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An experimental protocol to select nematode species from an entire community using progressive sedimentary enrichment

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ABSTRACT

The purpose of this study was to introduce free-living marine nematodes to the 'world of biomarkers'. Biomarkers are still not used in the monitoring and assessment of this highly diverse phylum, because the technique needs to be applied at the single species level. This incurs high costs and involves time-consuming procedures, which are currently the main pitfalls when it comes to the application of these techniques to marine nematodes. Consequently, this work proposes an innovative protocol for selecting a single species from an entire community of nematodes using two independent selection processes whereby the sediment of the microcosms is progressively and separately enriched with fine and coarse sediment fractions. During our experiment, the abundance and number of nematode species decreased discernibly with exposure to both finer and coarser sediment. Multivariate analyses revealed that deposit- and epigrowth-feeders were the most tolerant feeding guilds, probably due to their deposit-feeding and microvore behaviours. At the end of the experiment, *Terschellingia longicaudata* and *Ptycholaimellus ponticus* became the unique members of the community when the sediment was enriched with fine and coarse sediment particles, respectively. After the complete alteration of the community, and when the mono-species level had been achieved, it was possible to maintain these two species alive, and without any drop in numbers, under the same laboratory conditions considered during the selection process. Accordingly, the protocol adopted here lays new foundations for the study of nematodes in the biomarker field.

1. Introduction

Environmental pollution is one of the greatest problems facing the world today. Pollution can be either foreign chemicals/energies or naturally occurring substances that can disturb the stability of ecosystems and lead to volatility, disorder, harm, discomfort or even irreparable changes to the planet (Ramade and Papigny, 2000). The growing interest in these problems requires the assessment and adoption of low-cost, rapid, and reliable toxicity tests. Such tests could be applied to low or high levels of biological organization with the use of biomarkers or bioindicators, respectively.

Generally, biomarkers are chemicals, metabolites, susceptibility characteristics or physiological changes that relate to the exposure of an organism to a chemical. Accordingly, a selected biomarker, i.e. biological response, can be linked to a specific type of environmental exposure, and is representative of the health status of the species being studied (lysosomal biomarkers, immunotoxicity effects, energy metabolism impairments, endocrine disruption measures, and genotoxicity) (Arts, 2001). Therefore, biomarkers can be used as both diagnostic and predictive tools (Lagadic, 2002). In contrast, even though high levels of biological organization (individuals, populations or communities) have greater ecological relevance, they produce long-term responses to stressors.

Meiobenthic nematodes represent different trophic levels in the food chain (Gallucci et al., 2005; Moens et al., 2005). Accordingly, they can transfer pollutants to the higher trophic levels of the food web by being the prey for several macroinvertebrates and juvenile fish (Aarnio, 2000). Numerous investigations on the ecological quality of aquatic ecosystems are focused on the use of marine nematodes as bioindicators (Balsamo et al., 2012). Indeed, these nematodes are the most diverse, numerically dominant and widespread metazoans in such ecosystems, and are, like all the permanent members of the benthos, in direct contact with pollutants (Balsamo et al., 2010; Moreno et al., 2011; Boufahja et al., 2012). However, the results of studies on the structure and biodiversity of marine nematode assemblages do not always produce

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Table 1
Environmental parameters (±SD) considered at the 'Career Bay' site on January 1st and 8th 2014. *UTC*, Coordinated Universal Time; *PSU*, Practical Salinity Unit.

Sampling dates	January 1, 2014 (Preliminary sampling, PS)	January 8, 2014 (Sampling, S)	Student's *t*-test (*log*-transformed data)
Time (A.M. UTC)	7	7	
Air temperature (°C)	8.1	7.8	
Surrounding waters			
Temperature (°C)	8.2	8.0	
Salinity (PSU)	37.7	37.5	
Dissolved oxygen (mg l^{-1})	11.1	12.3	
pH	8.34	8.36	
Sediment			
Water content (%)	21.62 (0.31)	24.84 (0.75)	$p = 0.534$
Fine fraction (%)	50.25 (0.10)	49.41 (0.42)	$p = 0.621$
Mean grain size (mm)	0.82 (0.09)	0.77 (0.06)	$p = 0.238$
Organic matter (%)	8.13 (0.24)	8.97 (0.40)	$p = 0.587$

unequivocal results when it comes to environmental changes (Balsamo et al., 2012).

