities are able to respond quickly to these inputs because of their rapid growth rates and high physiological diversity (Wainright, 1987). Therefore, resuspension events and runoff from the margins are sometimes vented as possible explanations for high productivity at mid and inner estuarine sections (Hoppe *et al*., 1996).

Several studies have shown that mechanical resuspension of sediment particles either by tidal currents, wind forcing, wind-induced waves, dredging or bottom trawling, caused significant increase in seston content, particulate organic carbon, particulate organic nitrogen, chlorophyll *a* and bacterial biomass (Demers *et al*., 1987; Riemann and Hoffmann, 1991; Ritzrau and Graf, 1992). Different types of experimental approaches have confirmed these field observations. Bacterial biomass production significantly increased in water samples that were in physical contact with the sediment surface and the stimulation was more intense when sediments were heavily colonised by microphytobenthos (Middelboe *et al*., 1998). Bacterial abundance and total cell volume responded positively to amendment of natural seawater with resuspended particulate material (Wainright, 1987). Induced sediment resuspension in mesocosm experiments also increased chlorophyll *a* concentrations in the water and caused significant stimulation of bacterioplankton biomass production (Sloth *et al*., 1996).

Densely colonised salt marshes frequently found in the banks of estuarine channels may also export considerable amounts of materials to the main body of the estuary (Dame *et al*., 1986) through processes of advective transport of solutes and particles. However, the dissolved compounds may dominate the net flux of organic carbon from the marshes to the estuary (Taylor and Allanson, 1995).

Previous studies on the dynamics of bacterioplankton in the Ria de Aveiro allowed the identification of the shallow sections of different channels as sites of intense bacterial heterotrophic activity (Hoppe *et al*., 1996; Cunha *et al*., 1999; Cunha *et al*., 2000). Here, we report on a field approach to test the hypothesis that particles transported from the salt marshes and/or resuspended from the sediment may stimulate the heterotrophic activity of bacterioplankton communities and are ultimately related to the establishment of high rates of ectoenzymatic activity and glucose heterotrophic metabolism at an intermediate section of one of the main channels (Canal de Ílhavo) of the lagoon.

2. Materials and methods

Study site

Ria de Aveiro (Figure 1) is a branched estuarine ecosystem, also described as a coastal lagoon, in the Northwest coast of Portugal, with the maximum length and width of 45 and 10 Km, respectively. The tidal range at the mouth varies from 0.6 m in neap tides to 3.2 m in spring tides (Dias *et al.*, 2000). The average depth of the lagoon is 1 m and the shallow brackish water sections (< 3 m deep) account for approximately 95 % of the total wet area at high tide (83 Km^2) and together, encompass 75 % of the total volume of water of 161 Mm^3 (Silva, 1994; Dias *et al.*, 2000). The deeper marine zone varies in depth from 10 to > 20 m at high tide.

This study took place at the transect I6 located at the shallow brackish water section of Canal de Ílhavo, with its origin at the main navigation channel and extending to Southeast. Along the transect, 4 sampling sites were defined: S1 and S2 respectively at approximately 0.5 and 1.5 m from the west margin; S3 and S4 referred to the centre of the channel (taken as the deeper point) were located respectively at 5 m from the centre (measured westwards) and at the centre itself.

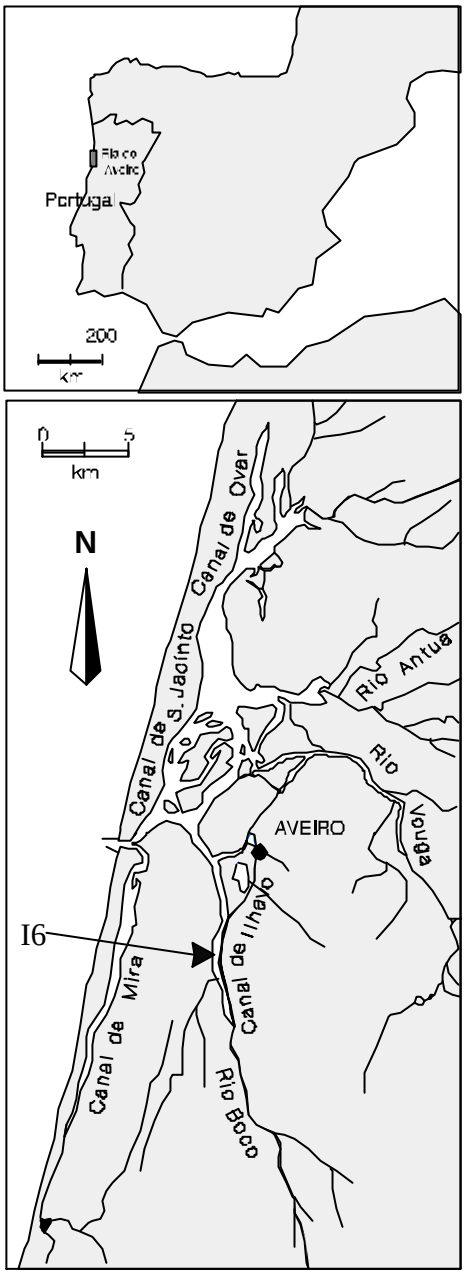

Figure 1: Iberian Peninsula and Ria de Aveiro lagoon (Portugal) with sampling transect I6 at Canal de Ílhavo indicated by the arrow.

Sampling

Transect I6 was visited 4 times from April to May 1997, under spring (6^{th} and 22^{nd} May) and neap (29^{th} April and 30^{th} May) tide conditions. Water samples were collected 0.2 m below water surface at all stations and also at 0.5 m above the sediment surface at the deeper sites (S3 and S4). In

each sampling day, samples were collected at 6 moments of the tidal cycle: low tide (LT), 2 hours after low tide (LT+2), 2 hours before high tide (HT-2), high tide (HT), 2 hours after high tide (HT+2) and 2 hours before low tide (LT-2). All samples were kept cold and in the shade during transport to the laboratory where they were processed within 2-3 hours from collection.

Physical and chemical characteristics

Temperature and salinity were measured with a WTW LF 196 Conductivity Meter. Depth of the water column was determined with a Sonar probe (Hondex PS-7 LCD Digital Sounder). Approximate distance to the west margin was visually estimated.

The concentrations of total (seston) and organic (POM) suspended solids were determined according to Parsons *et al.* (1989). Particulate organic carbon (POC) was assumed to be 50% of the POM (Rodier, 1996). Chlorophyll *a* was quantified fluorimetrically (Yentsch and Menzel, 1963) in a Jasco FP-777 espectrofluorimeter after extraction with 90% acetone.

Bacterioplankton characteristics

The densities of the total bacterial community (TBN) and of the particle-attached fraction (AB) were determined by direct counting under epifluorescence microscopy (Leitz Laborlux) after staining with 0.03% acridine orange of cells collected on Poretics 0.2 µm black polycarbonate membranes (Hobbie *et al.*, 1977).

Ectoenzymatic activity was determined fluorimetrically (Jasco FP-777 Fluorometer) as the maximum hydrolysis rate (Hm) of two model substrates (Hoppe, 1983), 4-methylumbelliferyl-?-D-glucoside for ?-glucosidase (BTGLase) and L-leucine-7-amido-4-methyl-coumarin for Leu-aminopeptidase (AMPase), added up to a saturating concentration of 1 mM. Samples were incubated 1-2 hours at *in situ* temperature. For the discrimination of the contribution of attached bacteria to total ectoenzymatic activity, water samples were fractionated by gentle vacuum filtration (< 50 mmHg) through 1.2 µm pore cellulose nitrate membranes (Sartorius). Determinations of ectoenzymatic activities were performed in the whole water samples and in the < 1.2 µm fractions.

The heterotrophic metabolism of glucose was described by the parameters Vm (maximum uptake velocity) and Tr (turnover rate) following the procedure described by Gocke (1977). Vm was determined after the addition of a final saturation concentration of

430 nM of ^{14}C-glucose (Amersham, SA 11.5 GBq mmol^{-1}, 310 mCi mmol^{-1}). Incubations were carried out for 2 hours at in situ temperature. Cells were later collected on Poretics 0.2 µm polycarbonate membranes and radioactivity was read in a liquid scintillation counter Beckman LS 6000 IC using UniverSol as the scintillation cocktail. For Tr determinations a tracer concentration of 43 nm of ^{14}C-glucose was used. The incorporated fraction was determined after collection of cells on 0.2 µm Poretics polycarbonate membranes and the respired fraction was determined after acidification of the sample and capture of the $^{14}CO_2$ on ethanolamine (Merck).

Statistical analyses

The significance of the effect of the sampling site on the variability of chlorophyll *a*, POC and bacteriological parameters was analysed by one-way ANOVA. Homogeneity of variances was verified by Levene tests. Tukey Honestly Significant Difference (HSD) was used for Post Hoc Multiple Comparison.

Multiple correlation analysis was performed with the aim of extracting statistically significant relations between biological parameters describing bacterial abundance and activity on one side, and on the other, either distance to the margin or to the sediments.

A multiple stepwise regression analysis was used to identify major environmental factors controlling tidal and transversal fluctuations of bacterial abundance and activity and to quantify their relative contribution to total variability. Temperature, salinity, seston, POC (total or as a percentage of seston) and chlorophyll *a* were used as independent variables.

All statistical analyses were performed with the SPSSWin 7.1 package.

3. Results

Environmental variables

The range of values of parameters related to water quality is summarized in Table 1. The numbers correspond to the average, minimum and maximum values registered at each sampling site during the series of six sampling moments during four tidal cycles. The 2 sub-tidal sites (S3 and S4) were characterized by depths with tidal averages of 2.2-2.6 m in spring tides and 2.2 m in neap tides. The inter-tidal site S2 was only slightly shallower (tidal averages of 1.9-2.7 m) while the spatially fluctuating site S1 was defined through a

fixed depth of 0.2 m.

Table I: Tidal average (Avg), minimal (m) and maximal (M) values of salinity, temperature, depth and seston concentration at surface (0.2 m) and bottom (0.5 m from sediment surface) layers of the water column, at 4 sampling sites along transect I6 (S1, S2, S3, S4) in 4 tidal cycles.

			Salinity (PSU)			Temperature (ºC)			Depth (m)			Seston (mg·L^{-1})		
			Avg	m	M	Avg	m	M	Avg	m	M	Avg	m	M
29 April	Surface	S1	27.7	23.7	31.1	20.9	18.8	22.9	0.2	0.2	0.2	53.3	50.0	60.0
		S2	27.8	23.8	31.1	20.9	18.8	22.8	1.9	0.5	2.8	53.0	49.0	63.0
		S3	27.8	23.8	31.1	20.8	18.8	22.7	2.2	1.7	2.8	52.8	46.0	71.0
		S4	27.7	24.0	31.0	21.1	18.8	23.5	2.2	1.3	2.8	51.5	46.0	65.0
	Bottom	S3	27.8	23.9	31.0	20.8	18.8	22.7				53.3	40.0	74.0
		S4	27.7	24.0	31.0	21.1	18.9	23.5				54.0	44.0	69.0
6 May	Surface	S1	30.6	26.8	33.6	17.6	17.0	18.0	0.2	0.2	0.2	74.2	64.0	106.0
		S2	30.8	26.7	34.0	17.5	17.0	18.0	2.7	2.0	3.6	72.7	57.0	89.0
		S3	30.6	26.7	33.6	17.5	16.9	18.0	2.6	1.7	3.6	70.0	46.0	95.0
		S4	30.7	26.7	33.5	17.6	17.0	18.0	2.6	2.0	3.3	65.8	44.0	84.0
	Bottom	S3	30.8	26.7	33.9	17.5	16.9	18.0				77.2	52.0	99.0
		S4	30.8	26.8	33.7	17.6	17.0	18.0				75.2	43.0	110.0
22 May	Surface	S1	24.9	18.2	32.3	19.8	18.5	21.1	0.2	0.2	0.2	54.5	45.0	62.0
		S3	24.9	18.3	32.9	19.8	18.7	20.9	2.2	1.7	2.7	57.5	49.0	66.0
		S4	25.0	18.8	32.9	19.8	18.8	20.9	2.3	1.7	3.0	55.8	48.0	63.0
	Bottom	S3	25,1	18,3	33,0	19,8	18,7	20,9				57,5	50,0	66,0
		S4	25,6	18,8	33,6	19,7	18,7	20,6				59,2	52,0	67,0
30 May	Surface	S1	20,0	11,4	28,5	22,4	21,3	23,2	0,2	0,2	0,2	50,2	42,0	55,0
		S3	19,9	11,4	28,0	22,5	21,7	23,3	2,2	1,5	3,0	48,7	38,0	57,0
		S4	19,9	11,4	28,6	22,5	21,5	23,3	2,2	1,8	3,0	47,2	36,0	55,0
	Bottom	S3	20,3	11,3	28,7	22,4	21,3	23,3				50,3	39,0	59,0
		S4	20,4	11,6	28,9	22,4	21,3	23,3				51,0	40,0	61,0

Temperature and salinity showed little variation along the transect as well as with depth. Seston varied within the range of 36-110 mg l^{1}. Maximal values were generally found at the bottom of the water column of the deeper site S4 while minima were often registered at surface.

