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A comparative analysis of benthic nematode assemblages from *Zostera noltii* beds before and after a major vegetation collapse

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A B S T R A C T

Benthic nematodes are widely regarded as very suitable organisms to monitor potential ecological effects of natural and anthropogenic disturbances in aquatic ecosystems. During 2008, the seagrass beds of *Zostera noltii* located in the Mira estuary (SW Portugal) disappeared completely. However, during 2009, slight symptoms of natural recovery were observed, a process which has since evolved intermittently. This study aims to investigate changes in patterns of nematode density, diversity, and trophic composition between two distinct habitat conditions: "before" the collapse of seagrass beds, and during the early recovery "after" the seagrass habitat loss, through the analysis of: i) temporal and spatial distribution patterns of nematode communities, and ii) the most important environmental variables influencing the nematode assemblages. The following hypotheses were tested: i) there would be differences in nematode assemblage density, biodiversity and trophic composition during both ecological conditions, "before" and "after"; and ii) there would be differences in nematode assemblage density, biodiversity and trophic composition at different sampling occasions during both ecological conditions. Nematode density and diversity were significantly different between the two ecological situations. A higher density was recorded before, but a higher diversity was evident after the collapse of *Z. noltii*. In spite of the disturbance caused by the seagrass habitat loss in the Mira estuary, the nematode trophic composition did not significantly differ between the before and after seagrass collapse situations. Despite the significant differences found among sampling occasions, a consistent temporal pattern was not evident. The response of nematode communities following this extreme event exhibited considerable resistance and resilience to the new environmental conditions.

1. Introduction

Benthic nematodes provide valuable information regarding ecosystem health (Sheppard, 2006). Sediment structure, chemistry, disturbance and availability of food, such as bacteria and microphytobenthos, are closely linked to nematode assemblage composition and distribution patterns (Giere, 1993; Heip et al., 1985; Moens et al., 2005), through the changes in density, diversity, structure and functioning (Danovaro et al., 2008; Norling et al., 2007; Patrício et al., 2012). Furthermore, several studies have highlighted the importance of the link between nematode diversity and ecosystem functioning, which may be important in the assessment of estuarine and marine biological integrity (Coull and Chandler, 1992; Danovaro et al., 2008; Fonseca et al., 2011; Moreno et al., 2008; Schratzberger et al., 2004; Steyaert et al., 2007).

Seagrass beds provide habitat for ecological communities and enhance biodiversity through their facilitative effects on associated species (Ellison et al., 2005), acting as ecosystem engineers by structuring pelagic and benthic assemblages (Bos et al., 2007). Seagrass beds are important in primary production, nutrient cycling, sediment and nutrient trapping, sediment stabilization, and their structural complexity is critical for the animals which live in them (Boström and Bonsdorff, 1997; Orth et al., 2006). Several studies that analysed the meiobenthic communities associated with seagrass beds have concluded that meiofauna in vegetated

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sediments is more abundant and diverse than in adjacent bare sediments (Castel et al., 1989; Fisher and Sheaves, 2003; Guerrini et al., 1998; Ndaro and Olafsson, 1999).

There have been numerous reports of seagrass decline around the world indicating that seagrass habitats are undergoing a global crisis threatening associated organisms and ecosystem functions (Hughes et al., 2009; Valle et al., 2014). Although sometimes caused by natural disturbances, most declines are attributed to anthropogenic disturbances (Short and Wyllie-Echeverria, 1996). Along the Portuguese coast, seagrass populations are also facing unprecedented declines in distribution, matching the general trends described for seagrasses worldwide (Cunha et al., 2013). The *Zostera noltii* (Hornem.) seagrass beds in the Mira estuary have been a prominent habitat since the 90s (Almeida, 1994; Costa, 2004; Costa et al., 1994, 2002). During 2008, however, these *Zostera* beds disappeared completely, leaving behind a bare muddy area (Cunha et al., 2013). Important changes in sedimentation dynamics were clearly observed during the last decade (Cunha et al., 2013) and have been identified as major drivers of seagrass loss elsewhere (Fourqurean and Rutten, 2004), but whether they are also at the basis of the disappearance of *Zostera* in the Mira remains unclear. During 2009, *Z. noltii* started showing slight symptoms of natural recovery characterised by growth pulses with an irregular spatial and temporal distribution of small-sized seagrass patches. Therefore, the spatial and temporal distribution of seagrass became strongly heterogeneous in both space and time (Cunha et al., 2013). This context of the seagrass collapse at the Mira estuary creates a unique opportunity to examine benthic faunal responses during the early natural recovery process of seagrass vegetation. Specifically, former data of the nematode assemblages from the Mira seagrass beds from two decades ago can be compared with data collected during the natural recovery process of seagrass.

