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Deep-sea bacterial communities in sediments and guts of deposit-feeding holothurians in Portuguese canyons (NE Atlantic)

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ABSTRACT

Deposit-feeding holothurians often dominate the megafauna in bathyal deep-sea settings, in terms of both abundance and biomass. *Molpadia musculus* is particularly abundant at about 3400 m depth in the Nazaré Canyon on the NE Atlantic Continental Margin. However, these high abundances are unusual for burrowing species at this depth. The objective of this research was to understand the reasons of the massive occurrence of these molpadiid holothurians in the Nazaré Canyon. To address this question we investigated possible trophic interactions with bacteria at sites where the organic content of the sediment was different (Setúbal and Cascais Canyons, NE Atlantic Continental Margin). The molecular fingerprinting technique of Denaturing Gradient Gel Electrophoresis (DGGE) with band sequencing, combined with non-metric multi-dimensional scaling and statistical analyses, was used to compare the bacterial community diversity in canyon sediments and holothurian gut contents. Our results suggest that *M. musculus* does not need to develop a specialised gut bacterial community to aid digestion where the sediment is rich in organic matter (Nazaré Canyon); in contrast, such a community may be developed where the sediment is poorer in organic matter (Cascais Canyon).

1. Introduction

Submarine canyons, deep incisions of the continental shelf and continental slope, are abundant along the European NE Atlantic Ocean and Mediterranean margins (Weaver et al., 2004; Canals et al., 2004). Over the last two decades, submarine canyons have received increasing attention as hotspots of benthic production (Vetter, 1994) and key habitats of commercially exploited species (Pagès et al., 2007). Compared to the surrounding continental slope areas, submarine canyons are characterized by a higher content of organic C and relatively high biogeochemical activity (Gage et al., 1995; Vetter and Dayton, 1998; Schmidt et al., 2001, Epping et al., 2002). The elevated benthic metabolic activity in canyons compared to adjacent regions is caused by the high delivery rate of organic matter (OM) (including fresh material) from the shelf directly to the deep sea (van Weering et al., 2002). These episodic inputs play an important role in deep-sea processes and also have an important effect on faunal distributions.

The composition and diversity of benthic communities reflect the stability of the systems they inhabit. Episodic events (slumps and turbidity flows) and tidal currents

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(reaching velocities of up to 2 m/s – Vetter and Dayton, 1998), cause resuspension and down-slope transport of sediments, creating significant disturbance to benthic ecosystems (Thorne-Miller and Catena, 1991). Faunal abundance and biomass in canyon systems are higher (e.g. Gage et al., 1995; Vetter and Dayton, 1998; Duineveld et al., 2001), and faunal diversity lower (e.g. Gage et al., 1995; Vetter and Dayton, 1998; Curdia et al., 2004), than in adjacent slope habitats. These differences have been attributed to the higher organic content of canyon systems (Gage et al., 1995; Duineveld et al., 2001).

The Portuguese margin has some of Europe's largest submarine canyon systems (Nazaré Canyon, Setúbal Canyon and Cascais Canyon – Fig. 1). As part of the EU Integrated Project HERMES (Hotspot Ecosystem Research on the Margins of European Seas), a number of research cruises have been undertaken to the Portuguese margin to investigate the composition, biomass and activity of benthic communities inhabiting these canyons. A large number of burrowing sea cucumbers (holothurians) is present in the central part of the Nazaré Canyon (3200–3400 m depth) (Weaver, 2005). On the other hand, the abundance of other benthic fauna commonly found at bathyal depths at continental margins is low. Burrowing sea cucumbers were also found in Setúbal and Cascais Canyons, albeit in low abundance. Two species of deposit-feeding holothurians were sampled in these canyons. One, *Molpadia musculus* Risso, 1826, is a sub-surface burrowing deposit-feeder with a mean abundance of 125 ind/m^2. The other, *Ypsilothuria bitentaculata* Ludwig, 1893, is a sedentary surface-feeder with a mean abundance of 136 ind/m^2. Their high densities are unusual for deep-sea burrowing species at this depth (Weaver, 2005).