Despite the great ecological interest in this phylum, the information available in the literature about the use of biomarkers on nematodes is still inadequate. Indeed, biomarkers have only been used in a limited way on transgenic strains of *Caenorhabditis elegans* (Power et al., 1998) and a few other soil species (Arts, 2001). However, these techniques, which should be applied to individuals of a selected species sampled in the field (e.g. Lagadic, 2002; Hynea and Maher, 2003; Durou et al., 2007; Halldórsson et al., 2008) using a high quantity of biological material, have been completely ignored with respect to marine nematodes. This is partially due to the difficulty to collect a high amount of selected species belonging to inconspicuous organisms such as meiofaunal organisms, but that strongly reflect the functioning of the marine ecosystems (Danovaro et al., 2008).

Thus, the purpose of this study is to analyze, by means of the creation of laboratory experiments, the possible use of marine nematodes as biomarkers. This will only be achievable if we can: move from pluri- to mono-species levels of the community without causing genetic damage; and, at the same time, collect a high enough number of nematodes to make the application of biomarker techniques possible. Consequently, an ideal protocol for the use of nematodes would not only produce high numbers of a single species, but would also incur relatively low labour costs. Thus, the species selection has been progressively carried out in a gradient of progressive granulometry variation. In particular, the following questions are addressed: (1) is it possible to create a low labour-cost protocol for selecting a single nematode species from an entire community? (2) can selected species be maintained alive under laboratory conditions? and (3) how could mono-species cultures derived through the selection procedure be utilized in biomarker studies?

2. Materials and methods

2.1. Study area and sampling collection

Preliminary sampling (PS) was conducted on 1st January 2014 at numerous southern intertidal sites in the channel zone of the Bizerte Lagoon in Tunisia. This zone was chosen because it is located between the fine sediment of the lagoon and the sands of the bay, thus providing the best opportunity to find a site with a roughly equal percentage of fine and coarse sediment fractions. After a granulometric analysis of all of the samples, the 'Career Bay' site (37°14.043′ N, 09°50.162′ E) was the only one chosen for further use in this study, as it had equal proportions of fine (<63 μm, 50.27 ± 0.16%) and coarse (≥63 μm, 49.73 ± 0.16%) sediment particles (Table 1).

On the sampling day, the sediment was removed at low tide following 5 cm-deep parallel tracks (Fig. 1). Nematode communities of the intertidal habitat were selected for the experiment because naturally adapted to vertical migrations. They generally prefer oxic conditions (Steyaert et al., 2005), and even short-term hypoxia may cause a decline in their abundance (Wetzel et al., 2001; Steyaert et al., 2007; Arroyo et al., 2012). This was also why the sediment was collected from the upper 2 cm of the intermediate zones using a large spatula, before being temporarily preserved in a plastic container. It was then divided into two parts in the laboratory: the first part was preserved with its natural meiofauna until the experiment was set up; and the second was used for the application of treatments. Only the biota inhabiting the part of the sediment to be treated were killed. The sediments were defaunated by repeated (3 times) freezing to −20 °C for 12 h and thawing at room temperature for 48 h (Schratzberger et al., 2004).

The dissolved oxygen of the water-sediment interface was also evaluated using an oxymeter (WTW OXI 330/SET, WTW, Weilheim, Germany) (Table 1). Temperature and salinity were measured using a thermo-salinity meter (WTW LF 196, Weilheim, Germany) and pH with a pH meter (WTW pH 330/SET-1, Germany) (Table 1).

For the nematode analyses, three plastic, cylindrical jars 4 cm i.d. (i.e. internal diameter) and 5 cm in height were filled with sediment (4 cm thick) collected in situ from the uppermost 2 cm layers at the study site.

On 8th January 2014, another sampling campaign (S) was carried out using the same methods applied during the preliminary survey.

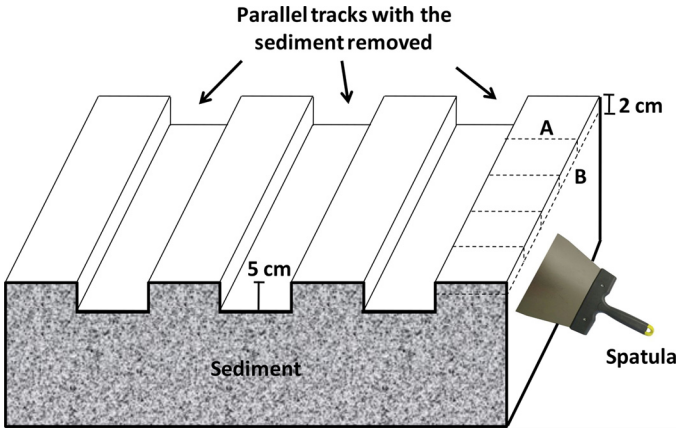

Fig. 1. Sediment collection method during the sampling: first, the sediment was removed following 5 cm-deep parallel tracks; and second, only the upper 2 cm of the intermediate zones were cut off with a spatula following the sketched lines A and B.