

## Impact of freshwater inflow on bacterial abundance and activity in the estuarine system Ria de Aveiro



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### ARTICLE INFO

#### Article history:

Received 30 July 2013

Accepted 24 December 2013

Available online 6 January 2014

#### Keywords:

bacterial dynamics  
particle-attached bacteria  
bacterial biomass productivity  
freshwater inflow  
Lagrangian model  
estuary  
Ria de Aveiro

### ABSTRACT

The influence of freshwater flow on bacterial communities in the estuarine system Ria de Aveiro (Portugal) was investigated at two sites differently impacted by river inputs, representative of the marine and brackish water zones of the estuary. Sampling events were clustered based on hydrological features. The hydrodynamic was simulated with a Lagrangian model and related to microbiological parameters. Estuarine bacteria responded to different freshwater regimes developing distinct patterns of abundance and activity at the marine and brackish water zones. A circulation pattern induced by high river inflow produced vertical stratification in the marine zone, promoting a seaward flux of bacterioplankton, and stimulating the import of riverine phytoplankton and particle-attached bacteria to the brackish water zone. Advective transport and resuspension processes contributed to a 3-times increase in abundance of particle-attached bacteria during intense freshwater inputs. Additionally, bacterial activity in the estuary was controlled by inorganic nitrogen, responding to different freshwater inputs, which, in association with different prevailing sources of organic substrates induced significant changes in bacterial production. The dynamic and main controlling factors of bacterial communities are clearly impacted by freshwater inputs. Therefore, significant changes in the recycling of nutrients by microbial activities can be expected from alterations in freshwater inputs either related to global climate change or regional hydrological regimes.

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### 1. Introduction

Estuaries are highly dynamic ecosystems, where the mixing between marine and freshwater produces steep gradients of salinity, as well as of organic and inorganic nutrients. Microbial communities adapt to this high variability of physicochemical factors and substrate availability with shifts in activity (Cunha et al., 2001; Almeida et al., 2001b), composition (Crump et al., 1998; Kirchman et al., 2005; Campbell and Kirchman, 2013) or/and life-style strategies (i.e., free-living or particle-attached) (Murrell et al., 1999; Karrasch et al., 2003; Lapoussière et al., 2011). The relative proportions of particle-attached to free-living bacterial abundance vary considerably with the aquatic environment, depending essentially on the abundance of particles (Simon et al., 2002). Although particle-attached bacteria represent less than 10% of total bacterial abundance in most of the pelagic environments, in

estuarine systems their contribution can be much higher, reaching up to 90% (Simon et al., 2002; Lapoussière et al., 2011). Moreover, particle-attached bacteria could be larger (Lapoussière et al., 2011) and more active (Crump et al., 1998; Crump and Baross, 2000) than free-living bacteria, playing an important role in the recycling of detrital organic matter in the estuarine environment (Crump and Baross, 1996, 2000; Crump et al., 1998). Particle-attached bacteria could be genetically distinct (Crump et al., 1999) or similar to free-living bacteria (Hollibaugh et al., 2000). This genetic differentiation is promoted by the organic rich particle microenvironment, which availability in estuaries is related to terrigenous inputs associated with particle loads from river runoff and, with the overall productivity of the system (Hollibaugh et al., 2000; Vallières et al., 2008).

In numerous coastal lagoons and estuaries, water circulation is mainly forced by river flow at the head of the estuary and tidal exchanges at its mouth (Vaz and Dias, 2008). Even when the water circulation is forced primarily by tidal exchanges, freshwater inflow also determines the general estuarine circulation and thermohaline patterns after periods of high precipitation (Dias et al., 1999).

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Additionally, freshwater flow is the predominant source of seasonal and interannual variability in estuaries (Skreslet, 1986), influencing the physics, geology, chemistry, and biology of estuaries through various pathways (Skreslet, 1986; Sklar and Browder, 1998; Almeida et al., 2007). Changes in freshwater flow impact the inundation of the flood plains, advective transport of materials and organisms, dilution or mobilization of contaminants, compression of the estuarine salinity field and density gradient, stratification, and residence time for water (Kimmerer, 2002) and ultimately, estuarine microbial processes.

Although the Ria de Aveiro hydrodynamic regime is tidally dominated (Dias et al., 1999), an exceptional high freshwater input to the estuary, during a particularly rainy year, led to unpredicted seasonal variations of bacterial abundance and activity, stimulated mainly by allochthonous substrates leached from the surroundings (Almeida et al., 2007). This episode reinforced a reactive behavior of bacterial activity to environmental changes (del Giorgio et al., 2006) and showed the many of environmental factors that controls microbial communities in estuaries (Apple et al., 2008).

This work aims to identify the factors that influence bacterial biomass productivity, abundance and lifestyle under different hydrological conditions in an estuarine environment. In order to achieve this goal, two sites with contrasting fresh and sea water influences in the estuarine system Ria de Aveiro were studied and compared, and the influence of rivers inflow on water residence time was assessed by a numerical model application. We consider that this study uses an innovative approach to analyze and interpret the variations of bacterial abundance and bacterial biomass productivity in an estuarine ecosystem, integrating statistical analysis with microbiological data and numerical model simulation of local hydrodynamics.