Chlorophyll *a* concentration varied from 0.2 to 7.0 µg l^{1} (Figure 2). In each tidal cycle, minima occurred generally at the bottom of the water column and maxima at surface but no well established vertical or transversal pattern could be noticed. The tidal pattern was clear with decreasing concentrations in flood tide and increasing levels in ebbing water. POC concentration varied within the range of 2.0-8.5 mgC l^{1} (Figure 2) with no distinct pattern of distribution with depth. Maxima were often found at site S1.

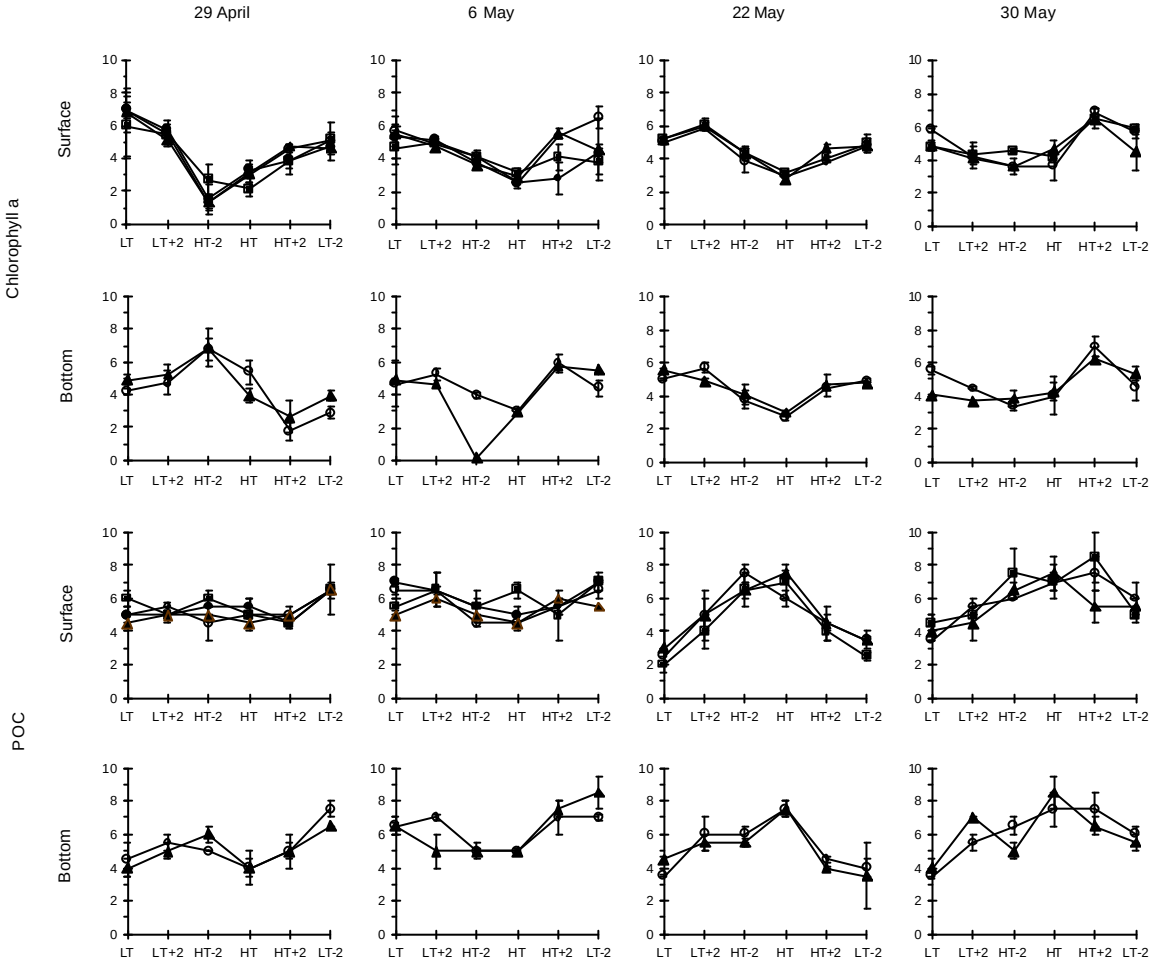

Figure 2: Fluctuation of chlorophyll *a* (µg l^{-1}) and POC (mg l^{-1}) at surface (0.2 m) and bottom (0.5 m from sediment surface) layers of the water column at 4 sampling sites along transect I6 (? ¦? S1, ? ☞? S2, ? ✍? S3, ? ? ? S4) in 4 tidal cycles.

Bacterioplankton variables

Total bacterial number (TBN) ranged 2.5-9.0 x 10^9 cell l^{-1} with maxima generally occurring at low tide and minima close to high tide (Figure 3). No distinct vertical or transversal patterns of variation were observed. The fraction of bacterial cells that were attached to particles (Figure 3) varied from 4 to 33 % of the total counts and was generally lower at high tide. Taking all the results respecting the 4 tidal cycles, the maximal percentage of attached bacteria was generally found in surface water.

Maximal ectoenzymatic hydrolysis rates varied from 396 to 4190 nmol l^{-1}h^{-1} for AMPase and from 14.5 to 489.0 nmol l^{-1}h^{-1} for BTGLase (Figure 4). AMPase maxima occurred generally in surface samples but BTGLase showed no distinct pattern in the

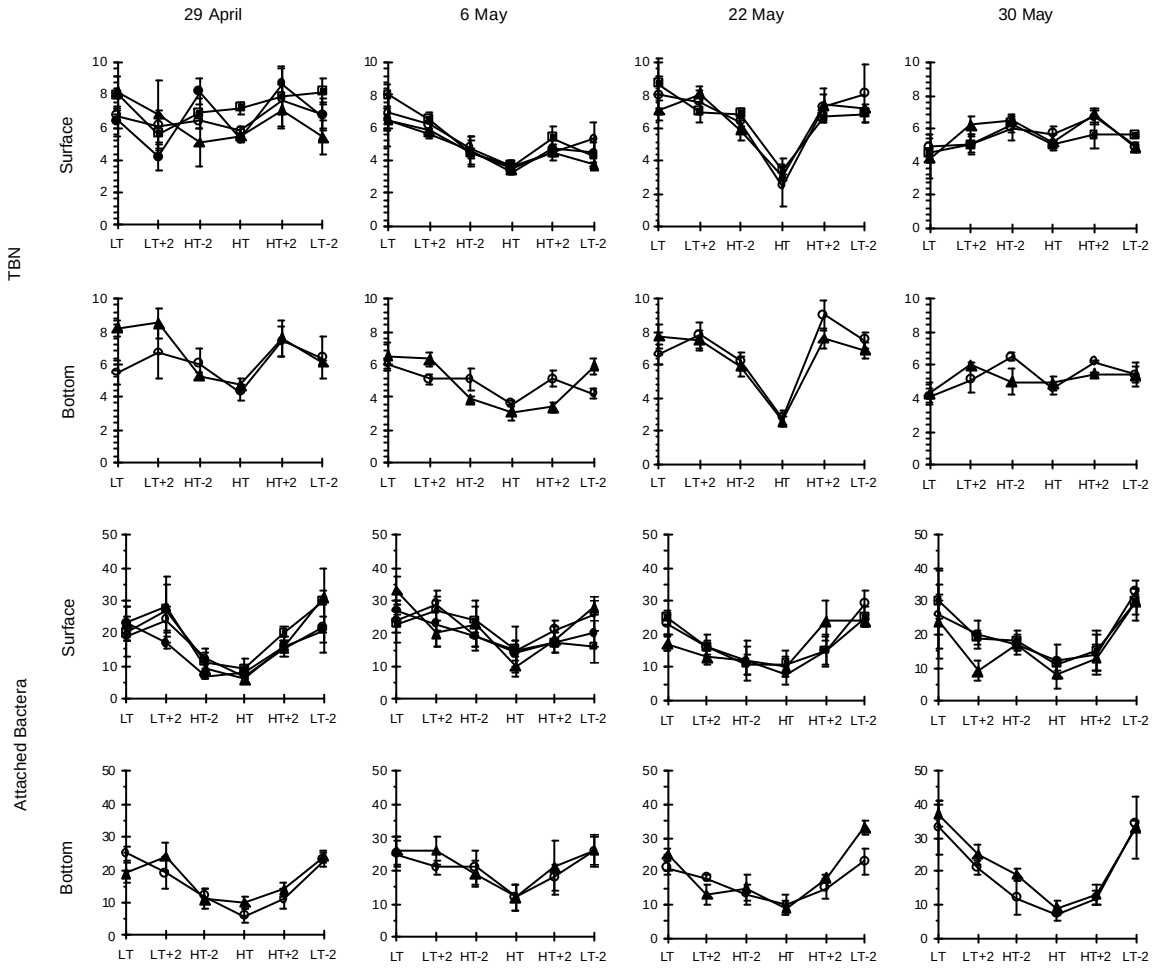

Figure 3: Fluctuation of TBN (10^9 cell l^{-1}) and attached bacteria (% of TBN) at surface (0.2 m) and bottom (0.5 m from sediment surface) layers of the water column at 4 sampling sites along transect I6 (? ¦? S1, ? ☞? S2, ? ✍? S3, ? ? ? S4) in 4 tidal cycles.

occurrence of maxima and minima. Higher activities of any of the two ectoenzymes were observed close to low tide.

The partitioning of ectoenzymatic activities between free (<1.2 µm) and attached (>1.2 µm) cells revealed that the contribution of the attached bacterioplankton could be either undetectable or compose up to 83 or 98 % of total AMPase or BTGLase activities, respectively (Figure 5). Maximal percentage of particle-associated ectoenzymatic activity was more often found in bottom water samples. The contribution of attached cells to AMPase activity showed a slight tendency to decrease during flooding and to regain importance during ebbing. This pattern was clearer on the 30th May. Tidal variation of the

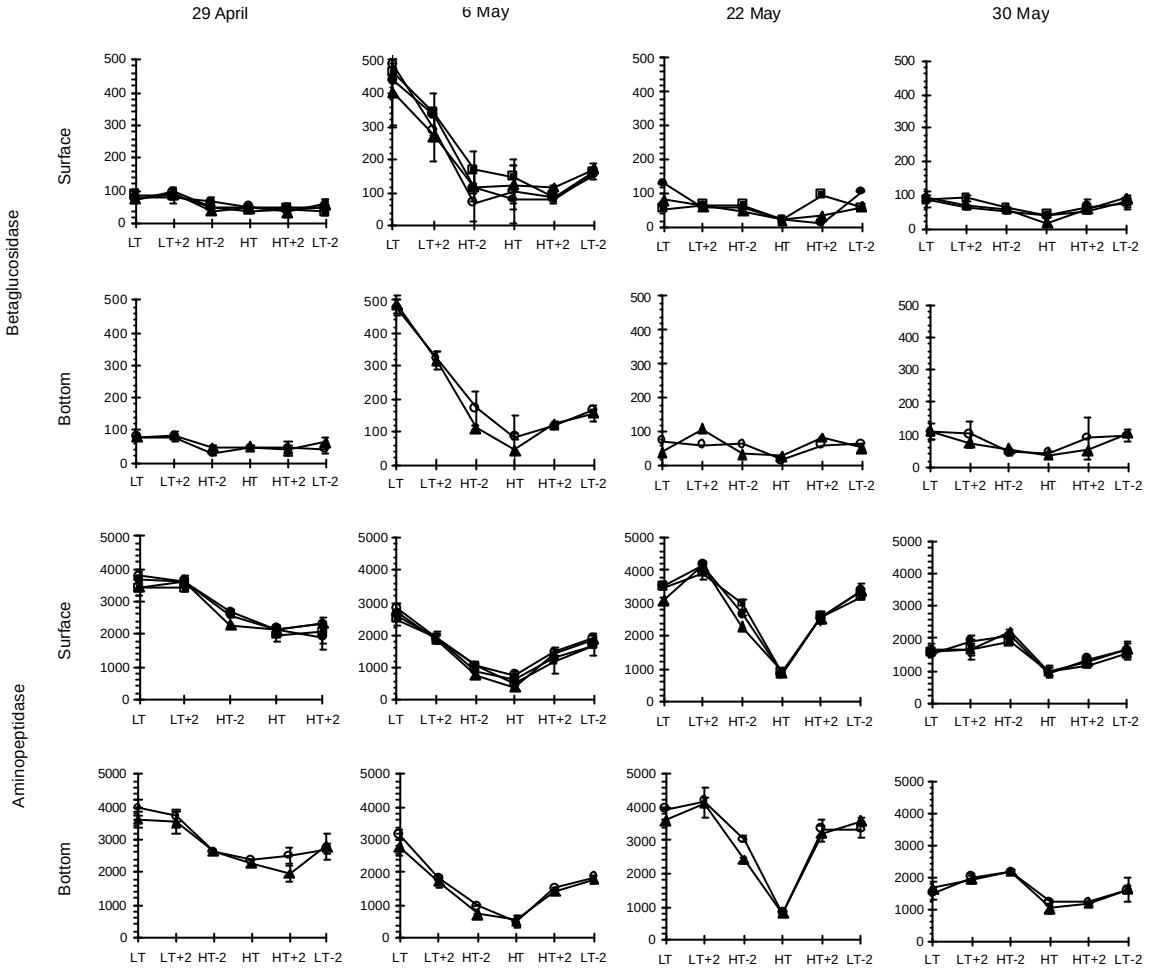

Figure 4: Fluctuation of aminopeptidase and betaglucosidase activities (nmol l^{-1} h^{-1}) at surface (0.2 m) and bottom (0.5 m from sediment surface) layers of the water column at 4 sampling sites along transect I6 (? ¦? S1, ? ☞? S2, ? ✍? S3, ? ? ? S4) in 4 tidal cycles.

proportion of BTGLase activity associated with the >1.2 µm fraction showed contrasting and non-conclusive tendencies in different tidal cycles particularly at surface water.