This study aims to investigate changes in patterns of nematode density, diversity, and trophic composition between two distinct habitat conditions: "before" the collapse of seagrass beds, and during the early recovery "after" the seagrass habitat loss, through the analysis of: i) temporal and spatial distribution patterns of nematode communities, and ii) the most important environmental variables influencing the nematode assemblages. The following hypotheses were tested: i) there would be differences in nematode assemblage density, biodiversity and trophic composition during both ecological conditions, "before" and "after"; and ii) there would be differences in nematode assemblage density, biodiversity and trophic composition at different sampling occasions during both ecological conditions.

2. Materials and methods

2.1. Sampling area and design

Sampling was performed at the Mira estuary at the southwestern coast of Portugal (37°40′N, 8°40′W), a small mesotidal system with a semidiurnal tidal regime (amplitude 1–3 m during neap and spring tides, respectively). Together with its surrounding area, this estuary is included in a protected area, the Natural Park of "Sudoeste Alentejano e Costa Vicentina'. In the Mira estuary the depth is usually lower than 5 m, the width of the water is usually much less than 400 m, and the tidal influence extends to *ca.* 40 km upstream. The physical and chemical fluctuations mainly result from natural pressures due to the estuary's morphology. Upstream tidal penetration is generally limited by the region's annual rainfall distribution concentrated between January and March, with the rest of the year being usually dry. In addition, the annual rainfall has a clear influence on the changes of sedimentation dynamics (Paula et al., 2006). Due to the low, seasonal and limited freshwater input, the lower section of the estuary has a dominant marine signature, and was characterised until 2008 by extensive, homogeneous *Z. noltii* meadows mainly in the intertidal area, with a strong seasonality and high biomass in the warm months. Moreover, *Zostera marina* vegetation was also present before 2008 in the adjacent subtidal area (Cunha et al., 2013). In 2008, *Z. noltii* meadows disappeared completely, however, indications of natural recovery have been observed since 2009 (Cunha et al., 2013).

To compare the temporal and spatial distribution patterns of nematode communities both "before" the seagrass habitat loss and "after" the loss, during the early recovery period of seagrass beds, samples were collected at two sampling sites located in the intertidal sediments of the *Z. noltii* beds; Site A, *ca.* 1.5 km from the mouth of the estuary, and Site B, 2 km upstream from the river mouth (Fig. 1). Sampling collections were carried out at neap low tide, at the same sites, and following a similar seasonal pattern. The former data was sampled in June 1994, September 1994, December 1994, February 1995 and June 1995. During the early recovery period, samples were collected in February 2010, June 2010, September 2010, December 2010 and February 2011. Former data was sampled three times at each sampling occasion, separated by 15 days, and in each time two replicates were taken, with a total of six replicates. In view of the high spatio-temporal variability in seagrass cover, in the 'after' sampling, it was decided to collect all samples of a given sampling occasion at the same day, taking three replicates per station as in many other environmental impact studies focussing on the composition and diversity of estuarine or marine nematode communities (Adão et al., 2009; Alves et al., 2013).

2.2. Sampling and sample treatment

2.2.1. Biological data

Nematode samples were obtained by forcing hand cores (3.6 cm inner diameter) to a depth of 3 cm. All samples were preserved in 4% buffered formalin solution. Nematodes were extracted from the sediment using a density gradient centrifugation in colloidal silica (Heip et al., 1985). The fixed samples were rinsed on a 1000 μm mesh sieve followed by sieving on a 38 μm mesh. The fraction retained was washed and centrifuged three times using the colloidal silica polymer LUDOX HS-40 (specific gravity 1.18 g cm^{-3}). The supernatant of each washing cycle was again collected on a 38 μm sieve. After extraction, all nematodes were counted under a

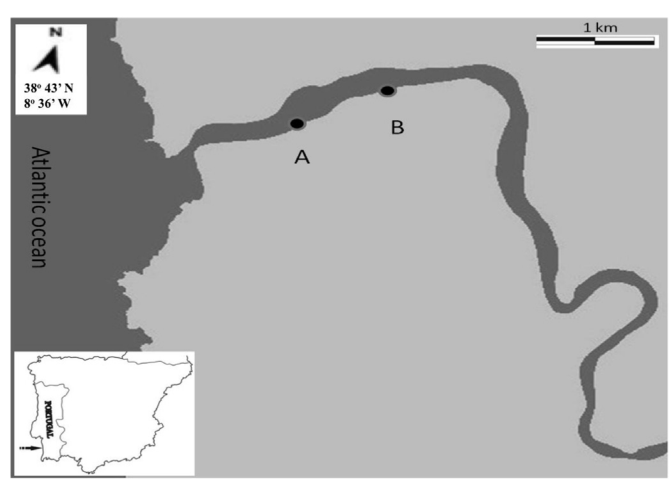

Fig. 1. Mira estuary (Portugal): indication of sampling sites (black circles) – (A, *ca.* 1.5 km from the mouth of the estuary, and B, 2 km upstream the river mouth).

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