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Macroinvertebrates communities associated with the decomposition of *Phragmites australis* and *Fucus vesiculosus* in transitional systems ☆

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ABSTRACT

The decomposition rates of a macrophyte (*Phragmites australis*) and an alga (*Fucus vesiculosus*) and the associated macrofauna communities were studied along a full salinity gradient, using the leaf-bag technique and four sampling times (days 3, 7, 15 and 30). A control was set up using an artificial substrate. A subsequent study conducted in the mesohaline part of the salinity gradient also included empty bags as procedure control. The decay rates of the alga and the macrophyte were significantly different, the alga decaying faster, and presented an opposite trend along the salinity gradient, with the faster decay rate for reed in the less saline areas and for the alga in the euhaline part of the gradient. The fauna associated with the decaying and the artificial substrate showed equally well the benthic succession from the marine to the freshwater areas, in all sampling times. Arthropods were dominant in all substrates along the estuarine gradient and replaced by annelids in freshwater. No significant differences were found between the benthic communities associated with *P. australis* and *F. vesiculosus*, despite the strong differences in the decay rates, suggesting that these do not seem to be primarily related to the benthic colonizers. Although the organic substrates sustained a more abundant fauna, the benthic communities did not show significant differences between the organic and the artificial substrates, especially at the level of the species composition, suggesting that the macroinvertebrates may colonize both substrates to feed on the biofilm and/or to seek shelter. The strongly impoverished benthic community sampled by the empty bags reinforced this idea.

1. Introduction

Benthic macroinvertebrates play an important role in many ecological processes in aquatic ecosystems (Griffiths, 1991; McCall and Soster, 1990). Transitional waters are extremely productive systems, with large quantities of organic matter available to decomposition (among others, McLusky and Elliott, 2004). In experimental studies conducted in the freshwater environment, litter decomposition starts with a weight loss due to the washing out of the organic matter's soluble constituents (leaching), followed by the modification of the organic matrix by micro-organisms as a result of enzymatic activities (conditioning) and finally the physical break down of the coarser detritus mediated by shredder feeding (fragmentation) (Hargrave, 1970; Mancinelli, 2012; Petersen and Cummins, 1974; Rossi, 1985; Webster and Benfield, 1986).

Macroinvertebrates have long received attention on impact assessment and water management studies, being one of the diagnostic biological elements included in the European Water Framework Directive (WDF, 2000/60/EC). Indices were developed namely based in the species tolerance/sensitivity to organic enrichment. However, transitional waters are naturally stressed and characterized by highly dynamic physical, chemical and hydro-morphologic conditions and by species with a higher level of tolerance to change, being more difficult to develop suitable quality indicators for these systems than for the marine environment (Elliott and Quintino, 2007). In recent years several biotic indices have been proposed to be used as ecological quality indicators for the marine and estuarine systems (AMBI, Borja et al., 2000, BENTIX, Simboura and Zenetos, 2002, BQI, Rosenberg et al., 2004, BOPA, Dauvin and Ruellet, 2007). The estuarine quality paradox however leads authors to suggest the importance to use functional as well as structural indicators of change in transitional waters (Elliott and Quintino, 2007).

In the freshwater environment, several bio-monitoring studies have included both functional and structural approaches, namely leaf-litter decomposition rates and structural characteristics of the associated benthic invertebrate community (Bergfur et al., 2007; Castela et al., 2008; Pascoal et al., 2003). In estuarine ecosystems, decomposition studies were far less common (Menéndez and Sanmartì, 2007; Rossi and Costantini, 2000; Sangiorgio et al., 2008; Twilley et al., 1986), and a recent study conducted in Mira Channel, Ria de Aveiro, Portugal, comprising the full salinity gradient and using *Phragmites australis* and *Fucus vesiculosus* as test species, indicated that the decaying substrate, the part of the salinity gradient where decomposition is taking place and the time interval for the calculation of the decomposition rate, all interfere with the

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final result and should be taken in consideration if using decomposition rates as a measure of estuarine ecosystem functioning (Lopes et al., 2011; Quintino et al., 2009). Here, we take that analysis further by studying the macrofauna associated with the decomposition of *P. australis* and *F. vesiculosus* in experiments conducted in Mira Channel, covering all levels of the Venice System (1959) classification of brackish waters, and in two other channels representing the mesohaline region, Canelas and Salreu. The study used a control, consisting of an artificial substrate made up of plastic pieces, and a procedure control, consisting of empty mesh bags. With these studies we aim to test the null hypotheses that from the euhaline to the limnetic area no significantly different macrofauna communities are associated with the experimental substrates at a given sampling time as well as along the study period and that no significant differences exist between the macrofauna community captured in the leaf-bags with the decaying organic substrates, with the artificial control substrate, and in the empty mesh bags.

2. Material and methods

2.1. Study area

Ria de Aveiro is located on the Northwestern coast of Portugal, between 40°38′N and 40°57′N and presents a maximum of 10 km width and 45 km length. This system is characterized by extensive intertidal mud and sand flats, salt marshes and islands. It includes four main channels, Mira, Ílhavo, Espinheiro and S. Jacinto, all of which receive freshwater inputs. This study was conducted in Mira, Canelas and Salreu Channels. Mira Channel is a narrow 20 km long channel, running south from the entrance (Fig. 1). It is one of the most pristine channels in Ria de Aveiro (Castro et al., 2006) and the salinity gradient ranges from fully marine at the mouth to freshwater at the head, receiving continuous freshwater input from a small system of ponds and drainage channels. Canelas and Salreu channels are smaller, connected with the Laranjo Basin, and located in the central area of Ria de Aveiro (cf. Fig. 1).