Glucose Vm and Tr showed irregular patterns along the vertical and transversal profiles of the channel. Glucose Vm varied within the range of 0.1 to 23.3 nmol $l^{-1}h^{-1}$ and Tr varied from 0.9 to 38.3 % h^{-1} (Figure 6). The heterotrophic metabolism of glucose showed stimulation close to low tide.

The results of one-way ANOVA show that the differences between sampling sites found in the values if chlorophyll *a*, POC and in parameters related to bacterial abundance and activity were generally not significant, with the exception of the specific Vm of glucose incorporation (Table 2). The means values of total and per cell ectoenzymatic activities and glucose incorporation at each sampling site are displayed in Figure 7.

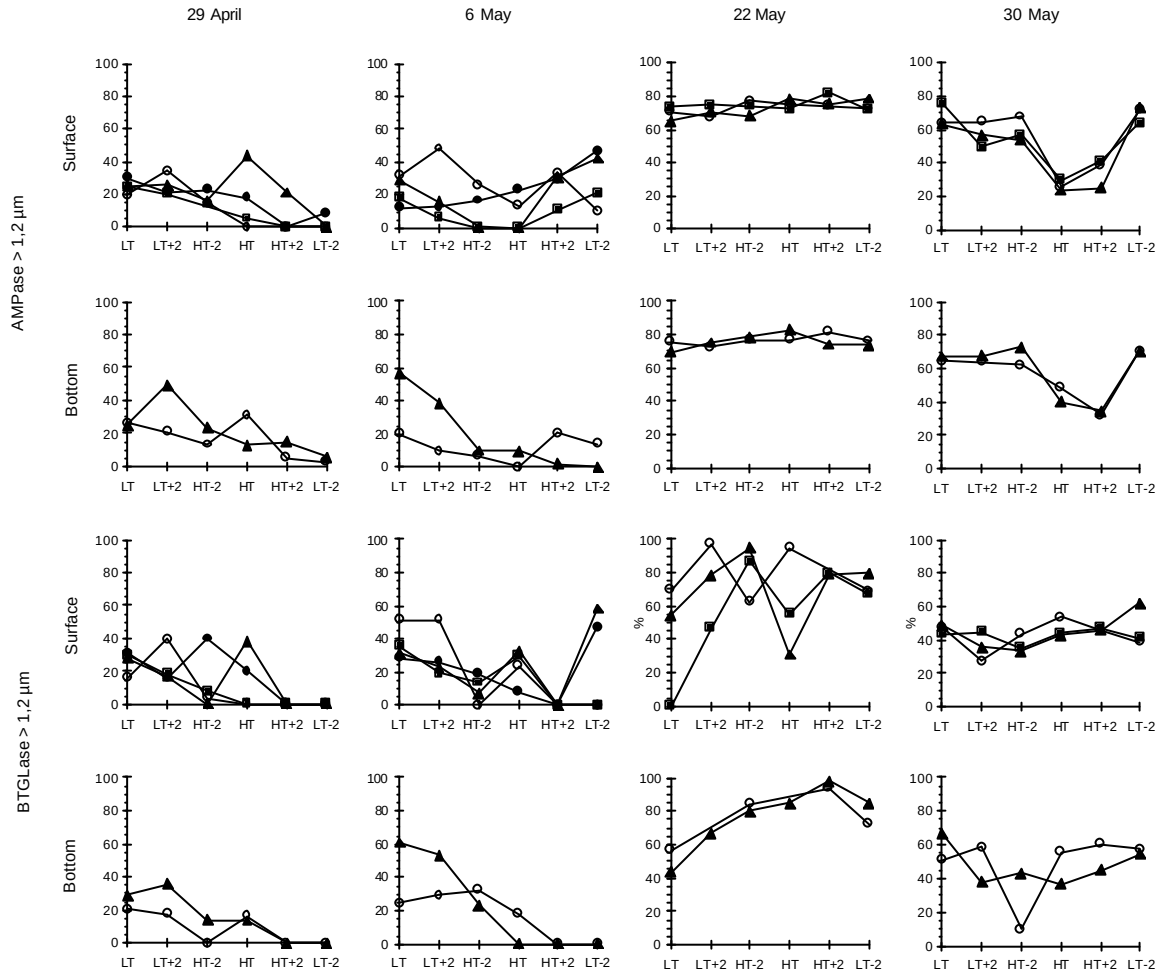

Figure 5: Fluctuation of the fraction (%) of aminopeptidase (AMPase) and betaglucosidase (BTGLase) activities associated to particles larger than 1.2 µm at surface (0.2 m) and bottom (0.5 m from sediment surface) layers of the water column at 4 sampling sites along transect I6 (? ¦? S1, ? ☞? S2, ? ✍? S3, ? ? ? S4) in 4 tidal cycles.

The multiple correlation analysis showed that seston, POC and chlorophyll *a* were not significantly related with to the proximity to the sediment or with the proximity to the margin in the salt marsh area (Table 3). However, the cell-specific AMPase activity of attached cells (obtained as the ratio between AMPase in the > 1.2 µm fraction and the abundance of attached cells), as well as cell-specific Vm of glucose incorporation (obtained as the ratio between glucose Vm and TBN), was inversely related to the distance to the sediment surface (Table 3). Cell-specific Vm of glucose incorporation was also negatively related to the distance to the margin.

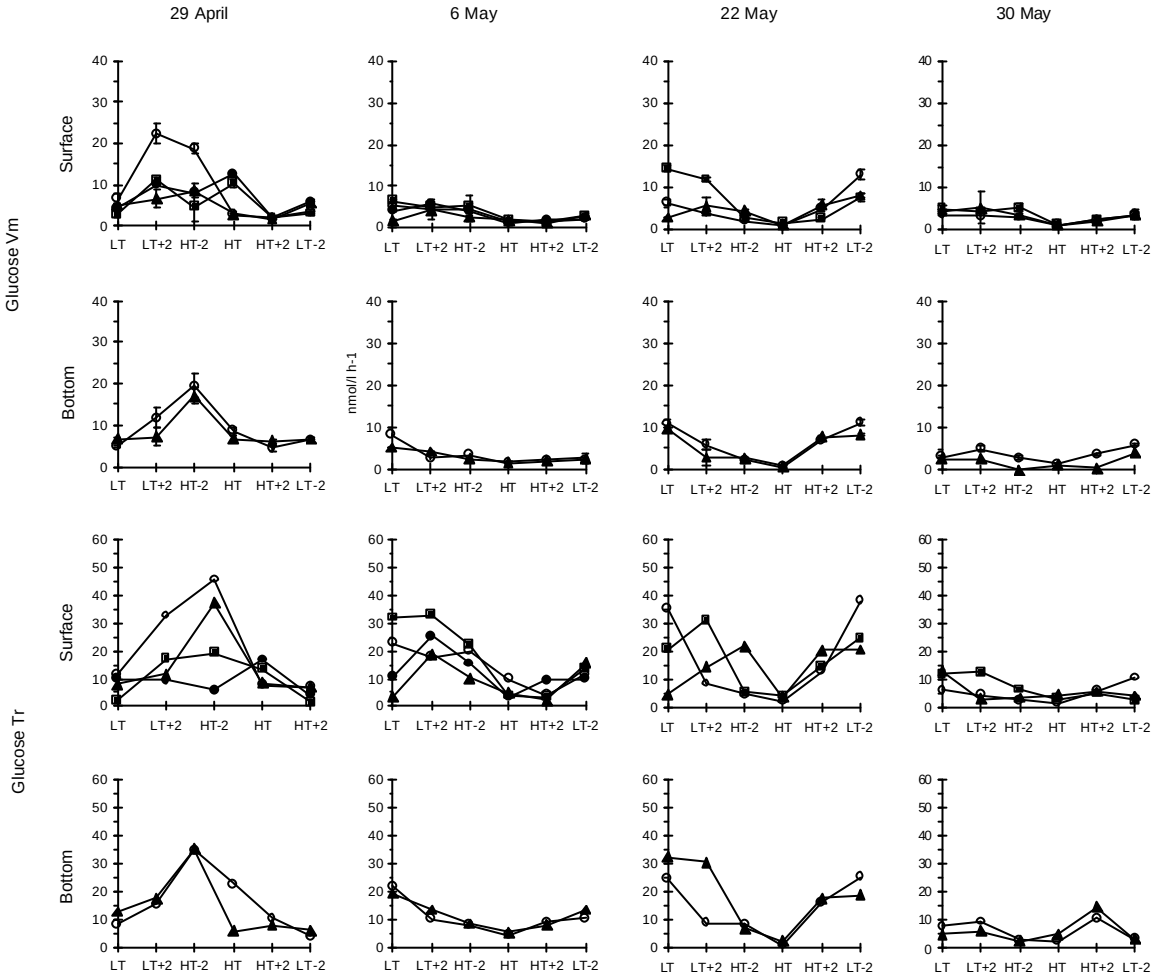

Figure 6: Fluctuation of the Vm of glucose incorporation (nmol l^{-1} h^{-1}) and of the turnover rate (% h^{-1}) at surface (0.2 m) and bottom (0.5 m from sediment surface) layers of the water column at 4 sampling sites along transect I6 (? ¦? S1,? ☞? S2, ? ✍? S3, ? ? ? S4) in 4 tidal cycles.

The results of the stepwise multiple regressions are presented in Table 4. The set of independent variables used for the analysis explained only a small proportion (11 – 43 %) of the observed variability in different bacterioplankton parameters with the exception of the abundance of attached bacteria (adjusted $R^2 = 0.665$).

4. Discussion

High variability of bacterial abundance and heterotrophic activity along longitudinal profiles of estuarine waters is a known and extensively reported feature of these

ecosystems (Karner *et al.*, 1992; Shiah and Ducklow, 1995; Hoppe *et al.*, 1998; Murrell *et al.*, 1999; Cunha *et al.*, 2000). In this study, we were focused on tidal and transversal sources of variability rather than on longitudinal gradients.

The results from ANOVA analysis indicated that in most cases, the differences in bacterial abundance and activity levels between distinct sampling sites were not significant after integration of data representing different tidal phases or tidal cycles.

Table 2: One-way ANOVA of biological variables at transect I6. The null hypothesis is that there are not significant differences between sampling sites. df = degrees of freedom; *MS* = mean square; F_s = *F* test value; *P* = probability value; TBN = total bacterial number; AB = % of attached bacteria; BTGLase = betaglucosidase; BTGLase > 1.2 µm = particulate betaglucosidase; Specific BTGLase = specific (per cell) betaglucosidase; AMPase = aminopeptidase; AMPase > 1.2 µm = particulate aminopeptidase; Specific AMPase = specific (per cell) aminopeptidase; Glucose Tr = glucose turnover rate; Glucose Vm = Vm of glucose incorporation; Specific glucose Vm = specific (per cell) Vm of glucose incorporation.

	df	*MS*	F_s	*P*
Chlorophyll *a*	3	0.575	0.347	> 0.75
POC	3	1.352	0.191	> 0.75
TBN	3	0.865	0.396	> 0.75
AB	3	11.415	0.215	> 0.75
BTGLase	3	3871.349	0.386	> 0.75
BTGLase > 1.2 µm	3	1394.943	1.791	0.25 > P > 0.10
Specific BTGLase	3	129.821	0.478	> 0.50
AMPase	3	318990.1	0.328	> 0.75
AMPase > 1.2 µm	3	1695.165	2.262	0.10 > P > 0.05
Specific AMPase	3	21436.11	1.484	0.25 > P > 0.10
Glucose Tr	3	32.369	0.384	> 0.75
Glucose Vm	3	10.639	0.683	> 0.50
Specific glucose Vm	3	14.331	4.100	< 0.05

Even though the availability of particulate substrates did not seem to be directly controlled by local sediment resuspension or runoff, the multiple correlation analysis confirmed that the proximity to the salt-marsh edge and to the sediment surface favoured monomer uptake and protein degradation at the level of individual cells. Considering that a clear transversal pattern of variation of particulate compounds could not be established and that the turnover of glucose, and even protein degradation, are better related to the dissolved pool of organic matter, the results might be interpreted as an indication that DOM rather than POM is regulating bacterial metabolism at these interfaces.

