Proceeding Paper

Diversity and Abundance Patterns of Benthic Invertebrate Assemblages on Intertidal Estuarine Seagrass Beds in Aveiro (Portugal) †

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Abstract: Seagrass meadows are productive ecosystems and many animal species are dependent on them, including a wide diversity of invertebrates. This study aims to explore spatial diversity patterns of benthic invertebrates associated to *Zostera noltei* meadows. Three meadows were sampled along the Mira Channel (Ria de Aveiro). At each meadow, two sites were selected and four core were taken at each site. Fauna was sorted, counted and identified to the lowest taxonomical level. Results showed significant differences on the number of taxa among meadows. It was also observed that some taxa presented differences in the abundance among meadows.

Keywords: macrofauna; intertidal; seagrass; Portugal

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1. Introduction

Seagrass meadows form one of the most productive ecosystems in the world [1] and fulfill key functions on coastal ecosystems [2]. Seagrasses are ecosystem engineers, due to their ability to modify environmental and biological drivers and enhance the local biodiversity [3,4]. Seagrass meadows are distributed in tropical and temperate waters around the globe, on marine and estuarine rocky, sandy and muddy substrates [2,3]. However, during the last decades they became one of the most endangered ecosystems on coastal systems [5]. This may be the result of their high sensibility to disturbances—such as water pollution, sediment resuspension, eutrophication, invasive species and even exploitation of animal species—that, for instance, increases the dispersion of their seeds [2,5,6].

Seagrass meadows establish complex habitats that shelter many marine organisms such as epiphytic algae, fishes, vertebrates, as well as a great diversity of invertebrates that can be associated with their leaves (epifauna) or their rhizomes in the sediment (infauna) [2]. Therefore, meadows may be tenfold more diverse than other similar systems not colonized by seagrasses and less structurally complex [2]. This enhanced invertebrate diversity plays a critical role in seagrass meadows. Epifaunal animals, such as amphipods and gastropods, feed upon epiphytic organisms that cover seagrass leaves, cleaning them and allowing to improve light incidence, oxygen and car-bon dioxide intake, while infaunal organisms increase sediment oxygenation [7], and filter-feeders, like annelids and bivalves, reduce water turbidity. Moreover, many of these animals serve as food for higher trophic levels [2].

The seagrass *Zostera noltei* creates extensive meadows along the Atlantic intertidal areas of the Iberian Peninsula, often dominant, especially in the Ria de Aveiro lagoon. The aim of this study is to explore changes in invertebrate diversity associated with *Z. noltei* meadows along an estuarine gradient.

2. Materials and Methods

2.1. Study Area

This study was done during July 2019 throughout the Mira channel of the Ria the Aveiro lagoon, a waterbody that runs parallel to the coastline for about 25 km. Freshwater supply, mainly on the upper area of the channel creates a salinity gradient, with low levels of salinity in the inner part and higher values at the mouth [8]. As a result of increasing anthropogenic influence, the channel has been transformed over the last decades becoming a more heterogeneous environment [9]. Several human activities, such as dredging, shellfish and bait harvesting, recreational navigation and oyster farming, have been advanced everywhere, but particularly along the margins.

2.2. Sampling and Sample Processing

To study the invertebrate diversity associated with *Z. noltei* meadows in the Ria de Aveiro lagoon, three areas were selected: (A, B and C, Figure 1). Meadow A was located upwards the waterbody and meadow C was positioned downwards, while meadow B laid in the middle close to oyster trestles of a commercial farm at approximately the same distance from the other sites (Figure 1).

Two sites, separated by 10 s of meters, were selected at each meadow; and at each site, four sediment replicates were randomly collected. Samples were retrieved with a 0.02 m2 corer to a depth of 30 cm, and subsequently sieved in situ through a 0.5 mm-mesh bag. Macrofauna retained was preserved in a 4% neutralized formaldehyde solution with Rose Bengal.

Fauna samples were sorted, identified to the lowest possible taxonomical level (mainly species) and counted, using a stereo microscope (Nikon SMZ800) and a microscope (Leica DM 2500 LED) for smaller organisms.