2.2. Field and laboratory procedures

The study in Mira Channel was performed during winter 2009 (January–March), in a total of fifteen sites arranged in five areas with three sites per area. The five areas spread across all levels of the salinity gradient according to the Venice System (1959) for the Classification of Estuarine Waters: euhaline (area 1); polyhaline (area 2); mesohaline (area 3); oligohaline (area 4) and limnetic (area 5) (Fig. 1). The mean salinity values (with standard deviation) obtained over a complete tidal cycle in the five areas were respectively 34.6 ± 1.52, 30.2 ± 3.48, 16.2 ± 6.59, 2.4 ± 2.12 and 0.0 (Quintino et al., 2009). An experimental field study of the decomposition of dry *P. australis* and *F. vesiculosus* was undertaken simultaneously in all fifteen sites (Lopes et al., 2011), using the leaf-bag technique (Petersen and Cummins, 1974). At the beginning of the experiment (day 0), all the 5 mm mesh bags with 3.0 g of *P. australis* leaves, of *F. vesiculosus* and of an artificial substrate used as control (plastic pieces) were placed in the field sites, at the bottom, in the subtidal. In each site, four replicates of each substrate were collected over time in days 3, 7, 15 and 30. Each replicate was placed in separate plastic containers, brought to the laboratory, washed through a 0.5 mm mesh sieve and the residue preserved in 70% ethanol. Macroinvertebrates were sorted and identified to species level whenever possible. At the end of the experiment, the biological data matrix included the macroinvertebrate species/taxa and their abundance per replicate, per site and per sampling time for each substrate. During winter 2011 (February–April) a confirmation study was conducted in the mid part of the salinity gradient, the mesohaline region, in two other channels of Ria de Aveiro, Canelas and Salreu. The mean salinity over a complete tide cycle was 13.8 ± 4.55 in Salreu and 12.8 ± 4.43 in Canelas. This confirmation study used the same experimental organic decaying substrates and the control substrate but included more samples and added a procedure control, consisting of empty mesh bags. Four areas per channel and two sampling sites per area were established (Fig. 1). In each sampling time, three replicates of each substrate and the empty bags were collected and treated as mentioned before for the study of the associated macroinvertebrate communities. In total, in the Salreu and Canelas Channels, 48 replicates per substrate were obtained per sampling occasion, whereas the mesohaline region of the Mira Channel was studied using a total of 12 replicates per experimental substrate and per sampling moment. The decay of *F. vesiculosus* and *P. australis* followed the same methodology used by Lopes et al. (2011) in the decomposition study of these two species in the Mira Channel. The decay rate (k) for each species was obtained by modeling the remaining biomass as a negative exponential decay function, from day 0 to 7 (k7), 0 to 15 (k15) and 0 to 30 (k30). Bottom water samples were also collected simultaneously every 30 min during a period of 12 h, in the three sites per channel, in order to measure salinity over a tidal cycle (sites Ca to Cc in Canelas and Sa to Sc in Salreu, Fig. 1).

2.3. Data analysis

The benthic macrofauna data were represented by the abundance of species per replicate, per site and per sampling time for each substrate and empty mesh bags. The resemblance matrix between samples was obtained with the Bray–Curtis similarity coefficient, following a square root transformation of the original data. Synthesis descriptors, namely the mean number of species/taxa and the mean abundance of specimens per study area, sampling time and substrate were calculated.

In Mira Channel, a few samples didn't have macrofauna associated and, in order to keep those samples in the analysis, prior to the calculation of the Bray–Curtis resemblance matrix, a dummy variable was added to all, with the abundance of 1 individual (Clarke et al., 2006). The macrofauna community data was analyzed in an experimental design with four factors, using the salinity classes, time and substrate as fixed and orthogonal factors and the sampling sites as a random factor nested in the salinity classes, under the null hypothesis of no significant differences in the benthic community among the levels of the main factors and their interaction terms. In this analysis, salinity had five levels (euhaline, polyhaline, mesohaline, oligohaline and limnetic), time had four levels (days 3, 7, 15 and 30) and substrates had three levels (macrophyte, alga and control).

The study conducted in Salreu and Canelas showed that many replicates had low species richness or no macrofauna, specially the empty mesh bags. Due to such low taxa richness, the use of a dummy variable would artificially increase the similarity between samples which could mask the potential differences. Instead, we opted to combine the three replicates from each site at each sampling time into a single sample. This minored the problem of having many replicates without fauna and increased the sample redundancy. Each site was then represented by a single sample from averaging the species abundance of the original three replicate mesh-bags. A second analysis averaged the species at the level of the area, using all six individual mesh-bags from the two sites sampled per area. This second approach produced a matrix where no single sample was devoided of species, but did not allow to analyze the data using areas nested in the channels, given that each area was represented by a single sample. In the first case, the data was analyzed in an experimental design with three factors, with channels (two) and substrates (four) fixed and orthogonal and areas (four), random, nested in channels. In the second case, a two-way analysis was used to test the null hypothesis of no significant differences between the macrofauna associated with the substrates and between channels, with channels and substrates as fixed and orthogonal factors. This same model was used following a presence–absence transformation, to analyze the selectivity of the macrofauna species for the organic substrates, the control substrate and the empty bags.