The next step was to check if variables describing the availability of particulate substrates were found among the major environmental factors controlling tidal and transversal variability of bacterial abundance, ectoenzymatic activity, glucose uptake and turnover. Stepwise multiple regression showed that the fraction of attached bacteria was negatively related to salinity and positively related to chlorophyll *a* (Table 4). These

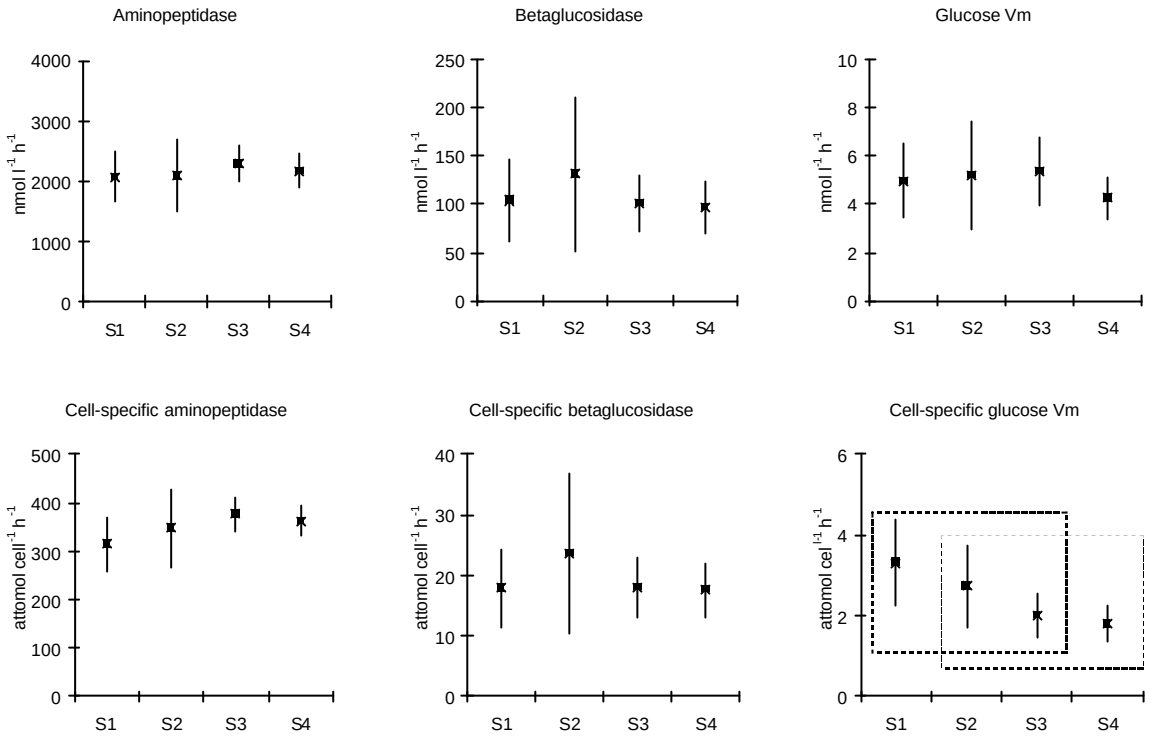

Figure 7: Means and 95 % confidence intervals (error bars) for the different sampling sites along transect I6. Homogeneous subsets obtained with the Tukey test are displayed only in the chart corresponding to the parameter for which significant differences between stations were found with one-way ANOVA (cell-specific glucose Vm).

relations were interpreted as an indication that processes of tidal transport of phytoplankton and colonized particles from upper sections of the estuary, rather than local inputs, are regulating the relative size of the attached bacterial community. The variability of the total size of the bacterial community remained mostly unexplained (adjusted $R^2 = 0.184$) even though it showed a negative relation to POC and positive relation to chlorophyll *a*.

Ectoenzymatic activities showed to be positively associated to chlorophyll *a* (Table 4) suggesting that primary production may be an important source of substrates. BTGLase showed negative relation to salinity as expected since it is generally considered that BTGLase is relatively more important in low salinity environments while AMPase is widespread along estuarine gradients (Murrell *et al.*, 1999; Cunha *et al.*, 2000). The absolute coincidence observed between factors related to total AMPase and BTGLase and the corresponding cell-specific activities indicates that the variations in total activity may correspond essentially to shifts in the levels of activity of the individual cells rather than in the total size of the community. The results also suggest that active attached bacterial cells were probably mostly transported from upper sections rather than locally imported from

Table 3: Results of the analysis of correlation between environmental and bacterioplankton variables and depth or distance to the margin. Significant correlations are signed with * ($p < 0.05$, $n = 132$) or ** ($p < 0.01$, $n = 132$)

	Depth	Distance to west margin
Seston	0.049	-0.78
POC	0.050	-0.076
Chlorophyll *a*	-0.044	0.020
TBN	-0.126	-0.056
AB	-0.096	0.011
AMPase	-0.025	0.031
AMPase >1.2µm	0.050	0.125
Specific particulate AMPase	-0.222*	-0.100
BTGLase	-0.065	-0.063
BTGLase>1.2µm	-0.024	0.146
Specific particulate BTGLase	-0.094	0.034
Glucose Vm	-0.010	-0.072
Specific glucose Vm	-0.720**	-0.303**

the margins, as the proportion of ectoenzymatic activity associated to the > 1.2 µm fraction was also negatively related to salinity (Table 4).

Although its source could not be directly related to the salt marshes or to the bottom sediments, POC showed significant, even though contrasting, relations to different variables describing heterotrophic bacterial activity. Carbohydrate degradation (BTGLase) was positively affected by the increase of POC but this last variable was negatively associated to the heterotrophic metabolism of glucose (glucose Vm and Tr). This indicates that inputs of particulate organic substrates were not accompanied by the corresponding increase in the availability of monomers and suggesting that bacterial utilization of organic-C was limited by ectoenzymatic degradation of complex substrates.

In the particular conditions of the shallow sections of the Aveiro lagoon, local resuspension and runoff may be less important as factors of substrate supply than the transport of particles from upstream. Considering that the nature of the sediment at transect I6, ranges from very coarse sand with 6 % (w/w) organic matter at the West margin, to fine sand with 2 % (w/w) organic matter at the East margin (Cunha *et al*., 1999) some transference of particles from the sediments to the water column could be anticipated. However, current speed at this section, as well as at most of the shallow sections of the estuary, is rather low, never exceeding 0.4 ms^{-1} (Dias *et al*., 2000). Low current speed may favour sedimentation during most part of the tidal cycle since susceptibility to resuspension is strongly driven by tidal forcing (Blanchard *et al*., 1997). In the conditions of the shallow brackish water section of the Ria de Aveiro, the benthic compartment as well as the salt marshes may well retain POM, as observed in other estuaries (Taylor and Allanson, 1995; Yin and Harrison, 2000).

Table 4: Regression equations for variations in microbiological parameters obtained from stepwise multiple regression analysis. Dependent variables: TBN (total bacterial number), AB (% of attached bacteria), aminopeptidase (AMPase), specific (per cell) aminopeptidase (Specific AMPase), particulate aminopeptidase (AMPase > 1.2 µm), betaglucosidase (BTGLase), specific (per cell) betaglucosidase (Specific BTGLase), particulate betaglucosidase (BTGLase > 1.2 µm), Vm of glucose incorporation (Glucose Vm), specific Vm of glucose incorporation (specific glucose Vm) and glucose turnover rate (glucose Tr). Independent variables: temperature, salinity, seston, POC (total concentration or % of seston) and chlorophyll *a*.

Dependent variable	Independent variables	Regression equation	Adj. R^2
TBN	POC (? = -0.272; p = 0.001) Chlor (? = 0.271; p = 0.001)	TBN = 6.161 – 0.152 POM + 0.311 Chlor	0.184
AB	Sal (? = -1.126; p = 0.000) Temp (? = -0.864; p = 0.000) Chlor (? = 0.145; p = 0.009)	AB = 98.139-1.181 Sal – 2.649 Temp + 0.719 Chlor	0.665
AMPase	POC (? = -0.372; p = 0.000) Chlor (? = 0.364; p = 0.000)	AMPase = 2447.5 – 137.6 POM + 279.8 Chlor	0.261
Specific AMPase	Chlor (? = 0.340; p = 0.000) POC (? = -0.279; p = 0.001)	Specific AMPase = 354.9 + 31.2 Chlor – 12.8 POC	0.183
AMPase > 1.2 µm	Sal (? = -0.526; p = 0.000)	AMPase > 1.2 µm = 105.2 – 2.5 Sal	0.271
BTGLase	Temp (? = -0.840; p = 0.000) Chlor (? = 0.253; p = 0.001) Sal (? = -0.496; p = 0.000) POC (? = 0.222; p = 0.004)	BTGLase = 959.1 – 40.5 Temp + 19.7 Chlor – 8.5 Sal + 8.4 POC	0.386
Specific BTGLase	Temp (? = -0.897; p = 0.000) Sal (? = -0.509; p = 0.000) POC (? = 0.257; p = 0.001) Chlor (? = 0.189; p = 0.010)	Specific BTGLase = 170.66 – 7.13 Temp – 1.44 Sal + 1.60 POC + 2.42 Chlor	0.430
BTGLase > 1.2 µm	Sal (? = -0.345; p = 0.000)	BTGLase > 1.2 µm = 78.86 – 1.68 Sal	0.112
Glucose Vm	POC (? = -0.352; p = 0.000)	Glucose Vm = 10.703 – 0.525 POC	0.117
Specific (per cell) glucose Vm	Depth (? = -0.488; p = 0.000) POC (? = -0.301; p = 0.000)	Specific glucose Vm = 5.53 – 0.872 Depth – 0.221 POC	0.356
Glucose Tr	% POC (? = -0.423; p = 0.000)	Glucose Tr = 29.3 – 0.890 % POC	0.173

Even if evidence for the transference of particles from the sediments and from the salt marshes to the water column was not found, the heterotrophic metabolism of glucose was stimulated in the water overlaying the sediment surface and close to the margins. The advection of simple organic substrates at these interfaces may explain these effects.

Contrasting to the removal of particles through sedimentation, considerable inputs of DOM to the water column may be expected since density displacement of interstitial water

causes the release of nutrients (Webster *et al*., 1996). This process has been directly related to the increase of dark oxygen uptake, glucose Vm and the turnover rate of amino acids by the bacterioplankton community in mesocosm experiments (von Bodungen *et al*., 1995). Hopkinson *et al*. (1998) could also establish a linear relation between bacterioplankton growth rates and the DOC concentration in the water, achieved after exposure to benthic sediments.

Globally, the results show some relations between availability of particulate organic substrates (POC, chlorophyll *a*) and levels of bacterial activity. However, resuspension and runoff, understood in the strict sense of processes of transference of particulate materials, do not directly explain the dense populations established at the shallow sections of the estuary or the high heterotrophic activity rates here observed. Therefore we believe, even though in this study the characterization of DOM was not attempted, that dissolved carbon may be the major factor controlling vertical and transversal variations of bacterioplankton heterotrophic activity.

Acknowledgements

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CHAPTER VI
FLUXES OF BACTERIOPLANKTON BETWEEN A TIDAL ESTUARY AND THE SEA DURING THE DRY SEASON: RETURNING TO THE "OUTWELLING HYPOTHESIS"

Cunha, M. A., Dias, J. M., Almeida, M. A., Lopes, J. F. and Alcântara, F. (2001)

Marine Ecology Progress Series (accepted)

Abstract - The tidal dynamics of bacterioplankton communities at the outer section of a shallow estuary (Ria de Aveiro, Portugal) were studied during 6 fixed-point tidal cycles. Bacterial numbers ($0.2 – 8.1 \times 10^9$ cells l^{-1}), aminopeptidase activity (189 – 1662 nmol $l^{-1}h^{-1}$), ?-glucosidase activity (1.7 – 67.0 nmol $l^{1}h^{-1}$) and potential glucose incorporation (0.48 – 3.99 nmol $l^{1}h^{-1}$) varied according to a consistent pattern of enrichment during ebb-tide and showing decrease during flood tide.
Fluxes of bacterioplankton populations and associated heterotrophic activities between the estuary and the coastal area during the time period equivalent to a tidal cycle were estimated from the water flux determined using a two-dimensional vertically integrated numerical model. The net fluxes estimated for the period equivalent to a tidal cycle ranged from -2.5 to $+26.0 \times 10^{16}$ bacterial cells. The net tidal fluxes of potential heterotrophic activities ranged from -80 to -10 mol h^{1} for aminopeptidase, -1.10 to -0.33 mol h^{1} for ?-glucosidase and -0.18 to +0.03 mol h^{-1} for glucose incorporation. Net fluxes were generally negative in sign indicating the transfer of phyto- and bacterioplankton, as well as potential capacities for the degradation and recycling of organic matter, from the outer estuarine compartment to the sea.