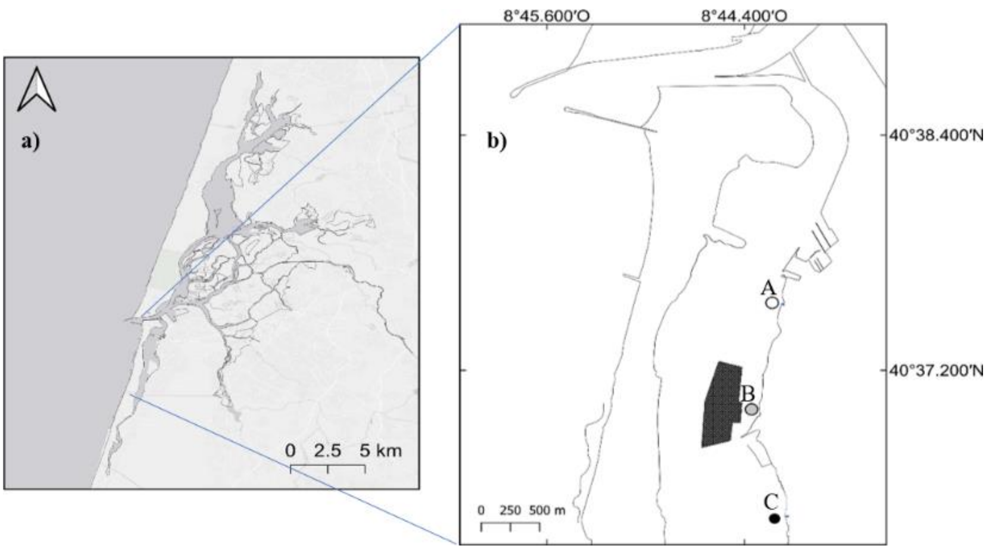

Figure 1. (**a**) Study area. (**b**) location of the three studied meadows along the Mira channel (A; B; C). Oyster farm is marked as dark grey polygon.

2.3. Data Analyses

Differences among meadows on total number of individuals (N), Shannon index (H′) and number of taxa (S) of macrofauna were explored by analysis of variance (ANOVA). These analyses considered two factors: Meadow (Me) as an orthogonal fixed factor with three levels (A, B, C) and Site (Si) as a random factor nested in Me with two levels (1, 2). Homogeneity of variance was tested by a Cochran's C test and data were log (x+1) transformed when necessary. Whenever ANOVA showed significant differences ($P < 0.05$), a post hoc Student–Newman–Keuls (SNK) test was done for a posteriori multiple comparisons. In order to explore differences among meadows in the abundance of dominant taxa, ANOVA analysis was used following the same design previously described.

3. Results

A total of 5560 individuals represented by 41 different taxa were sampled. Oligochaetes constitute 44% of this abundance followed by *Peringia ulvae* (20%). ANOVA results (Table 1) showed non-significant differences among meadows on the values of H′ and N but, significant variability among sites was detected for N (Table 1).

Table 1. ANOVA results for Number of Taxa (S), Abundance (N) and Shannon Diversity Index (H′).

		S			**N**			**H′**		
Source	**df**	**MS**	**F**	**P**	**MS**	**F**	**P**	**MS**	**F**	**P**
Meadow	2	103.625	11.840	**0.038**	2.771	4.320	0.131	0.584	2.910	0.198
Site	3	8.750	1.450	0.261	0.641	4.460	**0.017**	0.201	2.480	0.094
Residuals	18	6.028			0.144			0.081		
Total	23									

Values of S showed significant differences among meadows (Table 1) with significantly higher values on meadow A than meadows B and C (Figure 2).

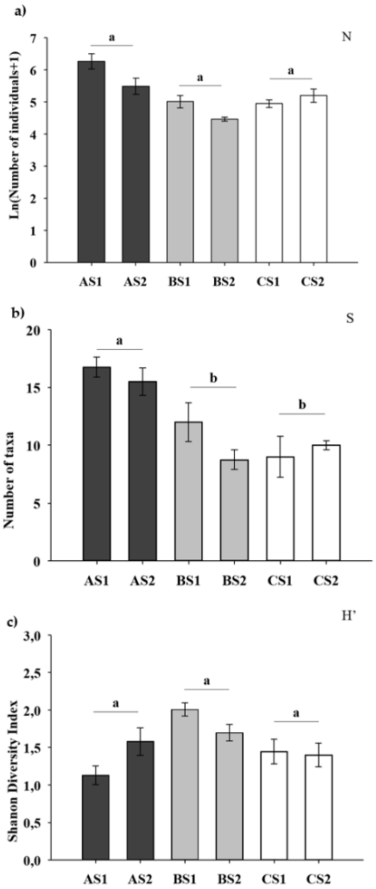

Figure 2. Mean + SE of (**a**) Abundance (N); (**b**) Number of Taxa (S); (**c**) Shannon Index (H′). Different letters indicate significant differences. In the *X* axis the first letter is the meadow (A, B and C) and sites within meadows are represented as S1 or S2.