## 2. Methods

### 2.1. Study site

The Ria de Aveiro (40° 38'N, 8° 45'W; Fig. 1) is a shallow tidal lagoon situated on the Northwest Atlantic coast of Portugal, separated from the sea by a sand bar. The lagoon covers an area ranging from 64.9 to 89.2 km<sup>2</sup> at low and high tide, respectively (Lopes et al., 2013). It exchanges with the sea a volume of water of  $137 \times 10^6$  for maximum spring tide and  $35 \times 10^6$  m<sup>3</sup> for minimum neap tide (Dias et al., 2000). The lagoon has a complex bathymetry, with four main channels spreading from the mouth, i.e., S. Jacinto, Espinheiro, Mira and Ílhavo. Due to their unique characteristics each one could be considered as an independent estuary connected to a common inlet (Dias et al., 2001). Freshwater is supplied to the lagoon mainly by the rivers Vouga, Antuã, Cãster, Gonde and Boco, with an average discharge of  $1.8 \times 10^6$  m<sup>3</sup> during a tidal cycle (Dias et al., 2003). Among these rivers, the major contributor is the Vouga River which discharges more than 66% of the incoming freshwater (Dias et al., 1999) and is connected to the Atlantic Ocean by the Espinheiro Channel. For this study two stations with distinct water column and hydrodynamic characteristics, as well as different impacts of freshwater inflow were selected. Station N1, located near the mouth of the estuary, is highly exposed to the oceanic influence and impacted indirectly by the whole freshwater input into the lagoon. Station I6, located at inner section of the Ílhavo channel, the narrower and shorter of the main channels (Dias et al., 2001), is directly influenced by the river Boco discharge. Due to their water column characteristics and location, these two stations are located in lagoon areas currently referred as the marine zone (station N1) and the brackish water (station I6) zone (Almeida et al., 2001a, 2002a).

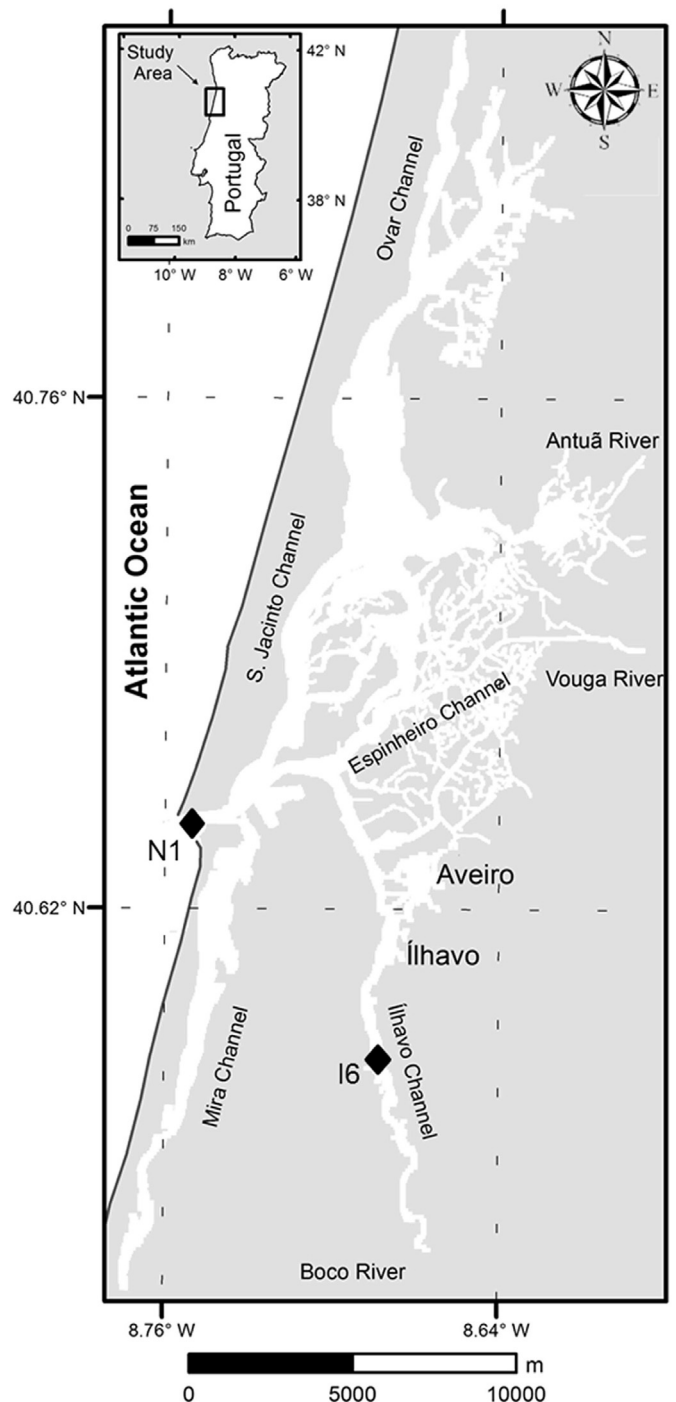


Fig. 1. The estuarine system Ria de Aveiro with indication of sampling stations. Station N1 in Canal de Navegação represents the marine zone, and station I6, in Canal de Ílhavo, represents the brackish water zone.

### 2.2. Sampling

Sampling was conducted at low tide, approximately every two months during two years (2006 and 2007). Water column samples were collected with a horizontal Von Dorn bottle at the fixed depths of 20 cm, 50 cm, mid-column and 50 cm above the sediment surface. Samples were kept at 4 °C during the transport to the laboratory and processed within 2–3 h after collection.

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