Key words: Estuarine bacterioplankton; tidal fluxes

1. Introduction

Estuaries are interface ecosystems that functionally couple continental and marine environments, receiving biogeochemically active inputs from land, rivers and the coastal sea. The estuarine environment is often reported as extremely productive, supporting dense bacterioplankton communities and high rates of primary production and bacterial heterotrophic activity (Barbosa, 1991; Fuks *et al*., 1991, Hoppe *et al*., 1996; Revilla *et al*., 2000). The basic model of salt-marsh estuaries as exporting systems usually referred as the "Outwelling Hypothesis" (Odum, 1968; Dame *et al*., 1986) can be extended to incorporate the passive transport of living cells and their associated heterotrophic activities.

Major transformations of the pools of organic and inorganic nutrients that reach the main body of the estuary are related to biological processes associated to bacterioplankton communities. (Billen and Garnier, 1997; Cloern, 2001). These, in their turn, strongly respond to environmental stimuli (Cunha *et al*., 2001), namely variations in nutrient availability (Hoppe *et al*., 1998). High fresh-water inputs and the corresponding increase in the concentration of labile organic compounds enhance the levels heterotrophic activity of estuarine bacteria (Murrell *et al.*, 1999) and impose a river-driven seasonality on the way the estuary interacts with the nearby ocean (Cloern and Nichols, 1985). However, in estuaries where the processes of transport of materials are mostly driven by tidal forcing, the variability associated to the semi-diurnal time scale prevails over seasonal effects (Smith, 2001). The tidal flooding of salt marshes and mud-flats, and the advection of dissolved nutrients from the sediments stimulate bacterial activity in the water column (Trousellier, 1993; von Bodungen *et al*., 1995; Ritzrau *et al*., 1997) and are probably major factors underlying the persistence of dense and active bacterioplankton communities in shallow estuarine sections (Hoppe *et al*., 1996; Cunha *et al*., 2000).

This work reports on an attempt to test in the field the hypothesis that in a shallow mesotidal estuarine system, such as Ria de Aveiro, exports of bacterioplankton and potential heterotrophic activities to the coastal ocean can occur under the summer regime of low fresh water input. With that aim, tidal dynamics of bacterioplankton were characterised in terms of (a) patterns of variability of abundance and activity and (b) sign and magnitude of the fluxes of cells and potential activities at the interface with the sea.

2. Materials and methods

Study area

Ria de Aveiro (Figure 1) is a bar-built estuary (Pritchard, 1989), also described as a coastal lagoon, in the Northwest coast of Portugal. It is a complex system characterised by narrow channels and extensive intertidal zones. During spring tides, the maximal wet area is 83 km^2 at high tide, reduced to 66 km^2 at low tide (Dias *et al*., 2000). The estuary encompasses a total volume of water of approximately 160 Mm^3 at high tide (Silva, 1994)

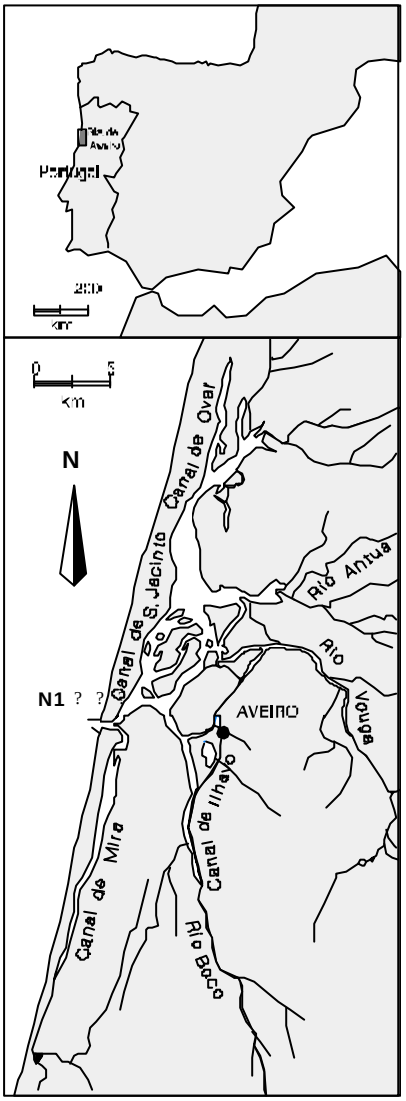

Figure 1: Ria de Aveiro (Portugal) with sampling station N1 indicated with arrow.

and the total river discharge during the period equivalent to a tidal cycle is 1.8 Mm^3 (Moreira *et al*., 1993). The tidal range varies between 0.6 m in neap tides and 3.2 m in spring tides, with an average value of about 2 m (Dias *et al*., 2000).

Study site

The investigation was conducted inside the lagoon assuming that a sampling station located close to the mouth would fairly represent the properties of the water masses moving in and out the estuary. Station N1 (40º33'N; 8º45'W) was located in the main navigation channel, approximately 2 Km from the mouth (Figure 1). Sampling was performed during day-time along 5 spring tide cycles (01-06-96, 03-06-96, 28-08-96, 30-08-96 and 02-09-97) and one neap tide cycle (10-09-97). In each tidal cycle, water samples were collected just below surface (0.2 m) at 6 tidal phases at approximately 2-hour intervals including one slack low tide (LT) and one slack high tide (HT), for no longer than 14 hours. Samples were kept cold and in the shade during transport to the laboratory.

Physical and chemical parameters

Temperature and salinity were measured with a WTW LF 196 Conductivity Meter and total depth of the water column was determined with a Sonar probe (Hondex PS-7 LCD Digital Sounder).

The determination of the concentration of suspended solids (seston) in estuarine samples was performed after filtration of 500 ml aliquots through Whatman GF/C (47 mm diameter) pre-weighted, pre-combusted filters. The filters were dried at 60 ºC for 24 hours and seston content was calculated as the increase in weight. Particulate organic matter

(POM) was determined through the difference in the weight of the dry seston filters after 4 hours incineration at 525 ºC of the dry seston (Parsons *et al*., 1989).

Chlorophyll *a* was quantified fluorimetrically (Yentsch and Menzel, 1963) in a Jasco FP-777 espectrofluorimeter. A total volume of 0.5 l of water was filtered through Whatman GF/C filters and later extracted with 10 ml of 90% acetone. Determinations were performed in 3 replicates of each sample.

Microbiological parameters

Total bacterial number (TBN) was determined by cell counting under epifluorescence microscopy after fixation of water samples with 2 % formaldehyde (final concentration). The samples (3 replicates) were then filtered through 0.2 µm black polycarbonate membranes (Poretics) and stained with 0.03 % acridine orange (Hobbie *et al*., 1977).

The determination of maximum uptake velocity of glucose (glucose Vm) followed the procedure described by Gocke (1977) in 3 replicates and 1 blank of each sample. A final saturation concentration of 430 nM of ^{14}C-glucose (Amersham SA 11.5 GBq mmol^{-1}, 310 mCi mmol^{-1}) added to 10 ml aliquots was chosen after kinetic analysis was. Incubations were carried out for 2-3 hours 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 scintillation cocktail.

Ectoenzymatic activity was determined fluorimetrically (Jasco FP-777 Fluorometer) as the maximum hydrolysis rate (Hm) of model substrates (Sigma-Aldrich) for ?-glucosidase (4-methylumbelliferyl-?-D-glucoside) and Leu-aminopeptidase (L-leucine-7-amido-4-methyl-coumarin) according to Hoppe (1983). Saturation was achieved with 1 mM of both substrates. Wavelengths for excitation and emission were respectively 380 and 440 nm for MCA (7-amino-4-methylcoumarine) and 360 and 450 nm for MUF (4-methylumbelliferone). Measurements were made in 3 replicates for each sample after 1-2 hours incubations at *in situ* temperature. Calibration was performed by adding a series of 8 concentrations of the fluorescent products (0-500 nM for MUF and 0-6 µM for MCA) to a pool of water from all the samples analysed in each day.

Estimation of estuarine fluxes

The tidal balance of several water properties was estimated as the difference between the integrated ebb and flood tide flows through a transverse section of the channel defined at station N1. In order to determine estuarine fluxes in time periodic current fields, water flow and properties concentrations are usually measured in cross-sectional structures along the tidal cycle (Taylor and Allanson, 1995). Due to the very difficult working conditions at the station location, in this work determinations were only performed at a fixed depth corresponding to 0.2 m below surface, and at approximately 2-hours intervals. Ria de Aveiro is, however, vertically well-mixed and laterally homogeneous (Dias *et al*., 1999), and therefore it can be considered that the measured values fairly represent the structure of the correspondent property. In addition, Ria de Aveiro is a very shallow environment which makes adequate the use of vertically integrated numerical models to determine the integrated water flow.

In this work a two-dimensional vertically-integrated numerical model (2DV) was applied. This solves the well-known shallow-water equations. This model was previously calibrated (Dias *et al*., 1998) and has been used in studies of tidal dynamics of the Ria de Aveiro (Dias *et al*., 2000), where it proved to be able to reproduce the tidal prism values computed by other authors for the main channels of Ria de Aveiro (Dias, 2001).

The 2DV numerical model was used to compute time series of water flow (time-step of 40 s) through the transverse section of the channel including station N1, during each one of the tidal cycles. The slack water times were also determined from the numerical results, allowing the estimation of the time of null flux. Instantaneous cross-sectional fluxes of salinity, chlorophyll, POM, ectoenzymatic activities, glucose incorporation and bacterial abundance were estimated as the result of the product of the field data measured at a given time by the simultaneous numerically computed water flow. New values corresponding to the slack water time (null instantaneous fluxes) were also included.

Both water flow and properties concentrations were considered to have nearly sinusoidal time evolution patterns in Ria de Aveiro (Hoppe *et al*., 1996; Cunha *et al*., 2000). Consequently, it was expected that the fluxes of most properties could reveal a parallel temporal variation. Therefore, time series of instantaneous fluxes during a tidal cycle were produced by polynomial interpolation of the value of the products of the field

data by the numerical results, using a third degree polynomial approximation. As expected, these curves fited very well with the numerically computed water flow curves. Estuarine fluxes through the transverse section were later calculated integrating the ebb and flood tide series.

3. Results

Tidal fluctuation of water properties and bacterioplankton abundance and activity

Data on the fluctuation of total water depth, temperature and salinity registered during 6 tidal cycles at station N1 are presented in Table 1.

Table 1: Values of total depth, temperature and salinity at station N1 determined during tidal cycles involving 6 sampling moments: LT (low tide), LT+2 (2 hours after low tide), HT-2 (2 hours before high tide), HT (high tide) HT+2 (2 hours after high tide) and LT-2 (2 hours before low tide).

		01-06-96	03-06-96	28-08-96	30-08-96	02-09-97	10-09-97
Depth (m)	LT	5.6	5.7	6.0	4.6	5.7	6.0
	LT+2	7.5	7.5	8.0	8.6	6.0	6.5
	HT-2	9.5	8.4	9.0	8.6	7.7	7.7
	HT	10.0	9.4	9.5	8.8	8.0	8.0
	HT+2	-.	7.5	7.0	7.0	7.3	7.7
	LT-2	8.0	6.1	6.1	6.3	6.3	7.0
Temperature (°C)	LT	17.0	17.0	18.1	17.0	18.7	20.6
	LT+2	16.4	16.9	15.6	16.5	18.6	20.3
	HT-2	15.3	15.5	15.6	15.5	16.5	17.4
	HT	15.5	15.5	15.6	15.4	16.8	17.6
	HT+2	-	15.7	15.9	15.5	16.7	17.7
	LT-2	16.0	15.9	18.6	16.4	17.3	18.7
Salinity (UPS)	LT	23.7	27.7	35.1	35.4	34.0	33.6
	LT+2	30.5	30.1	36.1	35.5	34.2	33.7
	HT-2	35.5	35.0	35.7	35.7	35.7	35.5
	HT	35.0	35.3	35.8	35.9	35.8	35.6
	HT+2	-	35.0	35.7	35.7	35.6	35.5
	LT-2	31.0	32.5	35.2	35.4	35.2	35.2

Depth at the station varied from a minimum of 4.6 m in low tide to 10 m in high tide. Water temperature (15.3-20.6 ºC) generally decreased during flood tide and increased during ebbing. Salinity varied within the range of 23.7-35.9 PSU. Salinity maxima and minima were always observed at high and low tide, respectively.