The most abundant taxa were: *Cyanthura carinata*, *Hediste diversicolor*, nematodes, *Notomastus latricerus*, oligochaetes, *P. ulvae* and *Scrobicularia plana*. However, ANOVA results only showed significant differences among meadows for *H. diversicolor*, nematodes and *P. ulvae* (Table 2).

Table 2. ANOVA results for the more relevant species.

		P. ulvae			***H. diversicolor***			**Nematodes**		
Source	**df**	**MS**	**F**	**P**	**MS**	**F**	**P**	**MS**	**F**	**P**
Meadow	2	3.157	18.690	**0.0202**	2399.542	19.800	**0.0187**	15.551	49.280	**0.0051**
Site	3	0.169	0.520	0.6728	121.208	3.170	**0.0495**	0.316	0.750	0.5370
Residuals	18	0.323			38.236			0.421		
Total	23									

Moreover, post hoc analysis showed very different patterns of abundance for these three species: *Peringia ulvae* had lower abundance on meadow B, higher on meadow C and in-termedial on meadow A (Figure 3a), while *Hediste diversicolor* increased its abundance significantly from meadow A to meadow B and C (Figure 3b). Finally, nematodes were significantly more abundant on meadow A (Figure 3c).

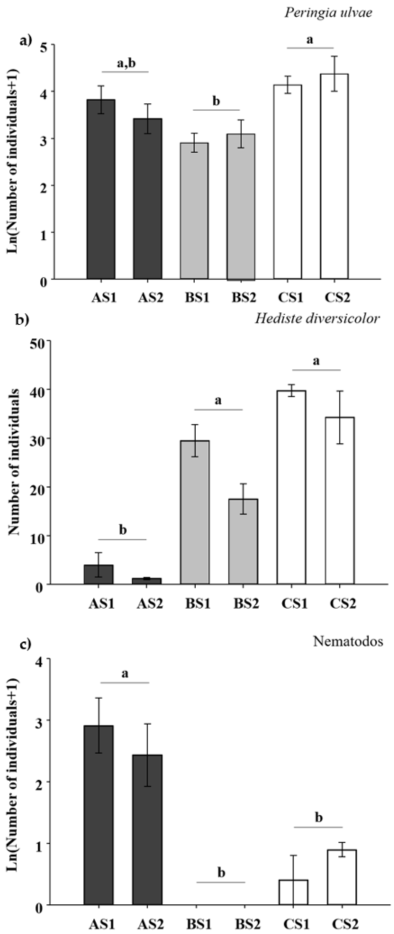

Figure 3. Abundance (Mean + SE) and significant differences among meadows for: (**a**) *P. ulvae*; (**b**) *H. diversicolor*; (**c**) Nematodes. In the *X* axis the first letter is the meadow (A, B and C) and sites within meadows are represented as S1 or S2.

4. Discussion

A total of 41 taxa were found in our study, while in a similar study, exploring invertebrate diversity associated to *Z. noltei* meadows in the Mediterranean, the authors found a total of 102 taxa with S values between 13 and 33 taxa per site and H values between 1.86 and 2.83 [10]. In general, these values were higher than those reported in this study and our highest values were nearly the lowest values found by [10]. This difference may be explained by a higher sampling effort done by [10] and because all their sampled sites were fully marine. However, *Z. noltei* populations in the Atlantic shores are frequently found in estuarine habitats like habitats like our study area. In this scenario, when we consider other studies done in the European Atlantic shores, we find contrasting results. In a study done in Belgium with a similar sampling effort, [4] found lower values of S (22 taxa) and H (1.24) than this study. On the other end, many studies done in Arcanchon bay (France) found similar values for the median number of taxa [11,12] and, in other cases, total or mean values of S were higher than in our study [3,13]. These differences may be the result of different sampling effort or due to variable structural heterogeneity of *Z. noltei* meadows [3]. Finally, many dominant species on our study were also found in other Atlantic localities. However, their abundance and distribution patterns were very variable spatially and temporally. This highlights the importance of investigating the community structure in stressful environments, particularly in estuarine seagrass meadows.

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Data Availability Statement: The data presented in this study are available on request from the corresponding author. The data are not publicly available because this data set will be included as part of other ongoing studies.

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

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