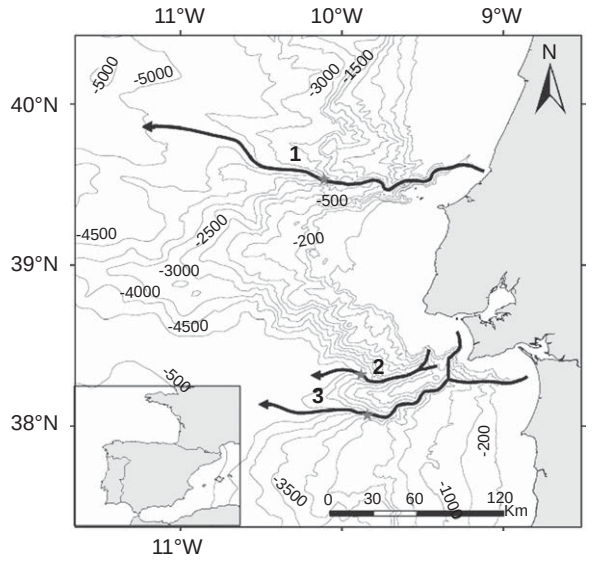

Fig. 1. Overview chart of the bathymetry of the Portuguese margin showing the location of the canyons: 1—Nazaré Canyon, 2—Cascais Canyon, 3—Setúbal Canyon. The ★ represents the stations (3500 m depth) sampled and the small square in the inset of Spain–Portugal the area zoomed in. Bathymetry from GEBCO, 2003.

Deposit-feeding holothurians dominate the deep-sea megabenthos in many areas, in terms of both biomass and abundance (Sibuet et al., 1982; Billett, 1991; Roberts et al., 2000). At the Porcupine Abyssal Plain (PAP; NE Atlantic Ocean), for example, holothurians account for ~76 and 93% of the megabenthos abundance and biomass, respectively (Billett et al., 2001). According to Sibuet (1985), food supply is an important factor controlling the abundance and the composition of the holothurian assemblages. The various species have each developed a different way of exploiting food sources. Individual holothurian species show both behavioural and morphological adaptations including habitat selection (Billett, 1991), differences in tentacle morphology (Roberts et al., 2000; Roberts and Moore, 1997) and gut diversification (Moore et al., 1995). The material ingested from the surface sediment by these deposit-feeding holothurians includes micro-organisms, meiofauna, decaying organic debris and inorganic components, as well as dissolved organic matter (DOM) (Roberts et al., 2001). The nutritional value of the sediments varies seasonally (Billett et al., 1983) and with distance from the source of organic material, which is generally produced photosynthetically in surface waters and sinks to the ocean floor as phytodetritus. The overall nutritional value of the phytodetritus is reflected by the biomass of both bacteria and invertebrate megafauna colonizing it (Roberts et al., 2001). High bacterial abundances have been recorded in the guts of deep-sea holothurians (Deming and Colwell, 1982; Sibuet et al., 1982; Roberts et al., 2001) and there is often a proliferation of microbes in their hindguts, suggesting that sediment and/or enteric bacteria play an important role in their nutrition. The deep-sea holothurians may use the bacteria directly as food source or they can use the bacteria indirectly to provide them with the essential nutrients not available otherwise (Deming and Colwell, 1982; Deming et al., 1981; Eardly et al., 2001).

This aim of this study is to understand how the high abundance of the holothurian *M. musculus* can be maintained in the bathyal Portuguese canyons (Nazaré, Setúbal and Cascais). We hypothesized that trophic interactions with enteric bacteria make the high organic content of the canyon sediment available to the holothurians. To address these issues, sediment samples and holothurian gut contents were collected in the three canyons and their composition analysed for bacterial diversity. We used denaturing gradient gel electrophoresis (DGGE) as a fingerprinting technique to characterize the bacterial community composition and sequencing, followed by multi-dimensional scaling and other statistical analyses, to evaluate bacterial diversity and potential differences between sample types.

2. Materials and methods

2.1. Study site

The Nazaré Canyon (Fig. 1) is a very narrow and deep canyon without a connection to a major river outflow. By