Seston (17-222 mg l^{1}) and POM (6-31 mg l^{1}) strongly fluctuated during the tidal cycles but a clear pattern could not be identified (Figure 2). Chlorophyll *a* ranged from 6.8 to 10.2 µg l^{1} being the maximal values achieved at low tide (Figure 2).

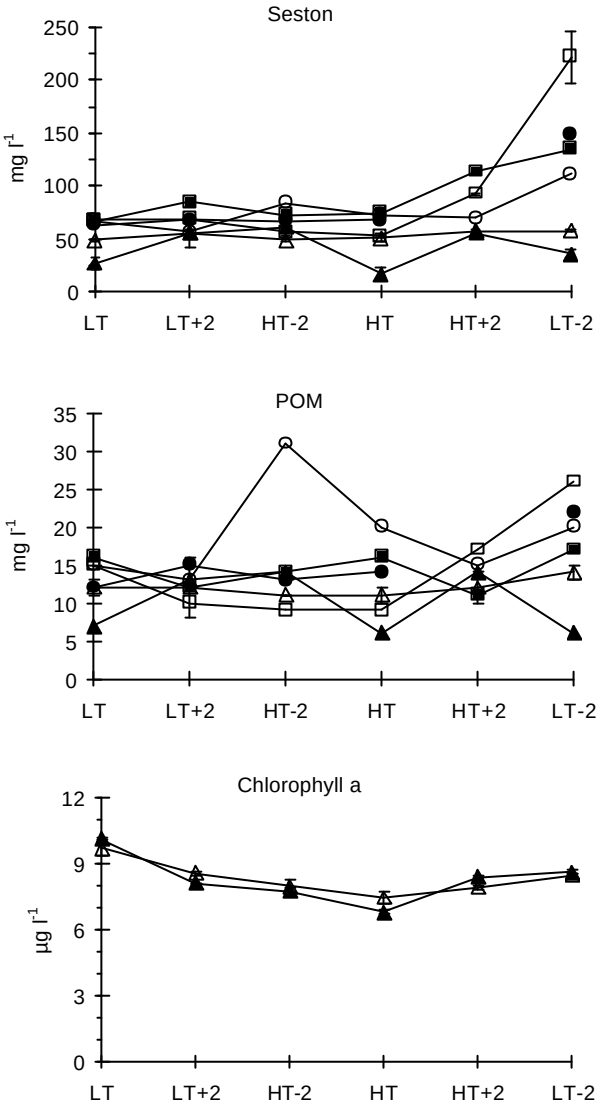

Figure 2: Variation of seston, particulate organic matter (POM) and chlorophyll concentrations determined at station N1 during tidal cycles involving 6 sampling moments: LT (low tide), LT+2 (2 hours after low tide), HT-2 (2 hours before high tide), HT (high tide) HT+2 (2 hours after high tide) and LT-2 (2 hours before low tide).
☞ - 01-06-96; ☞ - 01-06-96; ☞ - 28-08-96; ☞ - 30-08-96; ↴ - 02-09-97; ↴ - 10-09-97.

Total bacterial abundance varied between 0.2 and 8.1 x 10^{9} cell l^{-1} (Figure 3). Maximal values were more often found at low tide or 2 hours before.

Glucose incorporation Vm (0.48-3.99 nmol l^{-1} h^{-1}) followed a pattern similar to the variation of abundance with incorporation rates increasing towards low tide (Figure 3).

The ranges of variation of the maximum hydrolysis rate were 189-1662 nmol l^{-1} h^{-1} for aminopeptidase and 1.7-67.0 nmol l^{1} h^{-1} for ?-glucosidase (Figure 3). The tidal pattern of variation was again characterised by increasing hydrolytic activity during ebbing and a decrease of activity during flood tide.

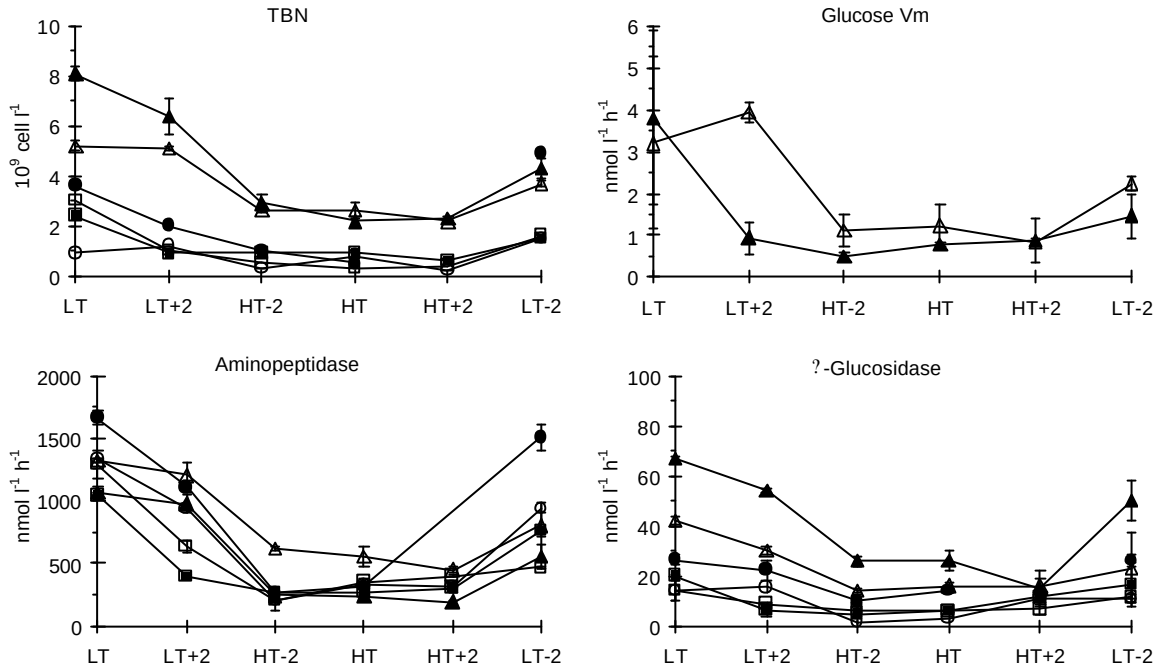

Figure 3: Variation of total bacterial number (TBN), glucose incorporation Vm, aminopeptidase and ?-glucosidase activity determined at station N1 during tidal cycles involving 6 sampling moments: LT (low tide), LT+2 (2 hours after low tide), HT-2 (2 hours before high tide), HT (high tide) HT+2 (2 hours after high tide) and LT-2 (2 hours before low tide). ☞ - 01-06-96; ☞ - 01-06-96; ☞ - 28-08-96; ☞ - 30-08-96; ↴ - 02-09-97; ↴ - 10-09-97.

Estuarine fluxes

The estimated values of the fluxes of materials and potential bacterial heterotrophic activities between the estuary and the ocean during the time period corresponding to one tidal cycle are represented in Figure 4.

Fluxes of salt were predominantly positive ranging from –73 to +727 tons. Negative fluxes of chlorophyll *a* corresponded to a net export to the sea of 9 to 55 Kg per tidal cycle. Fluxes of POM were variable in sign ranging from –781 to +478 kg per tidal cycle.

TBN and potential ectoenzymatic activities were consistently exported to the coastal sea. The absolute values of the net tidal fluxes were 3 to 26 x 10^{16} cells, 12 to 78 mol h^{-1} of potential aminopeptidase activity and 0.3 to 1.1 mol h^{-1} of potential ?-glucosidase activity, per cycle. The net flux of potential glucose incorporation was contrasting in sign between

the two 1997 cycles, but higher, in absolute value in spring tide (-0.18 mol h^{-1} per cycle) when compared to neap tide (+0.02 mol h^{-1} per cycle).

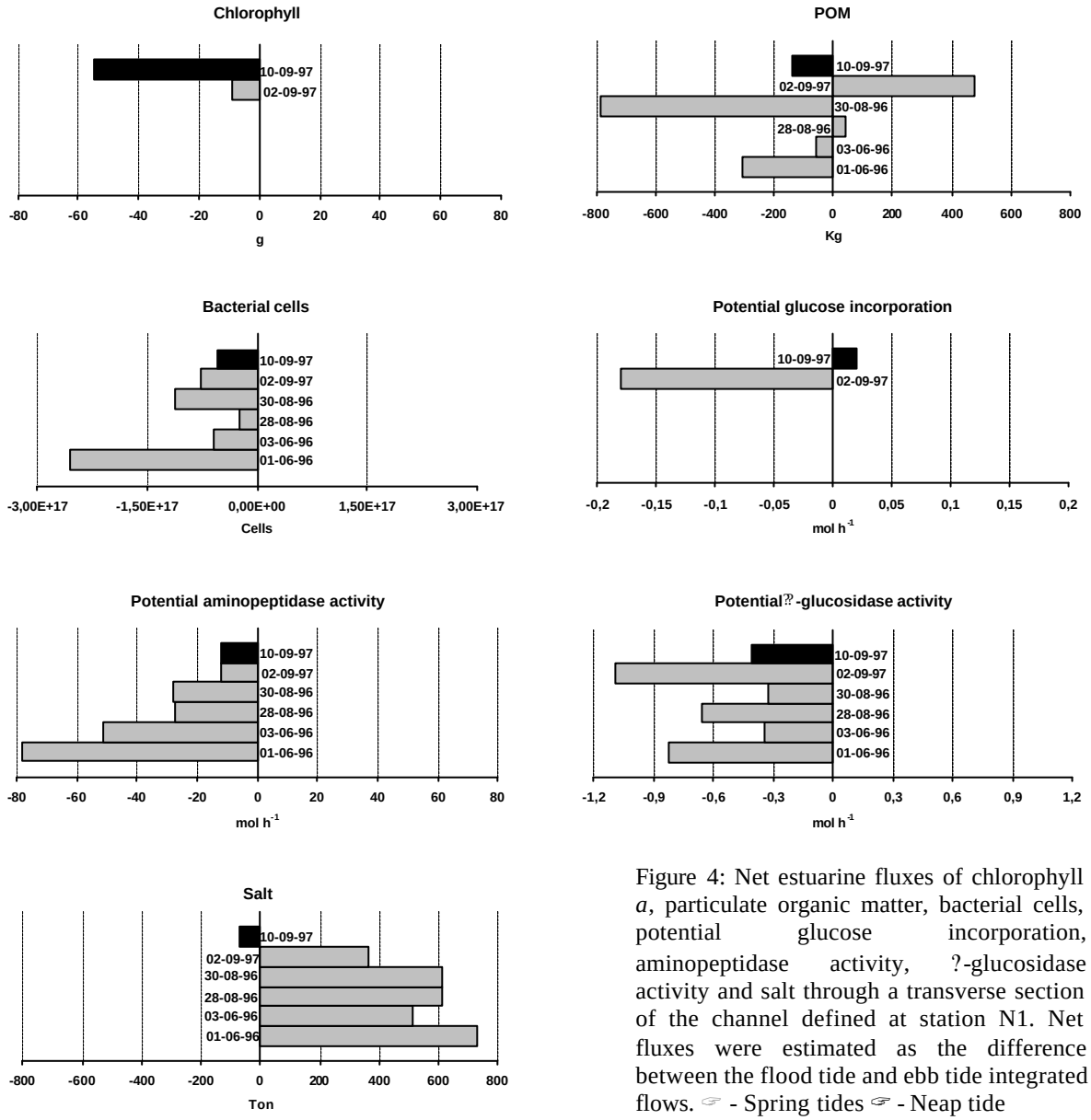

Figure 4: Net estuarine fluxes of chlorophyll *a*, particulate organic matter, bacterial cells, potential glucose incorporation, aminopeptidase activity, ?-glucosidase activity and salt through a transverse section of the channel defined at station N1. Net fluxes were estimated as the difference between the flood tide and ebb tide integrated flows. ☞ - Spring tides ☞ - Neap tide

TBN and potential ectoenzymatic activities were consistently exported to the coastal sea. The absolute values of the net tidal fluxes were 3 to 26 x 10^{16} cells, 12 to 78 mol h^{-1} of potential aminopeptidase activity and 0.3 to 1.1 mol h^{-1} of potential ?-glucosidase activity, per cycle. The net flux of potential glucose incorporation was contrasting in sign between

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4. Discussion

Tidal bacterioplankton dynamics at the interface with the sea

Ria de Aveiro is generally described as estuarine system with a complex topography. The location of station N1 was defined in order to allow the best approximation to the physical, chemical and biological properties of the water at the mouth of the estuary since the total volume of water that crosses this section corresponds to 90 % of the total tidal prism (Silva, 1994). Since fieldwork at the highly turbulent section between the origin of Canal de Mira and the mouth proved to be impracticable for security reasons, the contribution of this channel was left out of the current study.

The patterns of tidal variation of salinity and temperature observed at station N1 indicate that daytime warming occurred as salinity decreased during ebbing. The lagoon has an average depth of 1 m and the shallow sections (< 3 m) correspond to approximately 95 % of the high tide wet area (Silva, 1994). This allows significant warming of the water column due to insolation, especially during the warm season. The net export of heat from the estuary to offshore waters was previously reported by Dias *et al*. (1999).

The concentration of particulate matter did not follow a regular pattern along the tidal cycles. This uncoupling from salinity has also been reported for the variation of DOC at other temperate estuarine systems and is interpreted as an indication of tidally induced resuspension/sedimentation events that may exert a major control over the concentration of particles in the water column (Miller, 1999). However, in the Ria de Aveiro, like in other shallow estuarine systems, the sediment-bed and the salt marsh tend to retain particles and export solutes (Newell and Krambeck, 1995; Almeida *et al*., accepted for publication). Assuming that POC corresponds to 50 % of the POM (Rodier, 1996) and using a factor of 50 to convert chlorophyll to phytoplankton–C (Eppley *et al*., 1977), we can estimate that POC corresponds to 4-19 % of the seston and that phytoplankton biomass corresponds to 6-14 % of the POC. These values fit within the ranges usually reported for productive

coastal ecosystems (Charpy *et al.*, 1997; Canuel and Zimmerman, 1999). The high organic content of suspended matter may be related to inputs of inorganic nutrients and labile DOM at the mid- and inner-estuarine sections that cause the increase of phyto- and bacterioplankton biomasses to values that are up to 9 times higher than at the outer section (Cunha *et al*., 2000). Tidal transport of cells from upper sections of the estuary induced a regular pattern characterised by an ebb-tide enrichment in phyto- (1.3 – 1.4 fold) and bacterioplankton (1.1 – 10 fold).

Bacterial potential ectoenzymatic activity and glucose incorporation also increased at station N1 during ebbing and decreased during flood. However, tidal fluctuation of activity variables was less pronounced than the fluctuation of bacterial abundance. This conservative tidal fluctuation of activity parameters when compared with the fluctuation of abundance at station N1 may result from the anti-parallel fluctuation of total abundance and specific activity of bacterial cells. Experimental data referring bacterial communities of this estuarine system indicate that bacterioplankton heterotrophic activity strongly responds to changing water properties and is negatively affected by salinity (Cunha *et al.*, 2001). According to this scenario, the activity of individual cells and the viability of the community may decrease at station N1 during ebbing, as total abundance increases with upper estuarine inputs. On the contrary, when ocean water is entering the estuary, the density of the community is decreasing but cells are probably gaining vigour in response to the rising nutrient availability.

Fluxes between the estuary and the coastal oceanic area

Estuarine impacts on coastal ocean often result in the formation of plumes of elevated chlorophyll concentration, bacterial abundance, biomass production, nitrification, and total community respiration (Bianchi *et al*., 1999a; Bianchi *et al*., 1999b; Malone and Ducklow, 1990; Pakulski *et al*., 2000). The attempt to quantify fluxes of salt and biogenic elements between the Aveiro estuarine system and the adjacent Atlantic coast during the summer period showed a consistent seaward flux of bacterioplankton cells and potential heterotrophic activity.

The magnitude of the exports from the estuary to the sea reflects the total size and activity of estuarine communities and also the balance between marine and freshwater

inputs to the main body of the estuary during each tidal cycle. The fluxes of bacterial cells and AMPase activity calculated for the studied 1996 tidal cycles decreased from early to mid summer, when salt imports increased, probably as consequence of the reduction in freshwater inputs during the warm season (Simpson *et al*., 2001). ?-GLCase showed a more irregular temporal pattern. Since this ectoenzymatic capacity is often reported as being particularly associated to low-salinity bacterial communities (Murrel *et al*., 1999), processes of activation and deactivation of the corresponding enzymes may be superimposed on transport effects.

The fluxes of particulate materials at the estuarine-ocean interface are more irregular in sign probably reflecting physical processes of re-allocation of sediments by tidal and/or residual currents (Cloern and Nichols, 1985). Maximum current speed at the mouth of the estuary ranges 0.6-2.4 m s^{-1}, (average of 1.2 m s^{-1}) depending on tidal range and other hydrographical conditions (Dias *et al*., 2000). During the tidal cycle a succession of conditions for resuspension, transport and deposition of particles may occur. Also, the frequent disturbance of the channel bed by dredging works at this section of intense navigation traffic may interfere with tidal fluxes of suspended sediments.

The comparison between biogenic estuarine fluxes under spring and neap tide conditions is not conclusive. Results may indicate that other factors, rather than tidal range, are prevailing on the magnitude of the net estuarine fluxes. Neap tide exports, when compared to spring tide, corresponded to a 6-fold increase in chlorophyll, a 32 % decrease in bacterial cells and a 63 % decrease of potential ?-glucosidase activity. On the contrary, neap tide had no significant impact on the export of potential aminopeptidase activity, in relation to spring tide values, and coincided with a shift in the sign of the fluxes of salt and POM. Short-term variations of the same order of magnitude were also observed in the 1996 cycles, which corresponded to tides of similar range (2.8-3.0 m). The two pairs of cycles separated by 1-day intervals provide a perception of the amplitude of short-term variability not related to changes in tidal range. The results may indicate that other factors controlling the size and activity of bacterioplankton inside the estuary and/or the mechanisms of estuarine water circulation exert major influence on the processes of transference between the estuary and the sea.

In spite of the controversy on Odum's outwelling hypothesis (Odum, 1968) regarding the contribution of salt-marsh estuaries to the export of organic matter to the open marine environment (Dame *et al.*, 1986), this study brings evidence to the supply of the coastal area with enriched bacterial communities and increased potential capacity for the degradation and recycling of organic matter brought from the shallow estuary of Ria de Aveiro. It was demonstrated that these exports occur during periods of low fresh water inputs, when the salt balance corresponded to a net retention of marine water inside the lagoon. Estuarine fluxes of bacterial cells and associated heterotrophic activities are most probably supported by dense and active inner-estuarine communities and are, therefore, highly dependent on the reactivity of bacterioplankton communities and on processes of stimulation or deactivation of heterotrophic capacities during transport along the estuarine gradient.

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CHAPTER VII
DISCUSSION AND CONCLUSIONS

Preliminary assumptions and methodological options

The main feature underlying the field and laboratory work hereby reported is the metabolic reactivity of the heterotrophic estuarine bacterioplankton under unstable environmental conditions.

The strategies adopted and the design of the fieldwork were based on the fundamental assumption that estuarine interfaces with the riverine environment, the littoral zones, the bottom sediments and the coastal ocean were sites of enhanced instability. The collection of samples should be conducted is such a way that spatial, chemical and/or temporal variations could be fairly represented. However, the selection of the sampling sites is not exempt of criticism and some of the options are worth discussing in face of the particular morphological and hydrographical features of the study area.

The Ria de Aveiro is a bar-built estuary (Pritchard, 1967), sometimes referred as a coastal lagoon, that has a very irregular and complex geometry characterized by extensive intertidal zones and by an intricate net of narrow and shallow channels. The estuary is connected to the ocean by an artificial channel 1.3 km long, 350 m wide and ~20 m deep (Dias *et al.*, 2000). The Ria corresponds to a mesotidal estuary (Davies, 1964) with a tidal range that is minimum in neap tides (0.6 m) and maximal in spring tides (3.2 m) (Dias *et al.*, 1999). The tidal prim at the mouth and in spring tides is 83 Mm^3 and the estuary encompasses a total volume of water of ~160 Mm^3 in high tide (Silva, 1994). The total river discharge during the period equivalent to a tidal cycle is 1.8 Mm^3 (Moreira *et al.*, 1993). No significant signs of vertical stratification in terms of temperature and salinity are generally detected so the estuary is considered as well mixed (Dias *et al.*, 1999). Four channels originate from the main navigation channel with a correspondent distribution of the tidal prism of 38 % for Canal de S. Jacinto (North), 26 % for Canal do Espinheiro (Northeast), 10 % for Canal de Mira (South) and 8 % for Canal de Ílhavo (Southeast) (Silva, 1994). Maximum current speed at the mouth of the estuary ranges 0.6 – 2.4 ms^{-1}, depending on hydrographical conditions, but at the shallow sections of the channels it never exceeds 0.4 ms^{-1} (Dias *et al.*, 2000).

A first option corresponded to the definition of the extreme points of the estuary, which would represent the estuarine interfaces with the river and with the sea. The gateway to the sea should ideally be defined at the mouth of the Ria, right between peers. However, the strong currents, the frequent dredging of the channel bed and the intense navigation made anchoring at this section impracticable. According to Silva (1994) the volume of water corresponding to the tidal prism at the first section of the main navigation channel (83 Mm^3) is much larger that the volume that remains in low tide (28 Mm^3) so an extensive renovation of the water contained in this section during each tidal cycle was assumed. Therefore, a sampling station located approximately 1.5 km inwards to the mouth was selected to represent the interface with the sea (station N1). During tidal cycles, the small boat used during sampling was kept at a fixed position by attaching it to a navigation buoy, which was not positioned at the centre of the channel but slightly oriented towards the east margin. It is possible that during ebbing, the water properties at this site were more affected by the flux associated with Canal de Ílhavo than with either Canal de S. Jacinto or Canal do Espinheiro. Canal de Mira originates very close to the mouth and its contribution to the variability of bacterioplankton activity was excluded by the option of establishing the interface with the sea at station N1.

In order to characterize the responses of bacterioplankton to an estuarine gradient, and considering that the Ria has an intricate topography, it become necessary to define a model sub-estuary (Canal de Ílhavo) within the complex estuarine system. Some important attributes of Canal de Ílhavo were taken into consideration: (1) Canal de Ílhavo connects to a permanent fresh water stream at its upper end which, even though it is not a major fresh water affluent to the estuary (~5 % of total freshwater inputs), creates a distinct salinity gradient; (2) earlier studies developed along this channel provided preliminary information on the longitudinal patterns of variation of hydrographical variables such as temperature, salinity and sediment texture (Rodrigues, 1992); (3) although it is the shortest of the main branches of the lagoon (~15 km length), Canal the Ílhavo fairly represents the diversity of intertidal biotopes that occur within the estuarine system; (4) there is a marked shift in anthropogenic pressure associated to the margins from the outer (harbour facilities) to the inner sections (aquaculture ponds, industrial plants, diffuse domestic sewage inputs) of the channel; (5) the channel is accessible from the margins along almost all the extension and navigation is also possible in small recreation boats; (6) it is located within a short distance from the laboratory allowing a rapid processing of collected samples.

Bacterioplankton reactivity to changing water properties

Evidence for the high reactivity of bacterial communities to the changing properties of the water column was obtained from the analysis of field results and confirmed in laboratory experiments.

Assuming that Canal de Ílhavo represents the estuarine gradients of salinity and nutrient availability established in the Ria de Aveiro, the results indicate that the peaks of bacterioplankton abundance and activity occur at sections of intermediate salinity (20--30 PSU). This corresponds to a curvilinear pattern also observed in other temperate estuaries (Wright and Coffin, 1983; Palumbo *et al.*, 1984; Fuks *et al.*, 1991; Bordalo *et al.*, 1998). However, the salinity range corresponding to maxima in bacterial heterotrophic potential in the Ria is higher than in other estuaries where maxima occur at 3-10 PSU (Palumbo and Ferguson, 1978; Painchaud *et al.*, 1995) or at 5-25 PSU (Pakulski *et al.*, 2000). The longitudinal pattern obtained in Canal de Ílhavo may not accurately reflect the distribution of bacterioplankton in the complex estuarine system of the Ria. The annual average of the freshwater flux associated to Rio Boco during the period equivalent to a tidal cycle, corresponds to only ~1.5 % of the tidal prism at the outer section of this channel (Silva, 1994) and the unbalance between fresh and salt water inputs is enhanced during the warm season. Considering that the fieldwork along the estuarine gradient was conducted during late spring, the patterns of bacterioplankton abundance and activity are probably biased by the seasonal preponderance of marine influences.

The transition from the river to the main body of the estuary at the upper end of Canal de Ílhavo was characterised by a marked change in the characteristics of bacterioplankton communities, both in terms of size and of activity rates. The pattern of increasing abundance, potential peptide degradation and monomer uptake with decreasing salinity was interrupted between the two inner stations of the profile (I8 and Rio Boco). However, in spring tide, the strong marine influence associated to flood current produced a marked increase in salinity along the profile and extended the biological continuum to the upper station. Under different hydrological situations, total bacterial counts, rates of leucine-aminopeptidase (Leu-AMPase) activity and glucose uptake in Rio Boco were lower than in the adjacent estuarine section thereby confirming the mid-estuarine origin of the densest and more active bacterioplankton populations.

The pattern defined by the longitudinal variation of ?-glucosidase (?-GLCase) activity at the riverine interface was somewhat distinct. The highest activity rates were consistently achieved at Rio Boco indicating that in the Ria, like in other coastal systems,

?-GLCase activity was negatively related to salinity (Murrell *et al.*, 1999). It also indicates that the relative importance of carbohydrates as substrate sources decreased seawards. On the contrary, salinity played a minor role in the regulation of Leu-AMPase activity. The maximum rates of Leu-AMPase activity were always higher than the maximum rates of ?-GLCase as it also happens in other coastal and estuarine environments (Rath *et al.*, 1993; Christian and Karl, 1995; Murrel *et al.*, 1999). The shift in the pattern of polymer utilisation that occurred along the estuarine gradient was expressed as an increase of the ratio Leu-AMPase:?-GLCase. This ratio was 9-33 at the riverine interface and 35-48 at the interface with the sea. Changes in the relative importance of different ectoenzymes occur within estuarine gradients and reflect a change in the spectra of available substrates (Schulz Jr and Ducklow, 2000). Therefore, the transition from carbohydrate-utilising to protein-utilising communities was interpreted as an indication of the variation in the composition of the pool of utilizable organic matter between different estuarine sections.

The rates of Leu-AMPase in the Ria were in the high range of the values found in other estuarine and coastal waters (Hoppe, 1983; Rego *et al.*, 1985; Hoppe *et al.*, 1998). The exceptional potential for peptide degradation may result from the high anthropogenic inputs associated to the main body of the lagoon but may also reflect the utilisation of organic compounds as sources of nitrogen. The bacterioplankton of the Ria does not strictly depend upon phytoplankton primary production for carbon supply (Almeida* *et al.*, accepted) but may strongly compete with the dense phytoplankton communities for nitrogen (Chin-Leo and Benner, 1992). The high rates of peptide degradation may indicate that combined amino acids are intensely utilised as organic sources of nitrogen by the bacterioplankton of the Ria.

The variation of the rates of bacterioplankton-associated activities at the interface with the sea conducted to a consistent pattern of increase during ebb and decrease during flood tide. Along the longitudinal profile the tidal fluctuation of the variables describing heterotrophic activity was more pronounced than the fluctuation of cell abundance, reinforcing the non-conservative behaviour of planktonic bacteria during tidal transport.

The hypothesis that bacterial communities respond to changing water properties by shifting between levels of activity within time scales compatible with tidal effects originating the characteristic longitudinal patterns of activity, was tested in laboratory. The use of diffusion chambers allowed the assessment of the degree of environmental regulation of activity rates by reciprocally exposing bacterial assemblages from different estuarine sections to contrasting water properties. Activity rates of the tested communities markedly shifted within the 6-hour exposure periods whereas bacterial abundance

remained fairly stable. The results for ectoenzymatic activity (Leu-aminopeptidase and ?-glucosidase), glucose incorporation and biomass production after transference of the marine bacterial community to brackish water showed maxima in the range of 241-384 % of the control values. The opposite transference of the brackish-water bacterial community to marine water produced maximal decreases to 0.14-0.58 % of the control values. There was a concomitant increase in the CTC-active fraction of the marine community transferred to brackish water and a decrease in the proportion of active cells in the brackish water community transferred to marine water (Almeida *et al.*, 2001). This indicates that changes in total activity rates correspond to the superimposition of the variation of the size of the active fraction of the total community and of the activity levels of individual cells probably by shifting between enzymatic systems with distinct kinetic characteristics (Unanue *et al.*, 1999).

Ectoenzymatic activities were less affected by the experimental shifting in water properties, in coherence with the results obtained in the field, along the estuarine gradient and during tidal cycles at the interface with the sea. The parameters describing the heterotrophic metabolism of glucose (Vm and Tr) were more reactive to tidal transport than the potential rates of Leu-AMPase and ?-GLCase. Ectoenzymatic activity and monomer uptake reflect different metabolic processes and are differently controlled by the availability and quality of the pool of organic matter. The higher degree of reactivity of the uptake of monomers may indicate that the proportion of low molecular weight substrates within the DOM pool may vary significantly between the mid and outer sections of the estuary.

Major factors of regulation of bacterioplankton activity in Ria de Aveiro

Temperature and phytoplankton standing stocks were two of the major identified sources of variability of bacterioplankton heterotrophic activity. The combined influence of temperature, chlorophyll concentration and salinity explained ~80 % of the geographic variability of bacterial abundance. However, the contribution of these factors to the variability of parameters of bacterial activity was rather modest. The metabolic indicators related to monomer uptake, which were identified as the more reactive to environmental pressure, were not significantly associated to salinity or phytoplankton biomass, and temperature could not explain more than ~30 % of total variability. Under constant temperature, monomer uptake exhibited the strongest responses to experimental changing

of water. The control of bacterial activity in diffusion chamber experiments was restricted to the dissolved water properties supporting the thesis that bacterioplankton reactivity in the Ria is probably determined by short term variations of salinity and, in a major extent, by the size and composition of the DOM pool.

Phytoplankton standing stocks were positively associated with bacterioplankton abundance and efficiency of glucose incorporation. However, during the warm season, the secondary production of bacterial biomass is not limited by photosynthesis. In fact, bacterial carbon demand corresponded to ~20 % of the primary production in the water column determined in late spring (Almeida* *et al.*, accepted). The low dependence of bacterioplankton on autochthonous primary production and the high availability of organic substrates for bacterial growth during late spring and summer can also be inferred by the uncoupling between polymer degradation and monomer uptake. Maximum rates of glucose and leucine incorporation correspond respectively to 1-13 % of the ?-GLCase and 0.01-14 % of the Leu-AMPase associated supply of monomers, in terms of carbon. The uncoupling between phytoplankton primary production and bacterial secondary production as well as between polymer hydrolysis and monomer uptake occurs at high nutrient concentrations (Middelboe *et al.*, 1995; Hoppe *et al.*, 1998) and was interpreted as a consequence of the high degree of eutrophication of the Ria during the warm season.

Particulate organic matter inputs are sometimes related to high rates of bacterial activity in shallow environments (Chróst and Riemann, 1994) and estuarine turbidity maxima correspond to sections of elevated contribution of bacterial to total plankton biomass and organic matter degradation (Fuks *et al.*, 1991). No clear turbidity maximum could be defined within the high biological activity section corresponding to the 20-30 PSU range. However, the resuspension of bottom sediments and the particle-loaded runoff from the mud flats had been previously vented as on of the possible determinants of the dramatic increase of turbidity and of the high microbial activities and relatively low primary production in low tide (Hoppe *et al.*, 1996). The field approaches failed to demonstrate significant relations between POC concentrations and bacterioplankton heterotrophic activities. The fraction of particle-attached bacteria varied between 4 and 33 % of the total number, with no significant relation with either POC or chlorophyll. The relative contribution of attached cells (> 1,2 µm) to ectoenzyme activities was highly variable ranging from ~0 to 98 % of total Leu-AMPase and from ~0 to 83 % of ?-GLCase. Positive relations of bacterioplankton abundance and activity to phytoplankton biomass in the absence of clear relations with particulate sources of organic matter suggests that the

control is either exerted through algal exudates or that it corresponds to a parallel response of phyto- and bacterioplankton communities to gradients of inorganic nutrients.

No clear vertical or transversal patterns of POC or chlorophyll could be defined at the shallow fine-sediment mid-section of Canal de Ílhavo suggesting a minor contribution of local sediment resuspension and runoff to the supply of particles to the water column, under a slow tidal current regime. The indication that POM plays a secondary role in the regulation of bacterioplankton metabolism is coherent with laboratory evidence for the preponderance of dissolved factors in the reactivity of bacterial communities towards environmental variability. The advection of simple organic substrates at benthic and littoral interfaces, by density displacement of interstitial water (Webster *et al.*, 1996), may account for considerable inputs of DOM to the water column and contribute to the high rates of activity in shallow biotopes (von Bodungen *et al*., 1995) such as those prevailing in Ria.

The importance of biological processes in the water column in the context of a shallow estuarine system

For the assessment of the relative contribution of the water column to the total system metabolism it is important to consider that the estuarine system of the Ria de Aveiro has an average depth of 1 m, that the shallow section (< 3m) correspond to ~95 % the high tide wet area (estimated from Silva, 1994) and that the sediments are generally characterised by bacterial densities 3 orders of magnitude superior to those observed the water column (Alcântara *et al.*, 1996).

The magnitude of planktonic processes, standardised to surface units, exhibits a higher degree of short-term variability than that of the benthic compartment because of the tidal variation of depth. The sediments of the Ria are 9-71 times more active in organic matter aerobic mineralization than the water column, in a per volume unit basis (Alcântara *et al.*, 1996). However, the plankton accounts for 66-90 % of the total oxygen consumption per surface unit at sub-tidal areas. The contribution of bacteria to oxygen consumption, either in the plankton or in the benthos, was not established. In sediments, the contribution of microorganisms to total oxygen consumption tends to decrease towards shallower biotopes (Piepenburg *et al.*, 1995). Sulphate reduction seems to be the major pathway for organic carbon mineralization in shallow sandy sediments whereas denitrification gains relevance in finer and deeper sediments (Rysgaard *et al.*, 2000). The major contribution to oxygen consumption in estuarine waters is associated <1 µm size classes (Griffith *et al.*,

1990) but bacterial growth efficiency decreases with increasing eutrophication (del Giorgio and Cole, 1998). In the conditions of the Ria, and considering the evidences for the high availability of organic matter in the water column, an important contribution of bacterioplankton for total respiration should be expected.

Biogenic fluxes between the estuary and the sea

The patterns of organic matter utilisation along the estuarine gradient and the reactivity of heterotrophic bacterioplankton at the interface with the sea are major determinants of the impacts of the Ria on adjacent coastal waters.

Considerable amounts of labile DOC can be exported from estuaries where the residence time is short when compared to organic matter turnover time (Raymond and Bauer, 2000). The residence time estimated for the mid and inner sections of Canal de Ílhavo varies with season but generally corresponds to several weeks (Silva, 1994). In these conditions, the lability of organic substrates may considerably decrease with increasing proximity to the sea. Bacteria reaching the outer section of the estuary respond to higher salinity and to lower substrate quality by shifting to activity levels that are lower than expected by conservative dilution. On the contrary, a more favourable environment reactivates communities entering the estuary during flood tide.

The balance of the tidal exchange of water between the estuary and the ocean corresponded to a net seaward flux of bacterial cells and associated activities. The biogenic exports from the Ria to the sea persisted even during periods of net retention of marine water.

Unanswered questions

Two important aspects of the regulation of bacterioplankton activity in the estuarine system of the Ria de Aveiro could not be satisfactorily clarified.

The first refers to the role of DOM in the regulation of the processes of polymer hydrolysis and monomer uptake. The characterization of the DOM in terms of major estuarine sources, range of concentrations, spectra of compounds and bioavailability, as well as the evaluation of the relative importance of DOM and POM in the supply of nutrients for bacterioplankton growth are emerging investigation areas that will provide new insights into the dynamics of heterotrophic estuarine bacterioplankton.

The second aspect regards the co-existence of distinct communities highly adapted to the prevailing conditions at extreme sections of the estuary and the possibility that shifts in the levels of activity may correspond to changes in community composition. The experimental exposure to contrasting water properties revealed the plastic behaviour of mixed communities suggesting a high degree of environmental modulation of the expression of bacterial activities. However, the distinct reversibility of the effects caused by exposure to contrasting water of marine and brackish water communities may be interpreted as an indication of the existence of communities with different tolerance to salinity. The genetic characterization of the bacterioplankton communities of the Ria by culture-independent techniques would certainly bring decisive information on the subject.

Conclusions

The planktonic compartment is the major contributor to the aerobic degradation of organic matter in the sub tidal areas in the shallow estuarine system of the Ria de Aveiro. Although the Ria comprehends extensive areas of shallow channels and intertidal zones (salt marshes and mud flats) the resuspension of bottom sediments and the run off from intertidal zones appeared to play a minor role in the regulation of bacterial processes in the water column.

The bacterial component of the plankton responded intensely and within short-time intervals to shifts in environmental factors and the establishment of a clear longitudinal pattern of variation resulted from the reactivity of the expression of heterotrophic activity rates during tidal transport. The main body of the estuary, corresponding to the 20-30 PSU range, showed the highest bacterial abundance and heterotrophic potential for the ectoenzymatic degradation of polymers and for the uptake of monomers.

The levels of heterotrophic activity of bacterioplankton decreased at both riverine and oceanic boundaries, more than expected from conservative dilution, therefore confirming the reactive nature of bacterial metabolism in the highly unstable estuarine interfaces. There was also a shift in the patterns of organic matter utilization from a C-dependent metabolism at the less saline sections to an increasing relevance of N-dependence towards the outer section.

Laboratory experiments demonstrated that the environmental regulation of the activity of natural bacterial assemblages from contrasting estuarine sections is mainly

exerted through dissolved factors being salinity and substrate availability two of the main sources of variability.

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